THE SOUTH AFRICAN DENTAL JOURNAL

SADJ

MAY 2025
Volume 80 Number 4

ISSN No. 2519-0105 – Online Edition
ISSN No. 1029-4864 – Print Edition

SADA
THE SOUTH AFRICAN
DENTAL ASSOCIATION

Cape Agulhas Lighthouse

The Cape Agulhas Lighthouse, built in 1849, is the second-oldest working lighthouse in South Africa. Located at the southernmost tip of Africa, it marks the meeting point of the Atlantic and Indian Oceans. Its design was inspired by the Lighthouse of Alexandria, featuring a striking red and white tower. Originally oil-fueled, it now operates electrically, warning ships of the nearby Agulhas Reef. The lighthouse includes a museum and offers scenic views, attracting both tourists and maritime enthusiasts.

REGULATIONS ARE NOW HERE!

DRISA

IS YOUR PRACTICE COVERED? ACT NOW LOCK IN SAVINGS!

South African regulations are official as of March 31st, 2025. The **DFFE's Regulations for the Management of Mercury in South Africa** have been finalized and the time to comply is now. No mercury can be in the environment as of today, and DRI-SA has partnered with SADA to offer its members a complete solution to meet this regulation with incredible savings.

Don't delay - it costs nothing to sign up now and you can lock in your annual recycling pricing with 3 or 5-year term agreement available.
Sign up before September 1st and lock in your savings - your annual recycling payment is not due for 12 months!
A 25000 ZAR value - FREE with your SADA Membership.

GET YOUR FREE SOLUTIONS TODAY!

- Amalgam Separator
- Chairside Amalgam Kit

WHY SIGN UP NOW?

- Compliance Regulation in Effect Now
- Meet Regulatory Requirements for South Africa
- R25,000 Worth of FREE Products for SADA Members*
- Lock in Annual Recycling costs for 3 or 5 years
- No Recycling Payments till 2026 (12 mo after sign up)
- Easy Installation with Wright Millners partner
- DRI-SA handles all recycling and documentation

OFFER ENDS SEPT 1 2025 - ACT NOW!

R25,000 VALUE!

Lock in Pricing.
Start Complying.
Enjoy Peace of Mind.

SCAN ME

Learn More on Our Website: www.dri-sa.co.za

*Free amalgam separators and chairside kits are available with a signed service agreement of 3 or 5 years to lock in annual recycling pricing. This does not include installation of the Free unit,, as this service is provided by DRI-SA installation partner Wright Millners.

THE SOUTH AFRICAN DENTAL JOURNAL

SADJ

EDITORIAL OFFICE
Managing Editor
Prof NH Wood

Editorial Assistant
Mr Dumi Ngoepe
Email: Sadj@sada.co.za

Sub-editors
Prof N Mohamed
Prof P Owen
Prof L Sykes
Prof J Yengopal

Please direct all correspondence to:
South African Dental Association
Private Bag 1, Houghton 2041
Tel: +27 (0)11 484 5288
Fax: +27 (0)11 642 5718
Email: info@sada.co.za

Editorial Board

Prof V Yengopal	University of the Western Cape
Prof T Madiba	University of Pretoria
Prof S Singh	University of KwaZulu-Natal
Prof P Owen	University of Witwatersrand
Prof P Motloba	Sefako Makgatho Health Sciences University
Prof P Moipolai	Sefako Makgatho Health Sciences University
Prof NH Wood	University of Witwatersrand
Prof MPS Sethusa	Sefako Makgatho Health Sciences University
Prof L Sykes	University of Pretoria
Dr J Schaap	University of Pretoria
Prof J Morkel	University of the Western Cape
Prof E Rikhotso	University of Witwatersrand
Dr R Mthetwa	Sefako Makgatho Health Sciences University
Dr E Patel	University of Witwatersrand
Prof N Mohamed	University of the Western Cape
Prof H Holmes	University of the Western Cape
Prof H Gluckman	Private Practice
Dr C Nel	University of Pretoria

SADA OFFICE BEARERS
National Council

President :	Dr P Mathai
Vice President:	Dr S Ngandu

SADA Board of Directors
Board of Directors:
Dr F Meyer (Chairman)
Dr F Mansoor (Vice Chairperson)
Dr P Mathai,
Dr J Welgemoed,
Dr N Niranjan,
Dr YF Solomons,
Dr R Vermeulen,
Mr. KC Makhubele (Chief Executive Officer)

Published by:
CREATIVE SPACE MEDIA

On behalf of:
SADA

CONTENTS

Our Front Cover for this Issue...

Cape Agulhas Lighthouse

The Cape Agulhas Lighthouse, built in 1849, is the second-oldest working lighthouse in South Africa. Located at the southernmost tip of Africa, it marks the meeting point of the Atlantic and Indian Oceans. Its design was inspired by the Lighthouse of Alexandria, featuring a striking red and white tower. Originally oil-fueled, it now operates electrically, warning ships of the nearby Agulhas Reef. The lighthouse includes a museum and offers scenic views, attracting both tourists and maritime enthusiasts.

THE SOUTH AFRICAN DENTAL JOURNAL
SADJ

SADA OFFICE BEARERS
Chairpersons of Board Sub-Committees
Audit and Risk Committee: Dr F Mansoor
Strategy Ethics and
Remuneration Committee: Dr N Niranjan
Dental Practice Committee: Dr J Welgemoed

Chairperson of DDF Board of Trustees
Dr B Belinsohn

PRODUCTION OFFICE
Creative Space Media
Tel: +27 (11) 467 3341
Website: www.creativespacemedia.co.za

Publisher and Project manager
Leani Thomson – leani@creativespacemedia.co.za
Sizwe Zim – Sizwe@creativespacemedia.co.za

GENERAL AND ADVERTISING ENQUIRIES
James Chademana
Email: james@creativespacemedia.co.za
Tel: +27 (11) 467 3341

Design and Layout
Leani Thomson
Email: leani@creativespacemedia.co.za

Website smalls advertising / CPD Enquiries and Member contact detail update
South African Dental Association
Tel: +27 (0)11 484 5288
Email: marketing@sada.co.za

Published by:

CREATIVE SPACE MEDIA

On behalf of:

SADA
THE SOUTH AFRICAN DENTAL ASSOCIATION

CONTENTS

Editorial, Advertising and Copyright Policy
Opinions and statements, of whatever nature, are published under the authority of the submitting author, and the inclusion or exclusion of any medicine or procedure does not represent the official policy of the South African Dental Association or its associates, unless an express statement accompanies the item in question. All articles published as Original Research Papers are refereed, and articles published under Clinical Practice or Reviewed Notes are submitted for peer review.

The publication of advertisements of materials or services does not imply an endorsement by the Association or a submitting author, should such material feature as part of or appear in the vicinity of any contribution, unless an express statement accompanies theitem in question. The Association or its associates do not guarantee any claims made for products by their manufacturers.

While every effort is made to ensure accurate reproduction, the authors, advisors, publishers and their employees or agents shall not be responsible, or in any way liable for errors, omissions or inaccuracies in the publication, whether arising from negligence or otherwise or for any consequences arising therefrom.

The South African Dental Journal is a peer reviewed, Open Access Journal, adhering to the Budapest Open Access Initiative: "By 'open access' to this literature, we mean its free availability on the public internet, permitting any users to read, download, copy, distribute, print,search, or link to the full texts of these articles, crawl them for indexing, pass them as data to software, or use them for any other lawful purpose, without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. The only constraint on reproduction and distribution, and the only role for copyright in this domain, should be to give authors control over the integrity of their work and the right to be properly acknowledged and cited."

Accredited by the Department of Education SADJ is published 10 times a year by Creative Space Media,

Peer Review Matters: A Call to Strengthen South African Dental Scholarship

SADJ MAY 2025, Vol. 80 No.4 P179-180

Prof NH Wood, Managing Editor, SADJ – BChD, DipOdont(MFP), MDent(OMP), FCD(SA), PhD

Behind every credible scientific journal stands a dedicated community of reviewers who quietly uphold the standards of research, clinical relevance, and academic integrity. At the South African Dental Journal (SADJ), peer review is more than a procedural requirement; it is the backbone of our commitment to rigorous, relevant, and ethically sound publication. As we continue to grow, evolve, and respond to the needs of our profession, we issue a sincere call: we need even more competent academic reviewers to join our panel.

Why reviewers matter, especially now

The role of the peer reviewer has never been more important. In a world of rapidly emerging information, the responsibility to ensure that published research is methodologically robust, clinically applicable, and contextually relevant rests heavily on the shoulders of reviewers. Within the South African and broader African context, this responsibility takes on an additional dimension: ensuring that the evidence base reflects our unique clinical challenges, health system constraints, and population needs.

Our reviewers are critical gatekeepers. They help strengthen the scientific quality of articles, identify ethical concerns, and ensure that the research we publish reflects the evolving standards of academic and clinical excellence, and ensure our papers meet current standards and criteria accepted by the greater academic community. By offering thoughtful critique and constructive feedback, reviewers mentor authors, uphold professional standards, and shape the future of our discipline.

Who should consider reviewing?

We invite clinicians, academics, researchers, and postgraduate students with a strong grounding in their area of expertise to participate in the peer review process. Whether you are an experienced scholar or an early-career academic looking to build your professional profile, peer reviewing is an invaluable opportunity to:

- Stay current with emerging research in your field.
- Refine your own scholarly writing and critical thinking.
- Contribute to the quality and credibility of South African dental literature.
- Play a part in elevating the profile of local research on the global stage.

We are particularly seeking reviewers across all major dental disciplines including Oral Medicine and Periodontology, Prosthodontics, Paediatric Dentistry, Community Dentistry, Maxillofacial Radiology and Surgery, Orthodontics, and Dental Education, Dental Public Health, Endodontics, Aesthetics, Pain management, Basic Sciences, and many more.

A Note on misinformation and the integrity of local scholarship

It is unfortunate that in some academic circles, there are those who actively discourage publishing in the SADJ, often based on misinformed or misleading assertions. These types of statements are not only factually incorrect, but also ethically troubling, as it undermines efforts to build strong, credible, African-led academic platforms locally. The SADJ is currently on the DHET accredited journals list and be aware of those suggesting otherwise. The only way to continue to grow Dentistry in South Africa is to participate, and to facilitate development of the academic platforms such as the SADJ.

To be clear, the SADJ does not charge any author publication fees, except in cases where a manuscript significantly exceeds the standard page limits, a policy transparently communicated in our author guidelines and comparable to those of respected journals worldwide. Suggesting otherwise not only harms the journal, but also obstructs the development of a self-sustaining, respected local academic ecosystem, something we all share responsibility for strengthening.

Why your participation matters

Too often, the work of peer review is undervalued or left to a small number of dedicated individuals. To those we remain deeply grateful. But the responsibility for scholarly integrity must be shared across the profession. By participating in peer review, you are not only giving back, you are investing in the quality, credibility, and future of dental science in South Africa.

Reviewing is also an important part of your own academic journey. It strengthens your reputation, deepens your insight, and reflects a commitment to academic citizenship which is a principle we hope to model for both peers and students alike.

Join us!

If you are ready to contribute to the advancement of dental scholarship in South Africa, we invite you to apply to join our reviewer panel. Please send your academic profile and areas of interest to the SADJ Editorial Office at: sadj@sada.co.za

Together, we can ensure that the South African Dental Journal remains a trusted, respected, and proudly African platform for academic excellence.

Medical Aid Schemes Playing Judge, Jury, and Executioner: Who Really Decides Your Dental Treatment?

SADJ MAY 2025, Vol. 80 No.4 P18-182

Mr KC Makhubele – CEO, South African Dental Association

South Africa's Medical Aid Schemes /Third Party Funders have taken on a troubling role—one that places them in direct conflict with the very professionals responsible for delivering quality healthcare. Dentists and other oral health practitioners are finding themselves at the mercy of faceless assessors, often with questionable / unknown/ undisclosed qualifications, who dictate which treatments are "necessary" and which should be rejected.

This raises a fundamental and deeply disturbing question: Who is better positioned to make a clinical judgment—the dentist examining a patient in real-time or an administrator sitting behind a desk, assessing treatment claims based on paperwork alone?

The Dangerous Power of Medical Aid Schemes /Third Party Funders Assessors

It is common knowledge that medical aids review claims before approving or rejecting them. This process is meant to prevent fraud, a good goal, but more often used to ensure financial sustainability of the scheme. However, what is happening in South Africa's oral healthcare sector is something far more sinister—medical schemes are overriding the clinical decisions of trained professionals, often to the detriment of patients.

Our observation is that some claims are dismissed based on the opinion of an assessor who has never seen the patient, has no direct insight into their condition, and in some cases, may not even be qualified to evaluate complex dental procedures. There are unverified reports that even cases where oral hygienists and dental therapists—who do not have the same scope of practice as dentists—are placed in decision-making roles over the work of fully qualified dentists. If this is verified, it is not just concerning; it is a scandal that undermines both patient care and professional integrity.

What Qualifies These "Referees" to Overrule Dentists?

This brings us to an even more serious issue: What qualifies these so-called assessors to overrule the judgment of treating professionals? If a medical aid assessor is a dental therapist, oral hygienist, or, even worse, someone with no formal oral health qualifications, how can they be the ultimate authority on what treatment is appropriate for a patient?

Dentists spend years in intensive training, continuously updating their knowledge to keep up with advances in oral healthcare. Their expertise is built on hands-on experience with patients—not just theoretical knowledge. The idea

that a remote assessor, potentially with a lower level of qualification, can overrule a dentist's decision is not only absurd but a dangerous precedent that threatens the very foundation of ethical patient care.

The Human Cost: When Profits Come Before Patients

The impact of this system is not just bureaucratic; it has real, painful consequences for patients. A dentist may recommend a specific procedure based on clear clinical evidence, but if the medical aid refuses to cover it, the patient is either forced to pay out of pocket—often at great financial strain—or settle for a suboptimal alternative.

This practice prioritizes the financial interests of medical aid scheme over the health of patients. It turns healthcare into a numbers game, where the goal is to save money rather than provide the best possible treatment. And yet, medical schemes continue to sell themselves as partners in healthcare, while they act as cost-cutting corporations that interfere with professional judgment.

Time for Accountability: Medical Aids Must Answer to Practitioners

It is time for South Africa's oral health practitioners to demand accountability from medical aid schemes. There must be:

Transparency on Assessors' Qualifications – If a medical aid assessor is rejecting a treatment plan, they should be required to disclose their own qualifications and justify why they believe they are more competent than the treating dentist.

An Independent Review Process – Decisions that override a practitioner's treatment plan should be reviewed by an independent panel of experts, not just internal medical aid employees.

Professional Oversight – The Health Professions Council of South Africa (HPCSA) and the South African Dental Association (SADA) must investigate cases where unqualified or underqualified individuals are making treatment determinations.

To this end, the South African Dental Association will initiate discussions with Medical Aid Schemes to reveal who is responsible for making these decisions and how they are made. We will also express our serious concerns in the hopes of finding more effective solutions.

Enough is Enough

South Africa's oral healthcare system cannot continue to operate under a model where medical aids have unchecked power to dictate treatment decisions from behind a desk. Dentists are trained to heal, not to fight bureaucratic battles with people who do not share their level of expertise.

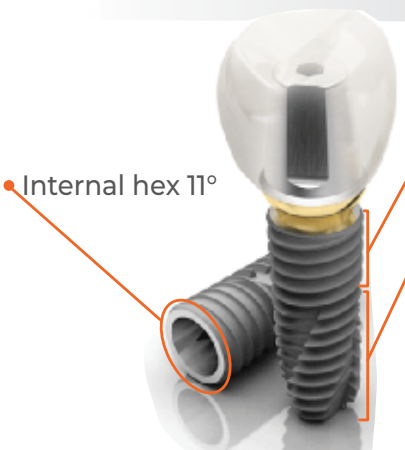
If the current trend continues, the message will be clear: In South Africa, your dental health is not decided by your dentist—it is dictated by a medical aid scheme that prioritizes cost-cutting over care. And that is something every healthcare professional, and every patient, should be outraged about.

OSSTEM IMPLANT

Why Osstem?
A Global Leader in Dental Implants

- Available in over 90 countries worldwide
- Top-selling implant brand (Global Rank #3 - 2022)
- Proven clinical track record (10+ years)
- Advanced surface & digital convergence technology
- 98.9% surgical success rate
- Easy certification access anytime, anywhere

Meet the TS III SA Implant
Submerged type implant with an internal hex 11° tapered connection structure



- Connection - Mini / Regular
- Excellent initial stability in soft bone with smaller threads in the upper section
- Corkscrew thread & cutting edge
- Sand-blasted Acid-Etched surface
- Cost-effecive

OSSTEM SA IMPLANT
021 883 2225
info@osstemsa.com
www.osstemsa.com

Precision Meets Performance

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.20799
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

Radiation protection and compliance with radiation safety standards by dental professionals and radiographers in rural Limpopo Province, South Africa: a cross-sectional study

SADJ MAY 2025, Vol. 80 No.4 P183-P190

RM Modiba[1], I Ntatamala[2], T Netshisaulu[3]

ABSTRACT

Introduction

Poor radiation protection practices in South African healthcare facilities have been reported on account of several factors including staff shortages, poor training on radiation protection, and inconsistent dosimeter use.

Aims and objectives

To investigate radiation protection practices of dental professionals and radiographers in Limpopo Province following the closure of two radiography units due to non-compliance.

Methods

A quantitative cross-sectional study was conducted among the eight rural hospitals in the Waterberg District of the Limpopo Province, South Africa, using a self-administered questionnaire comprised of five components exploring the knowledge and practices of dentists (36%), oral hygienists (18%) and dental therapists (18%) and radiographers (38%). Forty-five participants (*n*=45) completed the study questionnaire, representing a 75% response rate (45/60).

Results:

Dentists (63.0%) felt slightly at risk of radiation exposure compared to radiographers who felt at risk (59.0%). Only 58.8% of radiographers, and even fewer dental professionals (37.5% of dentists and 16.7% of oral hygienists & dental therapists) admitted to always wearing their personal radiation dosimeters in the workplace (p=0.03). Equally concerning is that only a third of radiographers (29.4%) reported always protecting patients before radiological examinations compared to most dentists (93.8%) and oral hygienists & dental therapists (83.3%) (p<0.001).

Conclusion:

Dental professionals and radiographers in this study underestimated the long-term health impacts of radiation exposure on themselves and patients, and should be better supported to comply with radiation safety protocols.

Authors' information:

1. Reshoketswe Mokgadi Modiba, Department of Health Sciences, University of Limpopo, School of Public Health, Turfloop Campus, P/Bag X1106, Sovenga, 0727, South Africa. ORCID: 0009-0004-3281-5156
2. Itumeleng Ntatamala, Division of Occupational Medicine and Centre for Environmental and Occupational Health Research, School of Public Health, University of Cape Town, Cape Town, South Africa. ORCID: 0000-0001-9799-0132
3. Thomas Netshisaulu, Department of Physics, University of Limpopo, Turfloop Campus, P/Bag X1106, Sovenga, 0727, South Africa. ORCID: 0000-0002-7792-8494

Corresponding author:
Name: Itumeleng Ntatamala,
Email address: Itumeleng.ntatamala@uct.ac.za (I. Ntatamala)

Contributors
RM and TN contributed to the conception or design of the work, the acquisition, analysis, and interpretation of the data. All authors were involved in drafting and commenting on the paper and have approved the final version.
Funding: This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author declaration and competing interests
The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval
Participation in the study was voluntary. Informed written consent was obtained from all participants and confidentiality assured. Ethical approval for the study was obtained from the University of Limpopo (MREC/H/12/2010:PG) and the Limpopo Department of Health's Research Ethics Committee (Ref 4/2/2).

Introduction

The discovery and use of ionizing radiation (hereafter referred to as radiation) has been both beneficial and detrimental to human health. The beneficial effects of radiation include the use of x-rays in diagnostic medicine and health sciences research, but also detrimental due to potential adverse health effects arising from acute and chronic exposure. Occupational exposure to radiation is mostly associated with low dose, chronic exposure which can result in adverse health outcomes such as cataracts and thyroid cancer.[1, 2, 3] As a treatment modality, ionizing radiation is intangible, neither seen or heard, which may contribute to inconsistent radiation protection and safety practices.[1] Healthcare workers such as dental professionals, who are exposed to ionizing radiation of over 20mSv per annum because of their occupation are defined as radiation workers. The recent COVID-19 pandemic has further highlighted that while there was a significant focus on using personal protective equipment and improving access to occupational health services to prevent radiation workers being infected with SARS-CoV-2, there was an equal need to promote continued compliance with radiation protection practices.[4,5]

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.20799
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

Several international bodies including the International Commission of Radiation Protection (ICRP), and International Atomic Energy Agency (IAEA) have highlighted the importance of adequate radiation protection and safety practice in radiation workers by making recommendations regarding radiography equipment quality, setting occupational radiation exposure limits, and detailing imaging standard operating procedures/ protocols.[6, 7] The International Labour Organisation (ILO) has also adopted the Radiation Protection Convention, 1960 (No. 115), and its accompanying Recommendation (No. 114) advocating for the reduction of ionizing radiation to the lowest practicable level and avoidance of any unnecessary exposure through collaboration between employers, workers, and governments.[8]

Various legislation in South Africa have been promulgated as early as 1973 to regulate the amount of ionizing radiation emitted by electronic products, including the Hazardous Substances Act, 1973 (Act 15 of 1973) and Regulations (No R1332 of 3 August 1973) that govern the safe use of electronic products, including medical x-ray equipment found in dental practices and radiography units.[9] The establishment of the Radiation Control Directorate in the National Department of Health (NDOH) and Code of Practice of Electronic Products have emphasized the importance of radiation protection and safety practice in all health facilities in the country based on the three main radiation protection principles of justification of the practice/investigation, optimization of protection (ALARA, keeping occupational and workplace exposures as low as reasonably achievable), and dose limitation.[10]

Dental professionals and diagnostic radiographers in South Africa must be registered with the Health Professions Council of South Africa (HPCSA) which has a Medical and Dental Professions Board and a Radiography and Clinical Technology Board that regulates the professional training and conduct of dental professionals and radiographers. Diagnostic radiographers perform radiation work such as general x-ray examinations, mobile radiography, fluoroscopy, angiography, computed tomography, magnetic resonance imaging, and mammography.[11]. The HPCSA notes for example that one of the key tasks radiographer's have include "understanding and observing health and safety at work as well as welfare issues, including infection control policies and ionising radiation regulations in order to protect themselves and others".[11] Radiation safety training for radiation workers such as dental professionals and radiographers takes place during undergraduate studies and in the workplace, commonly in the first year of professional work (compulsory community/ national service year) and thereafter in the public or private sector. The quality of the training received is however variable. Van der Merwe et al[12] has noted that undergraduate radiography students are often placed in clinical practice without proof of sufficient radiation safety knowledge, while Lewis et al[13] has also reported insufficient training even in qualified radiographers in practice. More recently, the *South African Health Products Regulatory Authority's Radiation Control Unit has hosted several* radiation safety training webinars online with a focus on improving radiation safety training for radiation workers, including those in *rural and remote areas who are able to livestream.*[14]

Despite these regulations, guidance documents and training, evidence of poor radiation protection practices in South African healthcare facilities have been consistently reported in largely urban and more economically advanced areas of South Africa.[15, 16, 17] There is an inequitable distribution of radiographers in South Africa, with more human and financial resources concentrated in the private health sector and larger central public sector hospitals compared to the often-under-resourced rural district and primary healthcare facilities.[16, 18] As of 2020, South Africa has a national average of 5.94 radiographers per 100 000 population, which constitutes 1.2% of the public health workforce, [19] While most radiographers in South Africa's nine provinces may be employed in either the public or private sector (or both concurrently), most are employed in more developed provinces such as the Western Cape (ratio: 9.36 per 100,000 public sector population) compared to more rural provinces such as Mpumalanga (ratio: 3.42 per 100,000). [19] Other systemic challenges faced in the health sector include not wearing registered dosimeters, poor quality control on x-ray machines, the lack of specialized radiologists, customized radiation guidelines and failing infrastructure in South African health facilities. [18, 20]

Significant challenges with compliance to set radiation protection standards have been experienced in largely rural and remote provinces which resulted in the closure of two x-ray/radiography units in Limpopo Province (Waterberg District) by the Radiation Control Directorate of the South African National Department of Health due to non-compliance. The factors contributing to these closures of radiation units and extent to which healthcare workers in rural healthcare facilities have adopted radiation safety practices are to be determined. The common work practices of the radiographers in the province consist of performing general and special radiological procedures that include an average of 45 x-rays per radiographer each day with a total of 1,700 x-ray examinations performed per month in the biggest hospital in the rural Waterberg District. The specialized examinations include assisting in/performing mammography, ultrasound, and computed tomography scans (CT scans) procedures. The oral hygienists, dental therapists and dentists do also assist in/perform 18 to 25 intra and extra oral radiography procedures each day including periapical, occlusal, bitewings x-rays (oral radiography) and panoramic and cephalometric x-rays (extra oral radiography). The dental professionals are not in the room during the patient exposure.

The study aims to investigate radiation protection practice and compliance with radiation safety standards by dental professionals and radiographers in rural health facilities of Waterberg District in Limpopo Province, South Africa.

Materials and methods

2.1. Study design and sampling

A quantitative cross-sectional study was conducted among the eight rural hospitals in the Waterberg District of Limpopo Province, South Africa. The study population included all radiographers and dental professionals in these institutions (one regional hospital and seven district level hospitals) to ensure a statistically significant sample size with adequate power. The total population therefore consisted of 60 radiation workers (namely 21 dentists, 6 oral hygienists and 8 dental therapists, and 25 radiographers) employed in the eight institutions. These health professionals work with both intra-oral and extra-oral x-ray machines serving a mostly rural population.

2.2. Study methods

An author generated questionnaire was developed from the

https://doi.org/10.17159/sadj.v80i04.20799
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

available evidence-based literature. The questionnaire was comprised of a combination of closed and open-ended questions divided into five components. The first component consisted of socio-demographic questions regarding participant's gender, age, level of education, designation/ occupation, and length of service. The second component evaluated participant's sources of exposure and knowledge about ionizing radiation, while the third component enquired about the ill effects of radiation exposure. The fourth component was organized as a series of statements, and participants were asked to express their opinion on a 4-point scale about compliance to radiation safety protocols. The last component consisted of was open-ended questions that probed further about radiation safety protocols and the risks associated with radiation exposure. The researchers hand-delivered the questionnaires to all participants at the eight rural hospitals after obtaining access into the health facility from the management.

2.3. Data analysis

The data were analyzed using SPSS (Statistical Product Service Solutions) Version 28. The responses to open-ended questions in the last component of the questionnaire were read, and re-read by the researchers, followed by coding, and forming of categories from similar responses that addressed radiation protection practice and compliance with radiation safety standards. Descriptive statistics for continuous variables was presented as frequencies and percentages. In addition, a Pearson's chi-square test was used to determine whether there was an association between categorical variables (at a p-value < 0.05).

2.4 Consent and ethical approval

Ethical approval for the study was obtained from the University of Limpopo (MREC/H/12/2010:PG) and the Limpopo Department of Health's Research Ethics Committee (Ref 4/2/2). The ethics approval letters were shared with the Chief Executive Officers/Clinical Managers of the eight participating rural health facilities. Participation in the study was voluntary and confidentiality assured.

Results

3.1. Socio-demographic variables

Forty-five (45) participants *(Table 1)* from the various rural hospitals in the Waterberg District completed the study questionnaire, representing a 75.0% response rate (45/60). The participants were largely female (67.0%) and below the age of 30 years (62.0%). The majority had received a bachelor/degree qualification (67.0%) and most had been employed for under 5 years (58.0%). Over a third (37.0%) of the participants were radiographers while remaining 63.0% were dental professionals (including oral hygienists [OH], dental therapists [DT] and dentists).

3.2 Knowledge, awareness, and perceived risk of ionizing radiation exposure

Understanding of safe dose of medical x-ray radiation

Most participants were able to identify x-rays as source of ionizing radiation (100% for both radiographers, OH & DT and 95% of dentists). The participant's understanding on whether there is a safe dose of x-ray radiation from medical imaging in a day was further explored (**Table 2**). The majority (64.7%) of all radiographers agreed (35.3% strongly agree and 29.4% agree) that there is a safe dose of radiation from x-rays that one can take in a day. Similarly, 83.3% of OH & DT (58.3% strongly agree and 25.0% agree) and 100% dentists (37.5% strongly agree and 62.5% agree) also shared a similar view. While differences in understanding of safe dose of x-ray radiation were observed amongst the professionals, these were not statistically significant

Table 1: Participants socio-demographic characteristics (n=45)

Item		Number of participants (%)
Gender	Male	14 (31)
	Female	30 (67)
	Unspecified/missing	1 (2)
Age (year)	Less than 30 years	28 (62)
	30 years and over	15 (34)
	Unspecified/missing	2 (4)
Level of education	Grade 12 plus Diploma	11 (24)
	Degree	30 (67)
	Postgraduate qualification	4 (9)
Length of service (year)	1 – 5	26 (58)
	6 – 10	7 (16)
	11 – 15	4 (9)
	16 – 20	2 (4)
	>21	2 (4)
	Unspecified/missing	4 (9)
Designation	Radiographers	17 (38)
	Dentists	16 (36)
	Oral Hygienists (OH)	6 (13)
	Dental Therapist (DT)	6 (13)
Total		**45 (100)**

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.20799
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

Table 2: Survey questions and participant responses

	Question: There is a safe dose of ionising radiation exposure					
Job category	**Strongly Agree**	**Agree**	**Disagree**	**Strongly disagree**	**Total**	**Pearson chi-square**
Radiographer	6 (35,3%)	5 (29,4%)	1(5,9%)	5 (29,4%)	17 (100,0%)	P=0.107
Dentist	6 (37,5%)	10 (62,5%)	0 (0,0%)	0 (0,0%)	16 (100,0%)	
OH&DT	7 (58,3%)	3 (25,0%)	0 (0,0%)	2 (16,7%)	12 (100,0%)	
Total	19 (42,2%)	18 (40,0%)	1 (2,2%)	7 (15,6%)	45 (100,0%)	
	Question: A pregnant radiographer can continue to perform her x-ray duties					
Job category	**Strongly Agree**	**Agree**	**Disagree**	**Strongly disagree**	**Total**	**Pearson chi-square**
Radiographer	2 (11,8%)	12 (70,6%)	1 (5,9%)	2 (11,8%)	17 (100,0%)	P=0.461
Dentist	4 (25,0%)	6 (37,5%)	3 (18,8%)	3 (18,8%)	16 (100,0%)	
OH&DT	3 (25,0%)	4 (33,3%)	3 (25,0%)	2 (16,7%)	12 (100,0%)	
Total	9 (20,0%)	22 (48,9%)	7 (15,6%)	7 (15,6%)	45 (100,0%)	
	Question: Frequency of the use of personal radiation dosimeters					
Job category	**Always**	**Sometimes**	**Rarely**	**Never**	**Total**	**Pearson chi-square**
Radiographer	10 (58,8%)	6(35,3%)	1(5,9%)	0 (0,0%)	17 (100,0%)	P=0.033
Dentist	6(37,5%)	4 (25,0%)	3 (18,8%)	3 (18,8%)	16 (100,0%)	
OH&DT	2 (16,7%)	3(25,0%)	1(8,3%)	6(50,0%)	12 (100,0%)	
Total	18(40,0%)	13 (28,9%)	5 (11,1%)	9 (20,0%)	45 (100,0%)	
	Question: Frequency of patient protection practices					
Job category	**Always**	**Sometimes**	**Rarely**	**Never**	**Total**	**Pearson chi-square**
Radiographer	5 (29,4%)	12 (70,6%)	0 (0,0%)	0 (0,0%)	17 (100,0%)	P <0,001
Dentist	15 (93,8%)	1 (6,3%)	0 (0,0%)	0 (0,0%)	16 (100,0%)	
OH&DT	10 (83,3%)	1 (8,3%)	1 (8,3%	0 (0,0%)	12 (100,0%)	
Total	30 (66,7%)	14 (31,1%)	1 (2,2%)	0 (0,0%)	45 (100,0%)	

(p=0.11).

Perceived risk of radiation exposure

When asked about their perceived level of exposure to ionizing radiation, radiographers felt more (35.0%) or highly at risk (24.0%) of radiation exposure than other health-care professionals while most dentists (63.0%) feeling only slightly at risk (**Figure 1**). Most radiographers attributed this perception to their daily continuous exposure to x-ray duties compared to other health-care professionals and the general population which would generally experience

Figure 1: Participants' responses on the risk of radiation exposure by health-care professionals

https://doi.org/10.17159/sadj.v80i04.20799
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

exposure only when medically warranted. Response to open ended questions identified possible factors that placed them at increased risk including the lack of access to personal dosimeters, uncertainty as to who should provide protective lead shields, poor maintenance of x-ray machines, use of mobile/portable x-ray units in theatre and when screening.

Performance of x-ray duties by a pregnant radiographer

The participant's knowledge of occupational health requirements for radiation workers, particularly vulnerable pregnant workers, was investigated (**Table 2**). Participants were asked whether a pregnant radiographer could continue performing her x-ray duties. Most radiographers (82.4%) agreed that a pregnant radiographer can continue to perform her x-ray duties, while dental professionals also agreed, though to a lesser extent (58.3% OH & DT and 62.5% dentists). The differences observed were however not statistically significant (p=0.46).

3.3. Radiation safety practice and compliance with radiation protection protocols

Compliance with wearing personal radiation monitoring badges/dosimeters

Participants were asked about the extent to which they complied with wearing a dosimeter (when supplied with this at the workplace). Only 58.8% of radiographers, 37.5% of dentists and 16.7% of oral hygienists & dental therapists indicated that they always wore their dosimeters (**Table 2)**. Even more concerning is that half of the oral hygienists & dental therapists (50.0%) and 18.8% of dentists indicated that they never wore a dosimeter. The differences observed in the use of dosimeters were statistically significant (p=0.03).

Compliance with radiation protection of patients

Compliance with protection of patients by covering them with lead apron and/or wearing a collar before procedures was enquired about (**Table 2**). Only a third (29.4%) of radiographers reported always protected patients before radiological examinations. This contrasted with responses from dental professionals (Dentists, OH & DT), majority of whom indicated that they always protect the patients before procedures (93.8% and 83.3%, respectively). The differences observed in the use of dosimeters were statistically significant (p<0.001).

The frequency with which participants repeat x-ray procedures on patients was also explored. A quarter (24%) of radiographers, 8% of OH&DT and 31% of dentists always repeat x-ray procedures because the radiograph images were not clear. Enquiry regarding compliance with radiation safety protocols to limit patient exposure including the safe filing or keeping of x-ray records revealed that 38% of dentists indicated to only sometimes keeping patients' records and 19% never keep the patients' records. Radiographers fared better in ensuring patients' records are kept, with 100% of all participants indicating that they comply with the requirement.

3.4. Perception of community's knowledge of radiation exposure risks

Finally, the study participants were asked about their perception of the patients/general public's knowledge of radiation exposure risks (**Figure 2**). A quarter (25.0%) of radiographers and the majority (75.0%) of dental professionals held a view that the public/patient community was not well informed about radiation exposure risks. Responses to an open ended question in the questionnaire indicated that possible reasons may include the low levels of literacy/education in the communities where the health facilities are located, patients not being familiar with the concept of x-rays, lack of appreciation of the health risk associated with ionizing radiation, and patients feeling disempowered to ask questions when procedures are being performed, and inconsistent/poor explanation of associated health risks by health professionals. Some participants however noted that health information, instructions and warnings are regularly provided to patients verbally and that the posters/signs on walls do explain risk associated with radiation exposure therefore patients should be better informed.

DISCUSSION

This study found that rural health facility-based dental professionals and radiographers in Limpopo Province (South Africa) were able to correctly recognize ionizing radiation as an important health hazard. More radiographers than dental professionals felt at increased risk of exposure to ionizing radiation due to their continuous occupational exposure while performing x-rays. Most radiographers agreed that a pregnant radiographer can continue to perform her x-ray duties. Most radiographers and even fewer dental professionals admitted to always wearing their personal radiation dosimeters while only a third of radiographers reported always protecting patients before radiological examinations. It was also common to repeat x-rays on patients and not keep adequate records of investigations completed. The participants perceived the public/patient community as not well-informed regarding radiation exposure risks.

The linear no-threshold (LNT) model stipulates that there

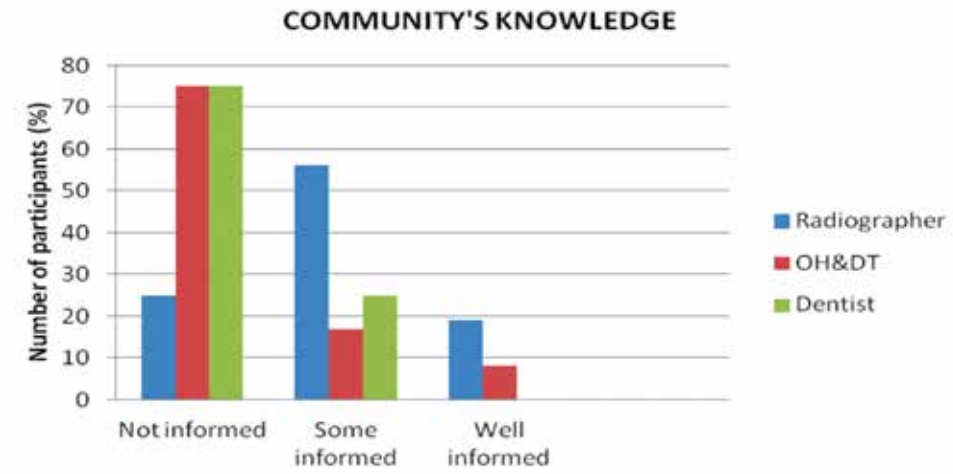


Figure 2: Distribution of responses on the community's knowledge to radiation exposure risks

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.20799
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

is no safe level of exposure to ionizing radiation [6], and it was therefore concerning that most participants (64.7% radiographers, 83.3% of OH & DT, and 100% dentists) believed that there was a safe dose of x-ray radiation from medical imaging. This belief may be due to several factors including the perception that ionizing radiation is unseen, insufficient training on the job and a lack of focused teaching on radiation protection during undergraduate university studies. [1, 13, 21, 22] The frequently stated occupational exposure dose of 20mSv per annum may have also led participants to believe that there is a safe radiation dose level. [10] These findings do however highlight the importance of ongoing education and training regarding the principles of radiation protection including justification, optimalisation of protection and limitation of all x-ray exposures (dose limits) that should be adhered to. Radiation worker's contact with the occupational health service, such as during entry medical examinations also provide an opportunity for this reinforcement to take place, particularly for new employees and those vulnerable such as those who are pregnant. [23]

The fetus in pregnant women who are radiation workers is particularly vulnerable to the effects of ionizing radiation.[10, 24] The participant's knowledge regarding occupational exposure to ionizing radiation for pregnant women was sufficient which is important observation in the context of this study as most of the respondents were young and female (67%) of childbearing age. Hussein et al.[25] in Sudan made similar observations in their cross-sectional study of dentists, where the majority (74.3%) recognized that dental radiography was not an absolute contra-indication in pregnant women. According to the Code of Practice for Radiation Workers [10], a pregnant radiographer can continue working but the maximum equivalent dose limit of 2 mSv to the abdomen should not be exceeded. They should be monitored and issued with a direct reading pocket alarm dosimeter. Women of childbearing age are further identified as a particularly vulnerable occupational group that should receive the requisite training and be well-versed with the use of ionizing radiation.[10, 24] In evaluating equivalent dose of ionizing radiation to the fetus in occupationally exposed pregnant workers, Osei and Kotre [24] confirmed that while radiation doses are generally low, it is important for pregnant staff to be encouraged to report the pregnancy early and to follow good radiation protection operational procedures for the protection of the fetus, co-workers, and patients.

Personal radiation monitoring dosimeters/badges are used for monitoring cumulative exposure to ionizing radiation that radiation workers receive. The finding that only 58.8% of radiographers in this study always wore dosimeters when performing radiation work is concerning. Though not exposed to the same extent as radiographers on a daily/continuous basis, it is also concerning that only 37.5% of dentists and 16.7% of OH&DT always comply with the wearing of a dosimeter. Globally, radiation protection compliance levels have also been found to be as low as 59.26% among radiographers and healthcare professionals in a meta-analysis conducted in four countries.[26] Other South African nationwide studies have also reported that only 16.3% of radiographers continuously used the radiation protection made available by the employer in the past[21], while in specific provinces (South Africa's Gauteng Province), only 41.67% of radiographers indicating that they always wore their radiation monitoring devices.[16] Other countries within the sub-Saharan region have noted concerning radiation protection practices amongst radiation workers with only 58.62% of medical imaging technologists in Rwanda having radiation-measuring devices, and 29% receiving dose readings inconsistently.[27] They further found that while lead rubber aprons were available in 99% of these government hospitals, over half (59%) of the medical imaging technologists had never checked the integrity of the aprons.[27] Fiagbedzi et al.[28] in Ghana similarly reported that while over 90% of radiographers in their study had personal radiation dosimeters, only 25% admitted to wearing them always. The low levels of compliance in our study could be attributed to a lack of/confusing messaging on when dosimeters should be worn, lack of recognition of radiographers in the healthcare team, having to perform x-rays on difficult patients, lack of resources, inadequate training, and a lack of management support.[16, 21, 27, 28]

Several national guidelines and reports[10, 20, 23] have detailed the importance of operators and patients wearing protective clothing however only a third (29%) of radiographers reported always protecting patients before radiological examinations. This practice could be due to several factors including having a poor understanding of the ill-effects of radiation exposure to patients or negligence/complacency in those who fully understand the risk of ionizing radiation. Previous studies in Gauteng, South Africa have reported that only 33% of radiographers knew the function of exposure indicators.[17] Shiralkar et al.[29] similarly reported that most doctors do not know the dose levels of radiation that their patients get exposed to during radiological investigations and that they routinely requested x-rays without weighing the necessity, thereby subjecting patients to more radiation exposure risk. The lack of providing patients with protective clothing may however also represent the recent paradigm shift away from the routine use of patient protection shielding during medical x-ray imaging as the contact shielding may interfere with the imaging process (resulting in a repeat test) or dose reduction technology, increased risk of radiation exposure should the shielding move during the examination (resulting in increased exposure), and reduced infection control practices.[30, 31]

This study provided evidence that patients are likely to receive more radiation exposure than necessary as a quarter (24%) of radiographers, 8% of OH&DT and 31% of dentists reported that they always repeated x-ray procedures. This routine repetition of x-ray imaging could result from poor record keeping, unnecessary requisition of x-rays and not paying attention and considerable care to ill-effects of chronic exposure to radiation. Our findings are in keeping with those by Arslanoglu et al.[32], who noted that 93% of the 177 doctors underestimated the actual patient radiation dose of various radiological examinations. Thirty eight percent (38%) of dentists indicated to only sometimes keeping patients' records and 19% never keep the patients' records which could lead to patients being subjected to further radiation. Requirements for dental radiography stipulate that a record/register of all patients undergoing x-ray examinations must be stored for a period of at least 5 years and that the repetition of x-rays is discouraged.[33]

Our study found a quarter (25%) of radiographers and the majority (75%) of dental professionals held a view that the public/patient community was not well informed about radiation exposure risks likely due to low levels of literacy/education, lack of familiarity with x-rays, and poor

https://doi.org/10.17159/sadj.v80i04.20799
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

risk communication by healthcare providers. Lewis et al. [34]observed that patient radiation protection knowledge influenced radiation protection practices. A study in a rural public sector hospital in Kwa-Zulu Natal, South Africa also revealed that very few patients (10.9%) had a fair knowledge about x-rays[35] while less than 50% of patients attending four Jordanian local hospitals had received information on radiation awareness before medical imaging examinations. [36] With improvements in access to modern technology and greater use of artificial intelligence tools such as ChatGPT and Meta AI, patients can search for information on radiation protection and treatment plans prescribed by themselves, becoming much more self-reliant and knowledgeable about the effects of ionizing radiation.[37] It is the responsibility of the health-care professionals to provide first-hand information to patients undergoing all radiological procedures and clearly articulate the risks and benefits.[10, 35, 36] Consent should also be sought from the patients before they undergo any radiological procedure to confirm that the patients understand the risks involved.

The study has some limitations. The study included rural health facilities in only one health district (Waterberg) out of five in Limpopo Province (Capricorn, Mopani, Sekhukhune, Vhembe) which reduced population sample size and limited generalizability of the study findings. While the focus of this study was the Waterberg District, future research should include all rural districts in the province to enable greater generalizability. The self-reported nature of the study does not make it possible to confirm the accuracy of responses regarding actual practices in health facilities, use of personal protective equipment, including the availability of equipment and resources. Social desirability bias may have reflected more positive radiation safety practices than are done in the workplace. The strength of the study is its focus on a remote and rural workforce in South Africa, in a province that has experienced closures of radiation units due to non-compliance. The study also focused on all exposed radiation workers including radiographers, and dental professionals.

This study highlights the need for several stakeholders at national, provincial and facility level to collaborate in addressing radiation protection concerns and avoiding further closures of radiation units in already resource constrained rural settings. Evidence-based radiation protection policies from the national department of health and related agencies are needed, with the requisite support provided to health facility managers and healthcare workers on their implementation.

The employer at each health facility must conduct a workplace health risk assessment as required by the Occupational Health and Safety Act 85 of 1993 to evaluate the risk to workers, and the public. The findings of the health risk assessment should inform risk mitigation strategies including building x-ray rooms compliant with set standards, procurement of required dosimeters and protective clothing in compliance with occupational health and safety legislation. Artificial Intelligence (AI) has also played an increasing role in radiation protection including dose optimization and reduction of radiation doses in select anatomical regions. [38] Patient education to improve radiation awareness should be an integral part of the health facilities daily practice to reduce unnecessary exposure from medical imaging examinations. [35, 36]

To improve compliance at a healthcare worker level, it is suggested that training at undergraduate training level and once fully qualified be provided to influence attitudes and practice. [16, 21, 22] Health-care professionals should be encouraged to present for their radiation worker medical examination/surveillance, ensure that personal dosimeters are worn daily/as prescribed, and that they submit these for analysis on a regular basis. [10, 20, 23] They should also take a keen interest in reading the reports sent from their dosimeter readings. Refresher courses, continuous educational programmes to occupationally exposed health-care professionals should be reinforced and should not only focus on radiation protection, but other important occupational hazards associated with radiation work including infections and chemicals in the workplace. [4, 5, 39]

CONCLUSION

This study demonstrated that though dental professionals and radiographers had identified ionizing radiation as an occupational hazard, their compliance with radiation protection and safety protocols highlights that they underestimate the long-term health impacts of radiation exposure on themselves and patients. More work is needed in drafting and implementing policies at national, provincial and health facility level to support radiation workers in better complying with radiation safety protocols. The patient population's low levels of knowledge on radiation safety should be addressed by all healthcare professionals involved in prescribing and performing x-rays and other radiological procedures.

Acknowledgements

The authors would like to thank the University of Limpopo for ethical approval, Limpopo Department of Health and hospital managers for granting access to collect data on hospitals in the Waterberg District. We thank all radiographers, oral hygienists, dental therapists, and dentists for participation in the study.

Declaration of competing interests:

The authors have declared that no competing interests exists.

Author's contributions

RM and TN were responsible for the overall study design, data collection and analysis, write-up and review of the manuscript. IN was responsible for the write up, and preparation of the manuscript. All authors were involved in commenting on the paper and have approved the final version.

Funding statement

This research received no specific funding grant from any finding agency in the public, commercial, or not-for-profit sectors.

Data availability

The data that support the findings of this study are not openly available because of reasons of confidentiality. Anonymised data are available from the corresponding author, RM., upon request.

Disclaimer

The views and opinions expressed in this manuscript are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.20799
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

REFERENCES

1. Rose A, Uebel KE, Rae WI. Interventionalists' perceptions on a culture of radiation protection. S Afr J Rad. 2018;22(1):1-0. S DOI: https://doi.org/10.4102/sajr.v22i1.1285
2. Chodick G, Bekiroglu N, Hauptmann M, et al. Risk of cataract after exposure to low doses of ionizing radiation: a 20-year prospective cohort study among US radiologic technologists. Am J Epidemiology. 2008 15;168(6):620-631. DOI: https://doi.org/10.1093/aje/kwn171
3. Zielinski JM, Garner MJ, Band PR, et al. Health outcomes of low-dose ionizing radiation exposure among medical workers: a cohort study of the Canadian national dose registry of radiation workers. International journal of occupational medicine and environmental health. 2009 Apr 1;22(2):149. DOI: https://doi.org/10.2478/v10001-009-0010-y
4. Hazell LJ, Stork LA. Radiographer experiences of personal protective equipment during COVID-19 in Gauteng, South Africa. Journal of Medical Imaging and Radiation Sciences. 2024 Jan 3. DOI: https://doi.org/10.1016/j.jmir.2023.12.002
5. Abuzaid M, Elshami W, Tekin HO. Compliance with infection control and radiation protection measures during COVID-19 in the UAE's radiology department. Journal of Medical Imaging and Radiation Sciences. 2022 Dec 1;53(4):S4.
6. International Commission on Radiological Protection. The 2007 recommendations of the International Commission on Radiological Protection. Oxford: Pergamon Press, 2007
7. International Atomic Energy Agency. Basic safety standards for radiation protection. GSR part 3. Vienna-IAEA-2014.
8. Niu S. Radiation protection of workers. International Labour Office; 2011 April. Available at: https://www.ilo.org/wcmsp5/groups/public/@ed_protect/@protrav/@safework/documents/publication/wcms_154238.pdf. Accessed 2024-04-02
9. Herbst CP, Fick GH. Radiation protection and the safe use of x-ray equipment: laws, regulations and responsibilities: opinion. S Afr J Rad. 2012 Jan 1;16(2):50-4. DOI: https://doi.org/10.10520/EJC122041
10. National Department of Health (South Africa). Code of practice for users of medical x-ray equipment. Radiation Control Directorate. 2016. Available at: https://www.sahpra.org.za/wp-content/uploads/2020/01/Code-of-practice-for-users-of-medical-x-ray-equipment-01-2015-2.pdf. Accessed: 2024-04-01
11. Health Professions Council of South Africa (HPCSA). Scope of practice: diagnostic radiography (22 May 2020). 2020. Available at: https://www.hpcsa.co.za/Uploads/professional_boards/rct/guidelines/RCT_Scope_of_Practice_Diagnostic_Rad_22_May_2020_for%20Current_Scope.pdf. Accessed 14 July 2024.
12. Van der Merwe B, Kruger SB, Nel MM. Developing training and assessment of radiation safety regulations. Journal for New Generation Sciences. 2017 Dec 1;15(2):108-18. DOI: https://doi.org/10.10520/EJC-f09b0a768
13. Lewis MS, Downing C, Hayre CM. South African radiography leadership co-constructing radiation protection change ideas. Journal of medical imaging and radiation sciences. 2022 Jun 1;53(2):248-55. DOI: https://doi.org/10.1016/j.jmir.2022.03.007
14. South African Health Products Regulatory Authority. Radiation Control. 2024. Available at: https://www.sahpra.org.za/radiation-control-news-updates/. Accessed [2024-07-14].
15. Nyathi T, Chirwa TF, van der Merwe DG. A survey of digital radiography practice in four South African teaching hospitals: an illuminative study. Biomedical imaging and intervention journal. 2010 Jul 1;6(1):e5. : https://doi.org/10.2349/biij.6.1.e5
15. Sethole KM, Ahrens E, Kruger U. The level of compliance with the use of personal radiation monitoring devices by qualified radiographers at provincial hospitals in the Tshwane District area. Health Physics. 2019 Oct 1;117(4):426-33. DOI: https://doi.org/10.1097/HP.0000000000001064
17. Moolman N, Mulla F, Mdletshe S. Radiographer knowledge and practice of paediatric radiation dose protocols in digital radiography in Gauteng. Radiography. 2020 May 1;26(2):117-21. DOI: https://doi.org/10.1016/j.radi.2019.09.006
18. Kabongo JM, Nel S, Pitcher RD. Analysis of licensed South African diagnostic imaging equipment. Pan African Medical Journal. 2015;22(1). DOI: https://doi.org/10.11604/pamj.2015.22.57.7016
19. National Department of Health, South Africa. 2030 Human resources for health strategy: investing in the health workforce for universal health coverage [online]. 2020. Available at https://www.spotlightnsp.co.za/wp-content/uploads/2020/08/2030-HRH-strategy-19-3-2020.pdf. Accessed 01 April 2024.
20. Office of Health Standards Compliance (South Africa). Improving the quality of healthcare in South Africa Annual Inspection Report 2016/17. Available at: https://knowledgehub.health.gov.za/system/files/elibdownloads/2023-04/OHSC-2016-17-ANNUAL-INSPECTION-REPORT-FINAL.pdf. Accessed: 01 April 2024
21. Lewis S, Downing C, Hayre CM. Radiation protection among South African diagnostic radiographers—a mixed method study. Health Physics. 2023 Mar 1;124(3):208-16. DOI: https://doi.org/10.1097/HP.0000000000001655
22. Van der Merwe B, Kruger SB, Nel MM. Radiation safety requirements for training of users of diagnostic x-ray equipment in South Africa. African Journal of Health Professions Education. 2017;9(3):123-7.
23. National Department of Health (South Africa). Guidelines - medical examinations for radiation workers. Radiation Control Directorate. 2012. Available at: https://www.sahpra.org.za/document/guideline-for-code-of-practice-for-users-of-medical-xray-equipment/ Accessed: 01 April 2024
24. Osei EK, Kotre CJ. Equivalent dose to the fetus from occupational exposure of pregnant staff in diagnostic radiology. The British journal of radiology. 2001 Jul 1;74(883):629-37. DOI: https://doi.org/10.1259/bjr.74.883.740629
25. Hussein RE, Hashim NT, Awooda EM. Knowledge, awareness and practice of Sudanese dentists towards oral radiology and protective guidelines. J. Dent. Med. Sci.. 2016;15:79-83. DOI: https://doi.org/10.9790/0853-1510047983
26. Sarman I, Hassan DH. Factors affecting radiographers' compliance with radiation protection on all areas of hospital settings worldwide-a meta-analysis. International Journal for Innovative Research in Science & Technology. 2016 Sep;3(4):433-8.
27. Maina PM, Motto JA, Hazell LJ. Investigation of radiation protection and safety measures in Rwandan public hospitals: Readiness for the implementation of the new regulations. Journal of Medical Imaging and Radiation Sciences. 2020 Dec 1;51(4):629-38. DOI: https://doi.org/10.1016/j.jmir.2020.07.056
28. Fiagbedzi E, Gorleku PN, Nyarko S, et al. Assessment of radiation protection knowledge and practices among radiographers in the central region of Ghana. Radiation Medicine and Protection. 2022 Sep 30;3(03):146-51. DOI: https://doi.org/10.1016/j.radmp.2022.06.001
29. Shiralkar S, Rennie A, Snow M, et al. Doctors' knowledge of radiation exposure: questionnaire study. Bmj. 2003 Aug 14;327(7411):371-2. DOI: https://doi.org/10.1136/bmj.327.7411.371
30. Samara ET, Saltybaeva N, Merce MS, et al. Systematic literature review on the benefit of patient protection shielding during medical X-ray imaging: towards a discontinuation of the current practice. Physica Medica. 2022 Feb 1;94:102-9. DOI: https://doi.org/10.1016/j.ejmp.2021.12.016
31. British Institute of Radiology. Guidance on using shielding on patients for diagnostic radiology applications. Available at: https://www.bir.org.uk/media/414334/final_patient_shielding_guidance.pdf . Accessed 15 July 2024
32. Arslanoglu A, Bilgin S, Kubali Z,et al. Doctors' and intern doctors' knowledge about patients' ionizing radiation exposure doses during common radiological examinations. Diagn Interv Radiol. 2007 Jun 1;13(2):53.
33. National Department of Health (South Africa). Guidelines – dental radiography. Radiation Control Directorate. 2017. Available at: https://www.sahpra.org.za/wp-content/uploads/2020/01/Dental-Radiography-Guidelines-2.pdf. Accessed: 01 April 2024
34. Lewis S, Downing C, Hayre CM. South African radiographers' radiation protection practices, a qualitative study. Radiography. 2022 May 1;28(2):387-93. DOI: https://doi.org/10.1016/j.radi.2021.12.008
35. Mung'omba B, Botha AD. Core competencies of radiographers working in rural hospitals of KwaZulu-Natal, South Africa. Afr J Prm Health Care Fam Med. 2017 Feb 22;9(1):1-8. DOI: https://doi.org/10.4102/phcfm.v9i1.1389
36. Alhasan M, Abdelrahman M, Alewaidat H, et al. Medical radiation knowledge among patients in local hospitals. Journal of Medical Imaging and Radiation Sciences. 2015 Mar 1;46(1):45-9. DOI: https://doi.org/10.1016/j.jmir.2014.09.002
37. Temple S, Rowbottom C, Simpson J. Patient views on the implementation of artificial intelligence in radiotherapy. Radiography. 2023 May 1;29:S112-6. DOI: https://doi.org/10.1016/j.radi.2023.03.006
38. Fiagbedzi, E.W., Gorleku, P.N., Nyarko, S., et al, 2022. The role of artificial intelligence (AI) in radiation protection of computed tomography and fluoroscopy: A review. Open Journal of Medical Imaging. 2022 12(1), 25-36. DOI: https://doi.org/10.4236/ojmi.2022.121004
39. Motiang KP, Chelule PK. Occupational health and safety in radiographic film processing in Limpopo province. Occupational Health Southern Africa. 2017 Jan 1;23(1):21-5. DOI: https://doi.org/10.10520/EJC-5843de548

CPD questionnaire on page 226

The Continuing Professional Development (CPD) section provides for twenty general questions and five ethics questions. The section provides members with a valuable source of CPD points whilst also achieving the objective of CPD, to assure continuing education. The importance of continuing professional development should not be underestimated, it is a career-long obligation for practicing professionals.

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.20985
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

The effects of two forms of commercially available denture adhesives on the growth of Candida albicans in vitro

SADJ MAY 2025, Vol. 80 No.4 P191-197

PN Gwala[1], SN Kabini[2], SR Mthethwa[3], EM Sekati[4]

ABSTRACT

Introduction

There has been an increased interest in the influence of denture adhesives on Candida albicans growth in denture wearers.

Aims and objectives

To compare the mean colony count and pH of Candida cultures grown in the presence of acrylic resin plates treated with two forms of a denture adhesive.

Design

A quasi-experimental design.

Methods

Two of three groups of 30 acrylic resin specimens were treated separately with two forms of a denture adhesive. The remaining group was left untreated. Ten specimens from each group were incubated for 6 hours, 24 hours and 48 hours following the addition of a diluted strain of *Candida albicans.* The number of colonies and pH of the culture media were recorded over three measurement occasions and analysed.

Results

Plates from the two forms of a denture adhesive had no colonies in the countable range in contrast to plates from the control group. The study found significant mean differences in pH values over three measurement occasions as well as significant group differences in terms of how the means vary over time. However, the results showed a significant time x treatment group interaction.

Conclusions

Both forms of the denture adhesive tested inhibited the growth of candida.

Authors' information:
1. Dr P.N Gwala, BDS. Sefako Makgatho Health Sciences University. ORCID: https://orcid.org/0009-0002-2505-5318
2. Dr S.N Kabini, BDS, MChD, MPhil. Sefako Makgatho Health Sciences University. https://orcid.org/ 0000-0001-7252-8451
3. Dr S.R Mthethwa, BDS, MPH, PhD. Sefako Makgatho Health Sciences University. ORCID: https://orcid.org/0000-0003-0420-808X
4. Ms. EM Sekati, ND Med Tech, BTech, BSc Med Honours, MSc. ORCID: https://orcid.org/0009-0000-0395-940X

Corresponding author:
Name: Dr S.R Mthethwa
Address: Medunsa Campus, PO Box D24, Sefako Makgatho Health Sciences University 0204
Tel: 012 – 521 5888
Fax: 012 -512 4274
Email: rocky.mthethwa@smu.ac.za

Authors' contribution:
P.N Gwala – 25%
S.N Kabini -25%
S.R Mthethwa – 25%
EM Sekati – 25%

INTRODUCTION AND BACKGROUND

A large and growing body of literature has investigated the influence of denture adhesives on *Candida albicans (C. albicans)* growth in complete denture wearers.[1-8] A tenuous link between denture adhesive use and denture stomatitis, the leading cause of which is *C. albicans* is suspected. The predisposing factors for denture stomatitis have been widely investigated. These can be categorised into denture wearer's characteristics; behaviour; medical history, and denture-related factors. Predisposing factors such as the denture wearer's age[9], gender[10] and salivary pH[11], behaviours such as high sugar intake[12] and smoking[13] as well as history of systemic diseases such as diabetes mellitus[14], xerostomia[15], hypertension[16], immunosuppressive therapy[17] and extended systemic antibiotic treatment[18] have been researched. The denture-related factors include the propensity of Candida to adhere to the denture base[19], the age of the dentures[20], poor denture hygiene[16,21], and sleeping with dentures.[10,21,22] The prevalence of denture stomatitis has been reported to range between 25% to 40%.[9,10,23,24]

Several studies investigating the in vivo effect of denture adhesives on *C. albicans* growth in denture wearers suffering and those not suffering from denture stomatitis have been carried out.[1,3,6,25-26] These have entailed counting the number of *C. albicans* colonies in saliva and swabs of dentures. Inconsistent results have been reported - whereas Scher et al (1978) reported a reduction in *C. albicans*, Borole et al (2016) found a statistically insignificant increase.[1,25] Kim et al (2003) reported that using a denture adhesive neither increased nor decreased the growth of Candida species.[3]

A great deal of the previous in vitro work in this field has described the adhesion and biofilm formation of *C. albicans* to denture base exposed to denture adhesives as well as measured the mean pH and number of colonies of Candida cultures grown in acrylic resin plates exposed to denture adhesives and incubated for varying periods of time.

Previous studies have reported that denture bases exposed to denture adhesives increased the adhesion and biofilm formation of *C. albicans*.[2,27-28] This increase was however not statistically significant. Darwish et al (2021) demonstrated this increase using denture bases manufactured using UDMA and PMMA resins. The difference in *C. albicans* count between bases manufactured using UDMA and PMMA resins was not statistically significant.[2]

Some studies have found that denture adhesives possess antifungal activity attributed to their effect on the pH of candida culture media.[4,7] Makihira et al (2001) reported that half of the six adhesives tested significantly suppressed the growth of candida species.[4]

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.20985
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

Several studies have reported that denture adhesives lower Candida growth rate in growth curves obtained in the presence of adhesives[8] or reduce the number of Candida colonies in Candida in resin specimen exposed to adhesives.[7,29]

The awareness and use of denture adhesives by denture wearers in South Africa has not previously been described. However, our experience at a tertiary dental teaching hospital in Gauteng indicate that patients use Corega powder or cream. There has been an increasing interest in the influence of Corega on the growth of *C. albicans*. The research to date has tended to focus on the influence of Corega on the adhesion of *C. albicans* to denture bases[2] as well as the growth rate/ number of *C. albicans* colonies in acrylic resin plates exposed to Corega[8] rather than on the influence of Corega on the pH of candida culture media. This is an important issue to research. This study was designed to determine the effect of Corega on the pH of candida culture media.

OBJECTIVES OF THE STUDY

To determine and compare the mean colony count and pH of candida cultures grown in the presence of acrylic resin plates treated with two forms of Corega over three measurement occasions

To determine whether there are significant mean differences in pH values over three measurement occasions
To determine whether there are group differences in terms of how the pH means vary over time.

MATERIALS AND METHODS

Study design

This was a post-test only non-equivalent control group design (a quasi-experimental design).

Study population

The study population consisted of acrylic resin plates

Sample size

The sample size was determined with reference to previous studies.[2,30] Ninety acrylic resin sheets (10 x 10 x 2mm) were prepared according to the manufacturer's instructions and kept in a flask containing normal saline and sterilized in an autoclave at 121^{0}C in line with a method described in a previous study.[31]

Allocation method

A non-random allocation method was implemented i.e., sampling units (sterile acrylic resin plates) were assigned by alternation - one to experimental group A, one to experimental group B, and one to experimental group C.

Interventions

The experiment was conducted according to the procedure used by Rajaram and Manoj (2017), with slight modifications.[7] An inoculum of the cultures of *C. albicans* strain ATCC 90028 was prepared by suspending colonies in 20ml of sterile normal saline and adjusting the turbidity to 0.5 McFarland standard, equivalent to 1.5 × 10^8 forming units. 1:10 serial dilutions of the 0.5 McFarland standard until 10^5 were prepared in sterile saline.

Ninety sterile acrylic resin plates were assigned to three groups of thirty. The sampling groups were labelled as follows: experimental group A, experimental group B, and experimental group C. An amount of 0.011g of Corega cream or powder was lightly applied to each acrylic plate in their respective groups. This amount (0.011g) was decided on with reference to a previous study.[5] No adhesive was applied to acrylic plates in the control group. The acrylic plates in their respective groups were individually placed at the flat bottom of a 25cm^2 tissue culture bottle. Fifty microlitres (0.050ml) of a wide range of dilutions (10^5, 10^3 and 10^2) of the *C. albicans* strain ATCC 90028 was added to each culture bottle and incubated at 37^{0}C for 1 hour to allow C. albicans to seed on the acrylic plates, under aerobic conditions. After the 1-hour incubation period, 2ml of Sabouraud dextrose broth was carefully dispensed into all the culture bottles, and each group was then divided into three equal subgroups i.e., three groups of ten, which were incubated at 37^{0}C for 6 hours, 24 hours and 48 hours respectively.

The pH of the resulting Candida broth cultures was measured at the end of the incubation periods using a calibrated pH meter. Repeated measurements were performed for a random sample of 20% of the cultures.

A calibrated nichrome inoculating loop was used to transfer 0.001ml of the Candida broth cultures (the 10^2 dilution was plated) onto separate Sabouraud agar plates at the end of the incubating periods. Streaks were produced on the agar plates using the loop, after which the plates were incubated at 37^{0}C for 24 hours. The resulting colonies were counted manually as colony forming units (cfu) by the trained principal investigator. The recommendations of Oregon State University were followed: only plates with 25-250 colonies were used; counts above 250 were considered Too Numerous To Count (TNTC) because it was impossible to tell whether colonies were separated, and plates with less than 25 colonies were deemed not to have a statistically significant number of colonies.[31] Repeated measurements were performed for a random sample of 20% of the acrylic plates. The research supervisors denied the principal investigator information that could identify the study groups.

Primary outcomes

The primary outcome measures were the mean colony counts and mean pH values. The effects of interest were the differences in mean colony counts and mean pH values over three measurement occasions between acrylic plates treated with two forms of a commercially available denture adhesive.

Definition of terms

Group A refers to acrylic resin plates which were treated with Corega cream
Group B refers to acrylic resin plates which were treated with Corega powder
Group C is a control group that refers to acrylic resin plates that were not be treated with any adhesive
Sampling unit refers to an individual acrylic plate
Time 1 refers to an incubation period of 6 hours
Time 2 refers to an incubation period of 24 hours
Time 3 refers to an incubation period of 48 hours

Data analysis

Collected data were subjected to univariate and multivariate analysis in Statistical Package for the Social Sciences (SPSS) software version 29. Measures of central tendency and dispersion were calculated.

https://doi.org/10.17159/sadj.v80i04.20985
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

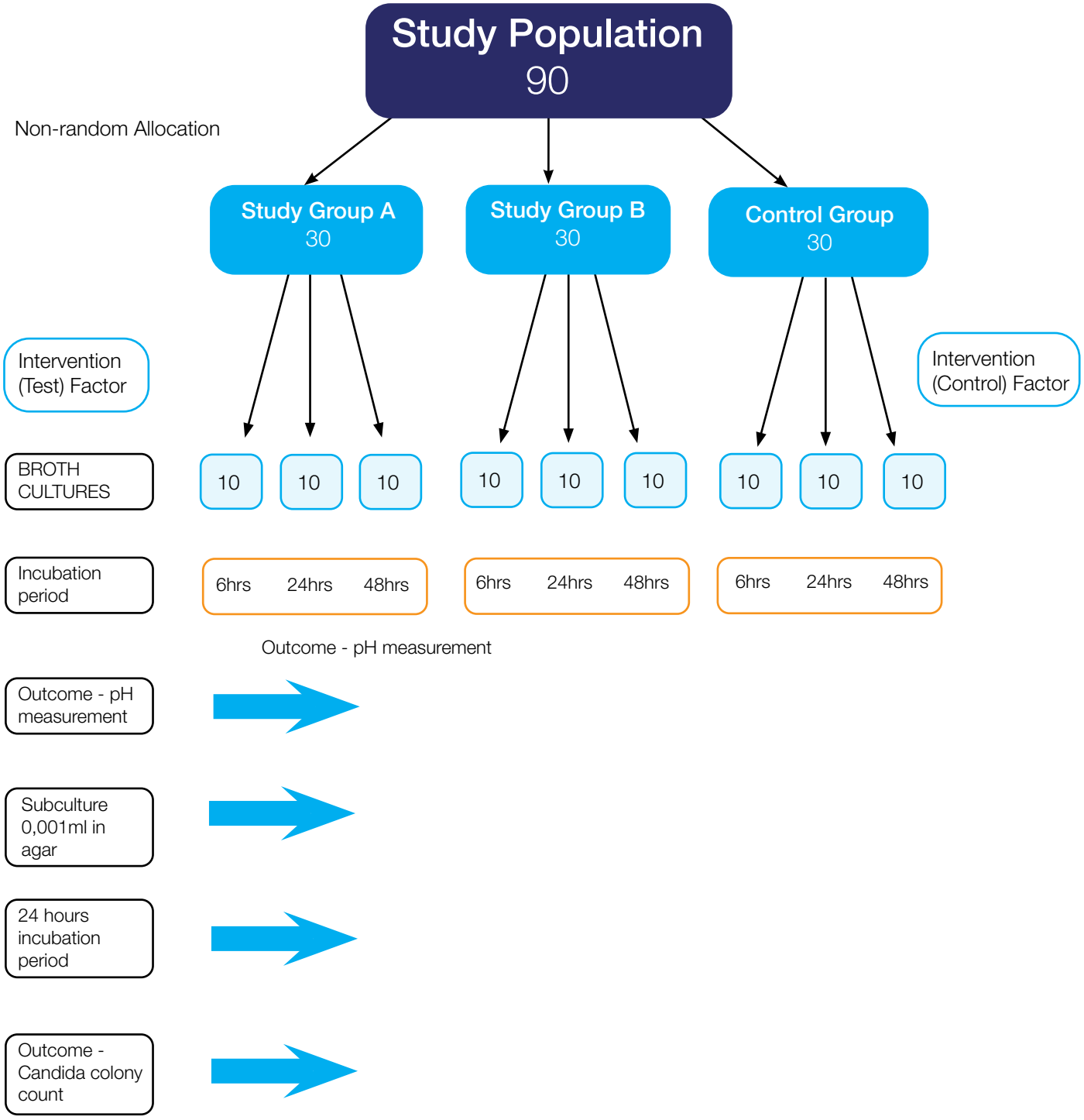
A two-way ANOVA (Analysis of Variance) was performed to test whether there are significant mean differences in pH values over three measurement occasions, as well as whether there are group differences in terms of how the means vary over time. The assumptions of two-way ANOVA were checked. These are: (1) inde endence of variables, (2) normal distribution of variables, (3) no outliers and sphericity i.e. constant variance across time points. Bonferroni adjusted pairwise t-tests were performed after significant effects were found. The significance level of the tests was a p-value less than 0.05.

ETHICAL CONSIDERATION

Ethical approval for the study was granted by the Ethics Committee of the institution (SMUREC/D/215/2023:PG). Permission to conduct the study was granted by the Chief Executive Officer (CEO) of the tertiary dental teaching hospital.

RESULTS

Colony counts and pH values recorded from cultures of *C. albicans* incubated at varying periods were analysed. Figure 1 below is a flow diagram of the progress through the phases of the study (that is, enrolment, treatment allocation, follow-up, and data analysis).



www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.20985
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

Table 1: The results of a growth curve of C. albicans grown at 37°C on Sabouraud broth

Time (hours)	Treatment group	Average cfu/ml
6	Corega cream	*
	Corega powder	*
	Control	*
	Total	
24	Corega cream	*
	Corega powder	*
	Control	4,6 x10^7
	Total	
48	Corega cream	**
	Corega powder	**
	Control	**
	Total	

*Statistically insignificant number of colonies.
** Too numerous to count

Not a single plate from any of the treatment groups had colonies in the countable range (25-250) during time 1 (6hours) as well as for the Corega cream and powder groups during time 2 (24 hour) from the 10^2 dilution.

A few number of plates either had a statistically insignificant number of colonies or had colonies that were too numerous to count during time 3 (48 hours). Consequently, average cfu/ml could not be calculated.

Table 2: Descriptive statistics of pH values for each group at each time interval

Time (hours)	Treatment group (tx.group)	Mean pH	Std. dev	Sample size (n)
6	Corega cream	7,0200	,10965	10
	Corega powder	7,1180	,21369	10
	Control	6,9290	,11949	10
	Total	7,0223	,16880	10
24	Corega cream	7,2200	,24490	10
	Corega powder	7,2360	,06150	10
	Control	7,2330	,04620	10
	Total	7,2297	,14318	10
48	Corega cream	,72280	,11612	10
	Corega powder	,72140	,17109	10
	Control	6,9740	,14470	10
	Total	7,1387	,18392	10

The mean pH of Corega powder was the highest during times 1 and 2. The mean pH of Corega cream was the highest during time 3.

Table 3: Output from two-way ANOVA (Mauchly's Test of Sphericity)

Within-Subject Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt
Time	,993	,171	2	,918	,994	1,000

Mauchly's test of sphericity tests the null hypothesis that the variances of the differences are equal.

The sphericity assumption is required for all univariate main effects and interaction tests.[33] Given Mauchly's test is impacted by non-normality and by sample size, it is not highly recommended when evaluating whether the sphericity condition has been met.

A Greenhouse-Geisser epsilon (ϵ) value < .75, suggests using the Greenhouse-Geisser adjustment with the univariate test of mean differences, whereas a value falling between .75 and 1 suggests the use of the Huynh-Feldt adjustment with the univariate tests. [(ϵ) = 1 is consistent with sphericity].

The sphericity assumed test was determined not to have been violated.

https://doi.org/10.17159/sadj.v80i04.20985
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

Table 4: Output from two-way ANOVA (Test of Within-Subject Effects)

Source	Sum of squares	Degrees of freedom squares	Mean squares	F ratio	P-value	Partial Eta Squared	Noncent, Parameter	Observed Power
time	,648	2	,324	13,731	<,001	,337	27,462	,997
time* tx.group	,247	4	,062	2,616	,045	,162	10,463	,695
Error	1,274	54	0,24					

The main effect of time on pH values is statistically significant, sphericity assumed F (2, 54) = 13.73, p<,001. This effect was qualified by a significant time x tx. group interaction effect, sphericity assumed.

F (4, 54) = 2,616, p <,05.

Table 5: Output from two-way ANOVA (Test of Within-Subject Contrast)

Source	Time	Sum of squares	Degrees of freedom squares	Mean squares	F ratio	P-value	Partial Eta Squared	Noncent, Parameter	Observed Power
Time	Linear	,203	1	,203	8,619	,007	,242	8,619	,808
	Quadratic	,445	1	,445	18,824	<,001	,411	18,824	,987
Time* tx.group	Linear	,070	2	,035	1,476	,246	,099	2,952	,287
	Quadratic	,177	2	,089	3,751	,037	,217	7,503	,635
Error	Linear	,636	27	,024					
	Quadratic	,638	27	,024					

Although the test of the linear component of the trend is significant (p <,05), the higher-order quadratic component was also significant [F (1,27) = 18,824, p<,001]. This suggests that across groups, the mean level of pH exhibited a quadratic trend over the three measurement occasions.

Despite the fact that the test of the interaction between the linear component of the trend and treatment group is not significant, the interaction between the treatment group and the higher-order quadratic component was significant [F (2,27) = 3,751, p <,05.].

Table 6: Output from two-way ANOVA (Levene's Test of Equality of Error Variances)

	Levene Statistic	df1	df2	Significance
pH at Time 1 (6 hours) based on means	4,639	2	27	,019
pH at Time 2 (24 hours) based on means	4,893	2	27	,015
pH at Time 3 (48 hours) based on means	,360	2	27	,701

The Levene's test results involve tests of differences in variances at each time point, an assumption of the univariate ANOVA. It turns out that the standard Levene's test based on means are significant for Time 1 and Time 2. Nevertheless, a violation of this assumption is less of an issue with equivalent sample sizes.

Table 7: Output from two-way ANOVA (Tests of Between-Subject Effects)

Source	Sum of squares	Degrees of freedom squares	Mean squares	F ratio	P-value	Partial Eta Squared	Noncent, Parameter	Observed Power
Intercept	4575,606	1	4575,606	228898,999	<,001	1,000	228898,999	1,000
tx.group	,341	2	,170	8,528	,001	,387	17,056	,947
Error	,540	27	,020					

The Tests of Between-Subjects Effects is a test of the main effect of the grouping variable on pH values on the repeated measure averaged over time. The result presented here is simply a test of group differences on the average of pH values (i.e. those values averaged over time for each Candida broth culture.

The main effect of treatment group on the average pH values across time is statistically significant, F (2,27) = 8,528, p <,05. Based on estimated marginal means. *. The mean difference is significant at the ,05 level. b. Adjustment for multiple comparisons: Bonferroni

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.20985
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

Table 8: Output from two-way ANOVA (Estimated Marginal Means)

Treatment group	Mean	Std.Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Corega cream	7,156	,026	7,103	7,209
Corega powder	7,189	,026	7,136	7,242
Control	7,045	,026	6,992	7,098

Corega powder had the highest mean pH followed by Corega cream.

Table 9: Output from two-way ANOVA [Pairwise comparison on the average pH (averaged over time) for each treatment group]

(I) Treatment group	(J) Treatment group	Mean Difference (I –J)	Std.Error	Sig.[b]	95% Confidence Interval	
					Lower Bound	Upper Bound
Corega cream	Corega powder	-,033	,037	1,000	-,127	,060
	Control	,111*	,037	,016	,017	,204
Corega powder	Corega cream	,033	,037	1,000	-,060	,127
	Control	,144*	,037	,002	,051	,237
Control	Corega cream	,111*	,037	,016	-,204	-,017
	Corega powder	-,144*	,037	,002	-,237	-,051

Based on estimated marginal means.*. The mean difference is significant at the ,05 level. b. Adjustment for multiple comparisons: Bonferroni

The pairwise differences between Corega cream and Control as well as between Corega powder and Control were significant p <,05. The pairwise difference between Corega cream and Corega powder was not significant p >,05.

Table 10: Output from two-way ANOVA (Estimated Marginal Means)

Time	Mean	Std.Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	7,022	,028	6,964	7,080
2	7,230	,028	7,174	7,285
3	7,139	,028	7,084	7,193

The mean pH was highest during time 2 and intermediate during time 3.

Table 11: Output from two-way ANOVA [Pairwise comparison on the average pH irrespective of treatment group]

(I) Time	(J) Time	Mean Difference (I –J)	Std.Error	Sig.[b]	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-,207*	,041	<,001	-,312	-,103
	3	-,116*	,041	,020	-,217	-,015
2	1	,207*	,041	<,001	,103	,312
	3	,091	,041	,074	-,007	,189
3	1	,116*	,041	,020	,015	,217
	2	-,091	,041	,074	-,189	,007

The pairwise differences between time 1 and time 2 and between time 1 and time 3 were significant p <,05. The pairwise difference between time 2 and time 3 was not significant p >,05.

https://doi.org/10.17159/sadj.v80i04.20985
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

Discussion

The present study was designed to determine whether there were significant mean differences in pH values over three measurement occasions from *C. albicans* cultures grown in the presence of acrylic resin plates treated with two forms of Corega, as well as whether there were group differences in terms of how the pH means varied over time.

Candida colony count

The current study found that colonies in the majority of plates plated with the 10^2 dilution were not in the countable range. This finding was unexpected and suggests serial dilution problems. It was surprising considering that a wide range of dilutions (10^5 – 10^3) were successfully plated. It is difficult to explain this result, but it might be related to air contaminants. Reynolds (2005) asserts that air contaminants can contribute significantly to a really low count.[34]

The most interesting finding was that in contrast to plates from the control group, plates from both the Corega cream and powder groups had no colonies in the countable range (25-250) during time 2. This result must be interpreted with caution. It suggests that both Corega cream and powder inhibit the growth of *C. albicans* albicans in contradiction to the previous research by Sampaia-Maio and colleagues (2012) which showed that among the different forms of Corega only the cream inhibits the growth of *C. albicans*.[8]

pH values

On the question of the main effects of time and treatment group on pH values, this study found significant mean differences in pH values over three measurement occasions (Table 4) as well as group differences in terms of how the means varied over time (Table 7) However, the results of this study showed a significant time x treatment group interaction. This indicates that, overall, the effect of time depended on the level of treatment group. In other words, the effect of time was different for different levels of treatment group. This means that interpretation of the main effects (time and treatment group) is incomplete or misleading.

The most interesting finding was that the pairwise differences in mean pH between Corega cream and Control as well as between Corega powder and Control were significant p <,05. This result has not previously been described. These findings suggest that both Corega cream and Corega powder raise the pH of the growth media. This action would inhibit the growth of *C. albicans* which prefers an acidic environment.[35]

Another important finding was that the pairwise difference in mean pH between Corega cream and Corega powder was not significant p >,05. This means that although Corega powder raises the pH of the growth media marginally higher (7,189 vs 7.156) than Corega cream the difference was not statistically significant.

Limitations of the study

Unanticipated number of plates with statistically insignificant number of colonies.

The Simple effects test was not performed to obtain more focused, specific information on where differences are in the interaction effect.

CONCLUSION

Both forms of the denture adhesive tested inhibited the growth of *C. albicans.*

REFERENCES

1. Borole A, Roopa KT, Khandelwal PV. "A comparative evaluation of the effects of different commercially available denture adhesives on the growth of *Candida* species in diabetic and nondiabetic subjects:" An *In vivo* Study. J Dent Allied Sci 2016; 5:63-9.
2. Darwish M, Nassani MZ, Al-Hallak KR, Kujan O. Effect of Denture Adhesives on Adhesion of Candida albicans to Denture Base Materials: An In Vitro Study. J Contemp Dent Pract 2021;22(11):1257-61.
3. Kim E, Driscoll CF, Minah GE. The effect of a denture adhesive on the colonization of Candida species in vivo. J Prosthodont 2003;12(3):187-91.
4. Makihira S, Nikawa H, Satonobu SV, Jin C, Hamada T. Growth of Candida species on commercial denture adhesives in vitro. Int J Prosthodont 2001;14(1):48-52.
5. Nomura T, Murakami T, Shimoyama Y, et al. Effects of denture adhesives on growth and morphological transformation of Candida albicans. J Prosthodont Res 2020;64(1):78-84.
6. Oliveira MC, Oliveira VM, Vieira AC, Rambob I. In vivo assessment of the effect of an adhesive for complete dentures on colonisation of Candida species. Gerodontology 2010;27(4):303-07.
7. Rajaram A, Manoj SS. Influence of 3 different forms of a commercially available denture adhesive material on the growth of Candida species: An in vitro study. J Prosthet Dent 2017;118(3):379-85.
8. Sampaio-Maia B, Figueiral MH, Sousa-Rodrigues P, Fernandes MH, Scully C. The effect of denture adhesives on Candida albicans growth in vitro. Gerodontology. 2012;29(2): e348-56.
9. Kossioni, A.E. The prevalence of denture stomatitis and its predisposing conditions in an older Greek population. Gerodontology 2011;28: 85–90.
10. Adam, R.Z.; Kimmie-Dhansay, F. Prevalence of Denture-Related Stomatitis in Edentulous Patients at a Tertiary Dental Teaching Hospital. Front. Oral Health 2021;2: 772679.
11. Chopde, N.; Jawale, B.; Pharande, A.; Chaudhari, L.; Hiremath, V.; Redasani, R. Microbial colonization and their relation with potential cofactors in patients with denture stomatitis. J. Contemp. Dent. Pr. 2012;13: 456–9.
12. Puryer J. Denture Stomatitis – A Clinical Update. Dent Update. 2016;43(6):529-35.
13. Al-Dwairi, Z.N. Prevalence and risk factors associated with denture-related stomatitis in healthy subjects attending a dental teaching hospital in North Jordan. J. Ir. Dent. Assoc. 2008; 54: 80–3.
14. Factors related to oral candidiasis in elderly users and non-users of removable dental prostheses. Rev. Inst. Med. Trop. São Paulo 2016, 58, 17. Bianchi CM, Bianchi HA, Tadano T, et al. Factors related to oral candidiasis in elderly users and non-users of removable dental prostheses. Rev Inst Med Trop Sao Paulo 2016;58:17
15. Ozaki, K.; Okada, K.; Matsushita, T.; Kondoh, M.; Arai, E.; Miura, K.; Yamazaki, Y. Clinical study of risk factors for adherence of Candida to dentures. J. Oral Maxillofac. Surg. Med. Pathol 2022;34: 653–60.
16. Qiu, J.; Roza, M.P.; Colli, K.G.; Dalben, Y.R.; Maifrede, S.B.; Valiatti, T.B.; Gonçalves, S.S. Candida-associated denture stomatitis: Clinical, epidemiological, and microbiological features. Braz. J. Microbiol 2023;54: 841–8.
17. Golecka, M.; Oldakowska-Jedynak, U.; Mierzwi´ nska-Nastalska, E.; Adamczyk-Sosi´ nska, E. Candida-associated denture stomatitis in patients after immunosuppression therapy. Transplant. Proc 2006; 38: 155–6.
18. Budtz-Jørgensen, E. Ecology of Candida-associated denture stomatitis. Microb. Ecol. Health Dis 2000;12: 170–85.
19. Zamperini CA, Machado AL, Vergani CE, Pavarina AC, Rangel EC, Cruz NC. Evaluation of fungal adherence to plasma-modified polymethylmethacrylate. Mycoses 2011;54(5):e344-51.
20. Al-Kebsi, A.M.; Al-Motareb, F.L.; Al-Hamzy, M.; Al-Shamahy, H.A.; Al-Sanabani, N.F. Multiple risk factors of Candida albicans associated denture stomatitis. On. J. Dent. Oral Health 2018;1: 1–15.
21. Aoun, G.; Cassia, A. Evaluation of denture-related factors predisposing to denture stomatitis in a Lebanese population. Mater.Socio-Medica 2016;28: 392.
22. Thilakumara IP, Jayatilake JAMS, Pallegama RW, Ellepola ANB. Denture-induced stomatitis and associated factors in a group of patients attending a university dental hospital in Sri Lanka. J Investig Clin Dent. 2017;8(2):10.1111/jicd.12211. doi:10.1111/jicd.12211
23. Sanitá, P.V.; Pavarina, A.C.; Giampaolo, E.T.; Silva, M.M.; de Oliveira Mima, E.G.; Ribeiro, D.G.; Vergani, C.E. Candida spp. prevalence in well controlled type 2 diabetic patients with denture stomatitis. Oral Surg. Oral Med. Oral Pathol. Oral Radiol.Endodontology 2011;111: 726–33.
24. Peri´c, M.; Živkovi´c, R.; Mili´c Lemi´c, A.; Radunovi´c, M.; Mili´ci´c, B.; Arsenijevi´c, V.A. The severity of denture stomatitis as related to risk factors and different Candida spp. Oral Surg. Oral Med. Oral Pathol. Oral Radiol 2018;126: 41–7.
25. Scher EA, Ritchie GM, Flowers DJ. Antimycotic denture adhesive in treatment of denture stomatitis. J Prosthet Dent 1978; 40: 622–7.
26. Ozkan YK, Uçankale M, Ozcan M, Uner N. Effect of denture adhesive on the micro-organisms in vivo. Gerodontology 2012;29(1):9-16
27. de Oliveira Junior NM, Mendoza Marin DO, Leite ARP, Pero AC, Klein MI, Compagnoni MA. Influence of the use of complete denture adhesives on microbial adhesion and biofilm formation by single- and mixed-species. PLoS One 2018;13(10):e0203951.
28. Silva MDDD, Nunes TSBS, Viotto HEDC, Coelho SRG, Souza RF, Pero AC. Microbial adhesion and biofilm formation by Candida albicans on 3D-printed denture base resins. PLoS One 2023;18(10):e0292430.
29. Alvim GC, da Silva CB, Oliveira VC, dos Reis AC, Watanabe E, Lepri CP, de Castro DT. Evaluation of the antifungal effect and adhesive strength of a denture adhesive supplemented with nystatin. Rev Pre Infec e Saúde [Internet] 2023;9:4337. Retrieved from: http://periodicos.ufpi.br/index.php/repis/article/view/4337. [Accessed 22/11/2024]
30. Işeri U, Uludamar A, Ozkan YK. Effectiveness of different cleaning agents on the adherence of Candida albicans to acrylic denture base resin. Gerodontology 2011;28(4):271-6.
31. Jafari, A.A., FALAH, T.A., LOTFI, K.M.H., Zahraeii, A. and Kazemi, A., 2012. Vinegar as a removing agent of Candida albicans from acrylic resin plates. Jundishapur J Microbiol 2012; 5(2): 388-92.
32. Oregon State University. Microbiology Writing Guide: Presenting Data. Retrieved from https://wic.oregonstate.edu/microbiology-writing-guide-presenting-data [Accessed 10/09/2024]
33. O'Brien RG, Kaiser MK. MANOVA method for analyzing repeated measures designs: an extensive primer. Psychol Bull 1985;97(2):316-33.
34. Reynolds, J. (2005) Serial Dilution Protocols. ASM Microbe Library. Retrieved from https://asm.org/ASM/media/Protocol-Images/Serial-Dilution-Protocols.pdf?ext=.pdf [Accessed 10/09/2024]
35. Chopde N, Jawale B, Pharande A, Chaudhari L, Hiremath V, Redasani R. Microbial colonization and their relation with potential cofactors in patients with denture stomatitis. J Contemp Dent Pract. 2012;13(4):456-9.

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

"Tissue Induction"

SADJ MAY 2025, Vol. 80 No.4 P198 - P212

U Ripamonti

ABSTRACT

This contribution to the induction of tissue formation starts with seemingly simple questions, "*Why Bone?*" and "*Why Cartilage?*", the essential ingredients to compose the skeleton ad thus the speciation of the vertebrates, the induction of long bone *via* endochondral ossification, the induction of the growth plate, body erection and the speciation thus of the *Homo* clade, walking upright toward the spectacular creativity of extant *Homo sapiens*. The title wishes to pay tribute to grand pioneer scientists such as Pollettini, Levander, Moss, Urist and Reddi who persevered to study the induction of bone formation as initiated by devitalized demineralized bone matrices. "*Tissue Induction*" is the title of a seminal paper by Gustav Levander in *Nature*, 1945. Levander hypothesized that unknown substances from heterotopically implanted bone matrices would activate recipient resident cells to initiate the induction of bone formation, where there is no bone. Levander went further by using the term "*Tissue Induction*" linking the induction of bone formation to embryonal development as described by Hans Spemann and Hilde Mangold, the 1935 Nobel Prize for Medicine and Physiology. Phylogenetically, bones were an ancestral character, and cartilage developed later, providing the growth plate, to growth vertebrate' long bones establishing body erection in selected hominid' clades. The TGF-β supergene family includes several osteogenic proteins endowed with the remarkable capacity to initiate the heterotopic induction of bone. Besides the sub-family of the bone morphogenetic proteins (BMPs), in primates and in primates only, the three mammalian TGF-β isoforms also initiate the induction of bone formation. Heterotopic implantation of recombinant hTGF-β_3 initiates the induction of bone formation by priming resident intramuscular cells, pericytes, myoendothelial cells and myoblastic cells to express and secrete *BMPs* genes and gene products; the expression and synthesis of BMPs initiate the induction of bone formation regulated by *Noggin* expression. Combined morphological and molecular analyses have indicated that doses of hTGF-β_3 in Matrigel®Matrix set into motion the *in vivo* development of multiple tissues and multicellular organoids within the implanted furcation bioreactors. Organoids form by gene expression pathways from available different cellular populations within the exposed furcation bioreactor. Our molecular and morphological data using undecalcified whole mounted sections cut by the Exakt diamond saw technique have indicated that hTGF-β_3 in Matrigel®Matrix induces distinct supracellular phases that together with morphological transformation and organogenesis result in the generation of intramuscular mineralized bone organoids. The generation of transformed periodontal bioreactors into organogenesis of alveolar bone is connected to a highly vascularized periodontal ligament system patterned by newly generated collagenic fibers. These attach into substantial cementogenesis with capillary sprouting and angioblastic activity that result in cementogenesis in angiogenesis with *de novo* cementoid formation.

Authors' Information

1. Ugo Ripamonti, *MD, DDS, MDent, MFS, PhD* (Med), *FBSE* The Department of Internal Medicine. School of Clinical Medicine. Faculty of Health Sciences University of the Witwatersrand, Johannesburg 2193 Parktown, South Africa ORCID Number: 0000-0002-6567-3594

Corresponding author:
Name: Ugo Ripamonti
Email: ugo.ripamonti@wits.ac.za

INTRODUCTION: "WHY BONE?"

With a seemingly simple question, Romer[1] asks: "*Why bone?*". This contribution to "*Tissue Induction*" would like to ask another seemingly simple question, that is "*Why cartilage*?".

These altogether interesting and certainly difficult question proposes the first digital images of the manuscript, the extraordinary induction of chondrogenesis by a coral-derived macroporous hydroxyapatite-based bioreactor when implanted *solo* in the dorsal musculature of the Selachian' fish, the dusky shark *Carcharhinus obscurus* (Fig. 1).[2,3]

The images presented in Figure 1 morphologically show the emerging era of cell engineering.[4] The work of Lim in *Science* proposes the new era of cell engineering whereby cells are used as building blocks to initiate cell differentiation and thus induction of tissue morphogenesis (Lim 2022). Cell engineering controllably "*push a cell's button*" to initiate desired morphological responses.[4]

In context, the induction of chondrogenesis by a coral-derived biomimetic biomaterial bioreactor when implanted *solo* in the dorsal musculature of the Selachian' fish the shark *Carcharhinus obscurus* figuratively shows how the macro- and micro-porous surface' characteristics of the intramuscularly implanted bioreactor ultimately "*push a cell's button*" that results in the induction of chondrogenesis (Fig. 1) by molecularly triggering invading myoblastic cells of into the macroporous spaces of heterotopically implanted bioreactors.[2,3]

In his lucid and clear contribution to the evolutionary development of the vertebrate skeletal tissues, Romer presents a concise assay on the "*Ancient history of bone*".[1] Tissue induction and the developmental biology of both cartilage and bone are controlled by a vast array of genes and gene products molecularly controlling cellular and extracellular matrices synthesis, deposition and gene expression pathways.[5-9]

Romer in his quest to address the question "*Why bone?*"[1] touch upon the hypothesis that bone formed as a storage of ions, particularly Ca^{++} and several pleiotropic proteins. These must include the structural collagenous proteins, i.e. collagens type I, IV and II, osteonectins, fibronectins together with an array of altogether different morphogenetic proteins, i.e. proteins initiators that *de novo* set into motion the extraordinary induction of bone formation, or, as *per* G Levander' classic paper in *Nature*: "*Tissue Induction*".[10]

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

The work or Romer has indicated that the induction of bone formation and thus "*Why bone?*" is for the development of the dermal skeletal armor.[1] "*It thus seems highly probable that the bony skeleton without which the evolution of the vertebrates could never have taken place, owes its origin close to half a billion years ago, to the threat of invertebrate predation on our feeble primitive ancestors*" (Romer 1963)[1].

Perhaps however, the grand contribution of Romer to both knowledge of the cartilaginous fishes and the emergence of the bony armor was that his classic assay presented evidence that bone has an ancestral character, and that cartilaginous fishes like sharks, skates and rays were not primitive when compared to phylogenetically ancestral sharks. [1] Indeed, Romer argues that the cartilaginous skeleton developed following a "*degenerative slump from bone bearing ancestors*".[1] Romer further states that ancient sharks were bone-bearing fishes, later "*degenerating*" the ancestral bone into newly developed cartilaginous endoskeletons.[1]

The work of Romer grandly shows that the origins and development of the vertebrates is "*the reverse of the truth*". [1] The cartilage as seen in vertebrates is only an embryonic adaptation to properly growth and expand the long bones of the axial skeleton. The development of the growth plate was only possible *via* the development of the cartilaginous ancestral matrix that not only retained but possessed the fundamental morphological and molecular mechanisms of the cartilaginous growth plate of mammals. Of note, these were ancestrally present within the induced cartilage by the macroporous spaces of the coral-derived bioreactor intramuscularly implanted in the shark *Carcharhinus obscurus* (Fig. 1). [2,3,11]

Intriguingly, high power images of chondrogenesis as induced by the coral-derived bioreactor reveal the columnar assembly of chondroblastic cells within the chondrogenic extracellular matrix as initiated within the coral-derived macroporous spaces implanted in the dorsal musculature of *Carcharinus obscurus* (Fig. 1). [2.11] The columnar chondroblastic assembly of the cartilaginous growth plate is the very mechanism of the longitudinal growth of the endochondral long bones in mammals, phylogenetically present within the ancestral matrix that diverged into cartilaginous Elasmobranchs' skeletons.

The uniqueness of the mammalian cartilaginous growth plate is a fundamental compartmentalized biological bioreactor that masterminds the three-dimensional growth of the mammalian axial skeleton. Osteogenesis and the induction of bone, form *via* endochondral ossification, i.e. *via* the development of the cartilage anlage. Intriguingly, high power images of chondrogenesis by coral-derived bioreactors implanted intramuscularly in *Carcharhinus obscurus*, reveal the columnar assembly of chondroblastic cells within the extracellular matrix as initiated by coral derived macroporous bioreactors when implanted in the dorsal musculature of the Selachian's' fishes (Fig. 1).

Ancestrally thus, the induction of chondrogenesis by a macroporous coral-derived bioreactor implanted in the dorsal musculature of the Selachian's fish *Carcharhinus obscurus* is a cartilaginous matrix that retains the molecular blueprints for the induction of endochondral bone formation in mammals. This developmental pathway was only possible by the development of the cartilaginous growth plate, phylogenetically however not predating the induction and development of the cartilaginous fishes or Chondrichthyes. [2,11]

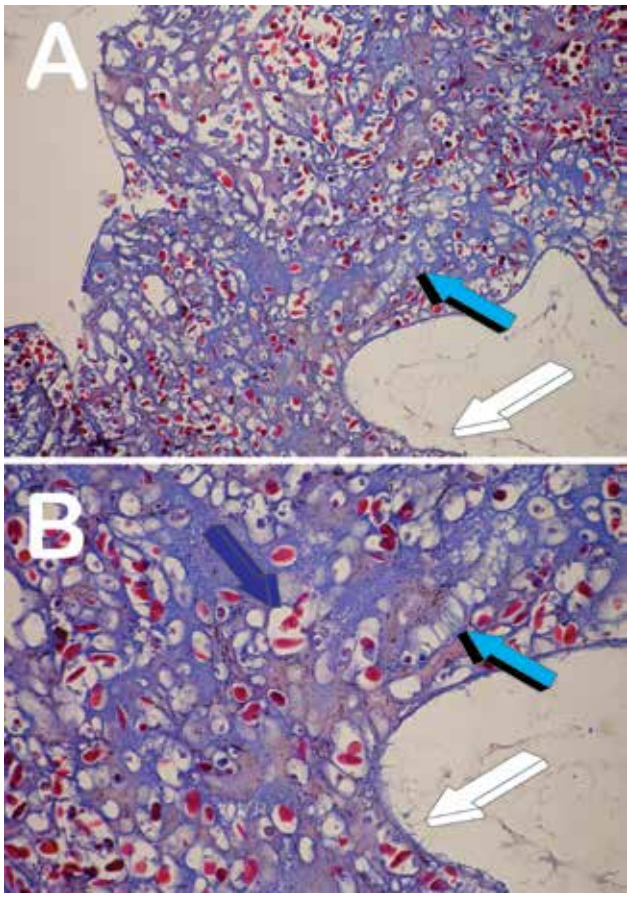


Figure 1. induction of chondrogenesis by a coral -derived calcium phosphate-based biomaterial biomimetic matrix after implantation of the calcium construct *solo* in the dorsal musculature of the Selachian' fish the shark *Carcharhinus obscuruus*.[2,3] Cell engineering [3] at the calcium phosphate interface (*white* arrows) facing invading Selachian' responding cells from the dorsal musculature of the Selachian' fish. How is chondrogenesis initiated by the calcium phosphate-based bioreactor? It is assumed that the macro- micro surface geometric configurations and topographies initiate cellular transformation and differentiation of invading Selachians' myoblastic cells which later attach to the micro surface topography that *per se* initiates chondroblastic cell differentiation and transformation into differentiating islands of cartilage within the macroporous spaces of the coral-derived constructs (Figs. 1 A,B). Remarkably, the induction of cartilage is additionally characterized by the presence of columnar chondroblastic cell differentiation (Figs. 1 A,B *light blue* arrows. Columnar chondroblastic cells typically characterize the embryonic growth plate of differentiating long bones in mammals. How possibly the vestiges of the mammalian growth plate is genetically and morphologically imprinted in *de novo* chondrogenesis by coral-derived bioreactors implanted intramuscularly in the Dusky shark *Carcharhinus obscurus* is still a matter of speculation,[2,11,25] save to say that evolutionary the first cartilage anlage for the development of the cartilaginous fishes the sharks, skate and rays and later for the development of the cartilaginous growth plate in mammals' evolution was a blue print cartilaginous matrix that retained the genetic and morphological blue print of both Selachians and mammals, developing thus from an ancestral matrix *ab initio* endowed with the columnar condensations required for mammalian bone growth, speciation, walking upright and thus differentiating the *Homo* clade. [84]

The development of a cartilaginous skeleton, not primitive but formed from ancestral bony skeletons inherited by sharks, skates and rays has been masterminded by genetic mutations that resulted in the ablation of angiogenic mechanisms controlling the evolution and development of the cartilaginous anlage.[2,3,11,] Such evolutionary pathways resulted in the expression and synthesis of powerful

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

inhibitors of angiogenesis[13-15] that blocked "*osteogenesis in angiogenesis*". [16-18]

The lack of cartilage vascular invasion or chondrolysis effectively blocked the induction of bone formation, as angiogenesis is a prerequisite for osteogenesis. [19] The lack of the induction of bone formation evolutionary speciated the Chondrichthyes or Elasmobranchs as genera with highly resilient cartilaginous skeletons to swim and feed in deep waters for proper swimming and hunting, altogether optimally surviving in the depths of the oceans.[2]

Why bone then? In a previous Chapter of a CRC Press Volume focused on the induction of bone formation by the transforming growth factor-β_3 morphogen (TGF-β_3),[20] we proposed that the evolutionary development of the growth plate was the morphological and molecular master key responsible for the longitudinal growth of the mammalian axial skeleton and for the induction of bone formation. Thus, the growth plate was the molecular and morphological bioreactor for the emergence of vertebrates and later of the *Homo* clade.[20]

The evolution of the skeleton, or rather the induction of bone formation and skeletogenesis, provided the biological tissues for the emergence of the vertebrates. As such, the skeleton acts as a "*giant molecular machine*" (AH Reddi personal communication 2011). Several key mutations and evolutionary adaptions resulted in the development of the pelvis for both ambulation, body erection and for fetal adaptations during hominins' speciation and the birth of man.

In a previous communication, we proposed that Nature's developmental biological and evolutionary plan was simply to provide "*Bone: Formation by autoinduction*", [21] skeletogenesis and body erection, pelvis adaptation to body erection and ambulation. This significantly contributed to enforcing industrious Homo-like activities by freeing the upper limbs for superior foraging, for the development of tools not limited to hunting and gathering but above all however for maternal care, physically and continuously guiding the newborn, contributing thus to the speciation of the *Homo* clade.[3]

The development of the skeleton, the induction of bone formation *via* the cartilaginous growth plate was Nature's master plan for the emergence of the vertebrates. The supramolecular assembly of the extracellular matrix of bone developed tissue forming substances or morphogens, first defined by Turing as "*forms generating substances*",[22] that initiate tissue morphogenesis, the genesis of form and function.

A variety of gene and gene products were thus required to set into motion the induction of bone formation and the initiation of skeletogenesis. It is noteworthy that Nature' parsimony in controlling multiple specialized functions or pleiotropy developed several osteogenic molecular signals with minor variation in amino acid sequence' motifs within highly conserved carboxy-terminal regions. [16,23]

Remarkably, gene products with ancestral sequences and amino acid motifs expressed in *Drosophila melanogaster* evolved for a billion years before the emergence of the vertebrates and the induction of skeletogenesis. Recombinantly generated DNA gene products of *decapentaplegic* and *60A* genes of *Drosophila melanogaster*, the boneless fruit fly, initiate the induction of endochondral bone formation when reconstituted with allogeneic insoluble and inactive collagenous bone matrix and implanted in extraskeletal heterotopic sites of rodents. [24]

Nature thus usurped phylogenetically ancient amino acid sequence' motifs controlling dorso-ventral patterning in *Drosophila melanogaster* to set the unique traits of the vertebrates, i.e. "*Tissue induction*"[10] and "*Bone: Formation by autoinduction*" [21] using minor modifications of amino acid sequence' motifs ancestrally deployed in *Drosophila melanogaster* for unrelated functions (Ripamonti 2006; Ripamonti 2019). [25]

Perhaps at the end of this sub-heading "*Why bone*?", reviewing the extraordinary developmental and molecular evolutionary plan that mechanistically frame the fundamental biological mechanisms of unique human biology,[26] it is perhaps worth to state again that Nature's plan for "*the induction of bone and osteogenesis was only to finalize the evolution of* Homo sapiens *on the planet earth*".[3]

SOLUBLE MOLECULAR SIGNALS AND THE INDUCTION OF BONE FORMATION

Last Century research has shown that intact demineralized bone matrices induce endochondral bone formation in heterotopic sites of animal model (for reviews: [16,27]). The critical experiments of Levander,[10,28] Urist, [21] Reddi and Huggins [29] and other showed that the extracellular matrix of mineralized tissues is the repository of differentiating morphogens tightly bound to the mineralized matrix.

In his classic work, "*A study of bone regeneration*",[28] Levander states that "*In the healing process of bone the new bone may be pictured as emanating from two different sources; partly from the end of the bone fragments and partly from the connective tissue surrounding the site of fracture. In the latter case, the connective tissue is considered transformed into bony tissue by virtue of a special process – the metaplastic theory of bone formation*". [28]

Levander' experiments show that after heterotopic implantation of autogenous bone grafts "*new bone is formed directly out of the mesenchymal tissue which surround the graft*". [28] Astutely, Levander understands that differentiation of bone from the mesenchymal tissue surrounding the graft "*must necessarily show that the process is influenced in some way or another by some specific agent*". He further states that such specific agents emanate from the grafted tissue. [28]

Levander thus hypothesizes that a "*specific bone forming substance is liberated from the implanted bone tissue and it is carried by the tissue lymph to the surrounding areas where it is able to activate the mesenchymal tissue in such a way that this becomes differentiated into bone tissue – either directly or by means of the embryonic pre-existing stage of bone and cartilaginous tissue*". [28]

It is our opinion that the above extraordinary statement summarizes with lucid and clear morphological and molecular insights "*The Bone Induction Principle*" (Urist et al. 1967), proposing that the extracellular matrix of bone is a reservoir of soluble and insoluble signals that initiate the induction of bone formation. [28]

https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

As a matter of semantic perhaps Levander' statements and insights were not perceived then worthy as claims to fame possibly because Levander' studies and publications did not propose a more precise or enticing definition of this unidentified "*bone forming substance*". This in spite of the major insights into the induction of bone formation, particularly by alcoholic extracts, and the vision of the "*bone forming substance*" as a soluble signal.[28] The above statements were paralleled by the statement that the morphological evaluation of the newly induced bone showed that "*fully formed mesenchymal cells ultimately emanate from the endothelial cells of the capillaries*".[28]

The above is a further challenging statement of Levander, who had the extraordinary morphological and somehow the molecular vision to understand "*The Role of the Vessels in Osteogenesis*" long before the classic paper of Trueta in *The Journal of Bone and Joint Surgery* [B].[19] Trueta defined the induction of *osteogenetic vessels* as essential morphological and molecular components for the induction of bone formation.[19] Several authors did already postulate the role of the vessels in osteogenesis and Aristotle even proposed that vessels and invading capillaries were organogenetic, constructing the frame of the body plan.[25,27]

Following Levander' studies (for details see [27] Urist recognized the importance of demineralized bone matrix (DBM) to induce reproducible heterotopic endochondral bone induction, [21,30] and later proposed the present terminology hypothesizing the presence of a bone morphogenetic protein complex (BMP) within the bone matrix .[31]

A *quantum* leap towards the mechanistic understanding of the phenomenon of "*Tissue Induction*", [10] has been the dissociative extraction and reconstitution of the bone matrix components which, when combined, trigger the bone induction cascade.[31,32] The experiments of AH Reddi, then at the NIH Bone Cell Biology Section[31,32] dissociatively extracted demineralized bone matrix in chaotropic agents such as 4M guanidinium hydrochloride or 6M urea resolving an insoluble and inactive collagenous matrix signal and solubilized extracted proteins, or soluble signals.[33]

Purified extracted proteins by gel filtration chromatography reconstituted with the allogeneic insoluble and inactive collagenous bone matrix restored the biological activity of the extracted proteins, initiating the induction of bone formation in the rodent subcutaneous assay.[31] Soluble signals, i.e. osteogenic proteins, need to be reconstituted with allogeneic inactive collagenous bone matrix, [32] since xenogeneic collagenous matrices as carriers block the bone induction cascade.[32]

The realization that the chaotropically extracted extracellular matrix of bone was a reservoir of structural and morphogenetic proteins set the scientific and biotech industry' race for the isolation and purification to homogeneity of the elusive yet to be isolated and characterized BMP complex postulated by Urist and Strates in 1971 as bone morphogenetic protein.[34]

A further incisive step ahead was again the work of Urist and co-workers published in PNAS describing the purification of bovine BMP by hydroxyapatite chromatography.[35] This experiment reported the adsorption or "*absorption*" of the BMP complex onto hydroxyapatite chromatography gels. The research experiment reported that a broad band of osteogenic fractions with BMP-like activity would adsorb onto hydroxyapatite chromatography gels.[35] Eluted fractions of 18.5 kDa induced large deposits of bone and newly formed ossicles in heterotopic sites of rodents.[35]

Using chaotropically extracted bovine bone matrices, Reddi' team at the NIH Bone Cell Biology Section purified osteogenin, an osteogenic protein with biological activity in the rodent subcutaneous assay.[36] Purification was by sequential hydroxyapatite adsorption, heparin-Sepharose affinity and S-200 Sephacryl gel filtration chromatography, reporting a molecular weight of 22 kDa with osteoinductive activity in heterotopic subcutaneous sites of rodents.[36]

Incisive work aided by continuous collaboration and contacts with leading scientists in the field allowed Genetic Institute, Boston US, to purify to homogeneity naturally derived bovine morphogenetic proteins (Wang et al. 1988). Purification steps included hydroxyapatite adsorption chromatography, affinity chromatography on heparine-Sepahrose gels, and Superose 6 and 12 columns connected in series to optimize gel filtration. Biologically active proteins were of approximately 30 kDa on SDS-PAGE.[37]

Genetic Institute' scientists decided to re-use the original term bone morphogenetic protein proposed by Urist and Strates in 1971[34] to define the newly purified and cloned proteins thus to ride all the biological *in vitro* and *in vivo* scientific background as formidably established by the Bone Research Laboratory at the University of California Los Angeles.[37,38]

Protein sequences were defined, obtaining amino acid motifs which were used to clone several human recombinant bone morphogenetic proteins (BMPs). *Science* reported the experiments as "*Novel Regulators of Bone Formation: Molecular clones and Activities*".[38] The contribution to *Science* primarily identified not one (Fig. 2) but several proteins with osteoinductive activity in the rodent bioassay, and that the newly isolated and cloned proteins were new members of the TGF-βs supergene family.[38,39]

MORPHOGENS, OR SOLUBLE MOLECULAR SIGNALS, INITIATE PERIODONTAL TISSUE INDUCTION

Purification to homogeneity of naturally derived BMPs, molecular cloning and expression of the recombinant human proteins [16,27] (for reviews) did appear, then, to resolve the "*Reality of a nebulous enigmatic myth*".[40] Tissue regeneration in postnatal life recapitulates events that occur in the normal course of embryonic development.[10,28,29] A highly conserved family of proteins, the transforming growth factor-β (TGF-β) supergene family, equally regulates both embryonic development and postnatal tissue induction.[16,17,18,23,29,41,42,43,44,45]

The pleiotropism of the TGF-β supergene family underlines the findings that the three mammalian TGF-β isoforms initiate endochondral bone induction in the non-human primate *Papio ursinus*.[46-49]

Pre-clinical studies in the Chacma baboon *Papio ursinus* showed the induction of bone by the bone morphogenetic proteins (BMPs), pleiotropic members of the TGF-β supergene family.[16,45,48,50] Mammalian naturally derived BMPs and recombinant human BMPs (hBMPs) induce *de novo* bone formation (Fig. 2). Proteins act as soluble

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

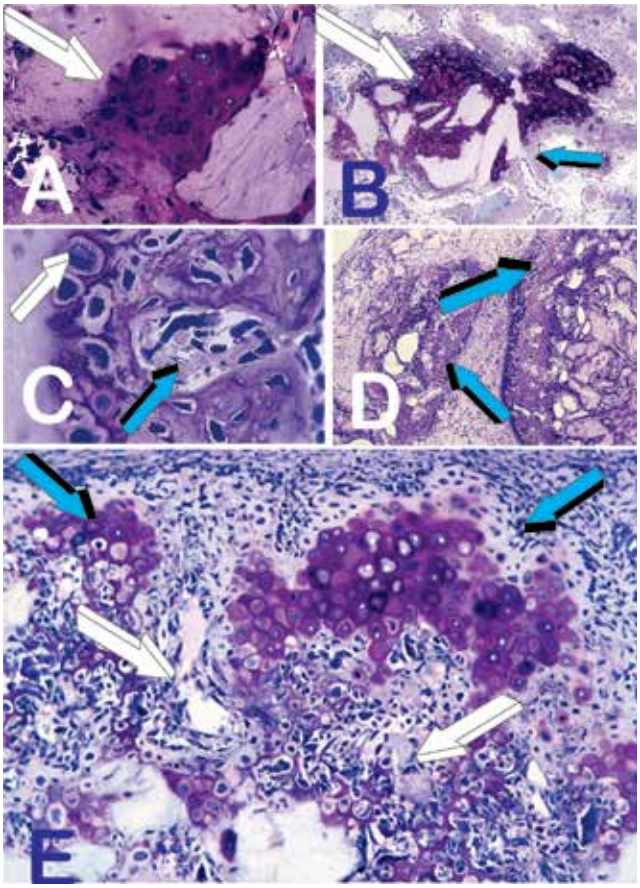


Figure 2. Operational reconstitution of the soluble signals with insoluble signals or substrata with restoration of the biological activity of chaotropically extracted bone matrix. Reddi' classic experiments chaotropically extracted bone matrices at the Bone Cell biology Section, NIH, Bethesda, MD. Extraction yielded an inactive insoluble collagenous bone matrix and soluble signals. Reconstitution of the inactive matrix with soluble signals after gel filtration chromatography to exclude high molecular weight contaminants, restored the osteoinductive activity of the chaotropically extracted soluble signals. The insoluble collagenous signals act as a carrier and delivery system for the osteoinductive activity of the soluble signals.[31,32,36] A. Induction of chondrogenesis (*white* arrow) by 0.1-0.5 µg osteogenin purified to apparent homogeneity reconstituted with allogeneic bone matrix.[63] B. Induction of chondrogenesis (*white* arrow) by 0.1-0.5 µg osteogenin reconstituted with allogeneic bone matrix. There is vascular invasion and differentiation of osteoblastic-like cells resulting in the induction of bone formation (*blue* arrow). There is recapitulation of embryonic development upon implantation of osteogenic proteins in heterotopic sites of rodents.[31,63] C. There is differentiation of hypertrophic chondrocytes (*white* arrow) and vascular invasion and chondrolysis of the newly formed cartilage (*blue* arrow). Vascular invasion sets into motion the differentiation of osteoblastic-like cells and the induction of bone formation.[29,31,32] D. Induction of bone formation (*blue* arrows) by highly purified osteogenin, 20 to 28 µg osteogenin after gel filtration chromatography reconstituted with inactive allogeneic insoluble collagenous bone matrix.[63] E. Induction of endochondral bone formation (*blue* arrows) with the induction of cartilaginous anlages (*blue* arrows) recapitulating embryonic development by 2.5 mg recombinant human osteogenic protein-1 (hOP-1) per gr of allogeneic bone matrix as carrier. Recapitulation of embryonic development with the induction of chondrogenic anlages that initiate vascular invasion (*white* arrows), chondrolysis and the induction of bone differentiation in the newly established heterotopic ossicle.

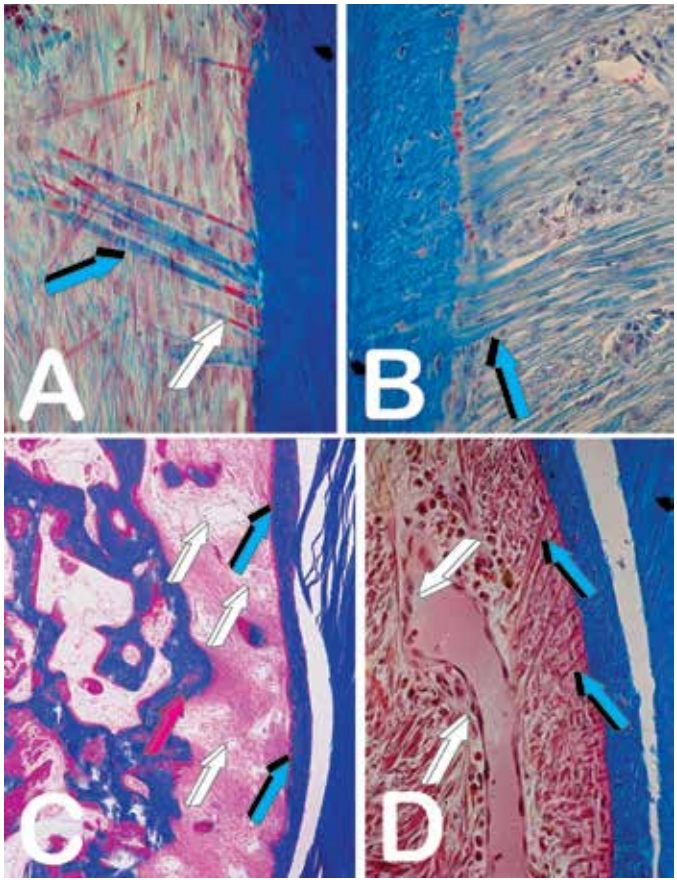


Figure 3. Tissue induction and regeneration of a new periodontal ligament system after application of 250 µg osteogenic protein fractions combined with 150 mg allogeneic insoluble collagenous bone matrix in Class II furcation defects of adult Chacma baboon *Papio ursinus*.[51] Undecalcified mandibular blocks containing both mandibular molars with surrounding lingual and buccal alveolar bone with the attached periodontal tissues were harvested as mineralized blocks and embedded undecalcified in K-Plast resin. Undecalcified sections cut at 2 to 6 μm were stained free-floating with a modified Goldner' trichrome.[16,51] A. Generation of Sharpey's fibers directly inserting into root planed mineralized dentine (*blue* arrows), with differentiating cementoblasts geometrically stacked between fibers (*white* arrow). B. Fibers insert into dentine also regulating the differentiation of cementoblasts-like cells along the planed root surface (*blue* arrow). The newly formed periodontal ligament space is highly vascular and hypercellular for differentiating phenomena at both cemental/dentinal surfaces. C. Complete regeneration of a highly vascular periodontal ligament space (*white* arrows), the alveolar bone (*magenta* arrow) and induction of cementogenesis (*blue* arrows). The induction of cementogenesis was unexpected in these early and first experiments in the Chacma baboon *Papio ursinus* and indicated the vast pleiotropic activity of the newly isolated bone morphogenetic proteins' fractions,[51] then labelled as osteogenin.[63] D. Detail of the regenerated periodontal ligament space by osteogenic proteins and the newly formed capillary with plump hypertrophic endothelial cells (*white* arrows). Note fibers that attach to the endothelial basement membrane emanating from the newly formed cementum (*blue* arrows). The morphological set up of the capillary and its relationships with the periodontal ligament fibers indicate cellular migration and riding from the vascular angiogenic to the cemental/dentinal microenvironment providing a continuous flow of endothelial, pericytes and other cells in perivascular niches for tissue induction and morphogenesis.

signals for tissue morphogenesis, sculpting the multi-cellular mineralized structures of the periodontal tissues with functionally oriented periodontal ligament fibers inserting into newly formed cementum (Fig, 3).[16,25]

BMPs induce the complex tissue morphologies of the periodontal tissues in the non-human primate *Papio ursinus* in Class II mandibular furcation defects treated with naturally – derived and recombinantly produced BMPs.[51,52,5354] The presence of multiple forms of BMPs has a therapeutic significance and the choice of a suitable factor is a formidable challenge to the practicing periodontologist and skeletal reconstructionist-.[55,56]

Tissue morphogenesis induced by hOP-1 and hBMP-2 is qualitatively different when the morphogens are applied

https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

singly. This indicates that the structure/activity profile amongst BMPs is controlling pleiotropic tissue induction and morphogenesis (Fig. 4). [25,56,57,59,] Furcation defects of *Papio ursinus* with root surfaces long-term exposed to periodontal disease implanted with doses of gamma-irradiated hOP-1 resulted in complete regeneration of the furcation defects with prominent induction of cementogenesis with Sharpey's fibers embedded within the newly formed cementum.[58,59]

Short-term studies delivering 125 µg hOP-1 combined with xenogeneic gamma-irradiate bovine bone matrix reported the induction of cementogenesis by day 60 after implantation along the exposed root surfaces of Class II furcation bioreactors (Fig. 4f). [52] Induction of cementogenesis was evident 6 months post-implementation in Class II furcation defects of *Papio ursinus* (Figs. 2.4g,h).[58]

A novel regenerative approach but in primates only is the induction of periodontal tissue regeneration with substantial cementogenesis by doses of the recombinant human transforming growth factor-β_3 (hTGF-β_3). [25,56,60] In the non-human primate *Papio ursinus* periodontal tissue induction and regeneration develops as a mosaic structure in which the osteogenic proteins of the TGF-β superfamily singly, synergistically and synchronously initiate and maintain tissue induction and morphogenesis.[43,56,59]

An alternative bone induction strategy and regenerative approach is to induce in heterotopic sites newly formed ossicles by recombinant hTGF-β_3 later transplanted as morcellated autogenous bone grafts into Class II furcation defects of *Papio ursinus* [59,61] and with hTGF-β_3 in Matrigel®Matrix with *rectus abdominis* responding cells.[62]

Research experiments analyzed both the morphological and gene expression studies of periodontal tissue induction and morphogenesis of selected osteogenic proteins of the TGF-β supergene family. [56,60] Results showed that hOP-1 and hBMP-2 singly or in binary application show pronounced morphological regenerative differences (Fig. 2.4). Recombinant proteins, singly or in binary applications where implanted reconstituted with insoluble collagenous matrices into Class II furcation defects of *Papio ursinus*. [25,57,59] The results highlighted the site tissue specificity and the structure activity profile of each recombinant hBMP when applied singly to root planed surfaces recapitulating embryonic development of the expressed and secreted proteins (Figs. 4a,c; Figs. 4b,d,e). [25,43,52,56]

We defined the capacity of mammalian BMPs to initiate a programmed cellular cascade resulting in the induction of bone [63] as well as cementogenesis [25] as "*a functionally conserved process utilized in embryonic development, recapitulated in postfetal osteogenesis, and can be exploited for the therapeutic initiation of bone formation*" [63] as well as cementogenesis.[25]

Previously identified challenges in periodontal tissue regeneration [55] are still unsolved; this despite several research work on cellular populations allegedly initiating periodontal tissue induction. [64,65]

The biological significance of redundancy is a still unresolved challenge. [55] Experimentation in non-human primates has shown that the presence of multiple forms of BMPs has a therapeutic significance. [56,59] Limited research addressed this challenge since "*the choice of a suitable morphogen is still a formidable challenge to the practicing periodontologist*".[55] We have proposed that critical research experimentation would have been to study optimal combinations and developing a structure-activity profile amongst the members of the BMPs family. [51,55,59]

Our Unit has been amongst the first to propose heterotopic and orthotopic regenerative studies combining homologous

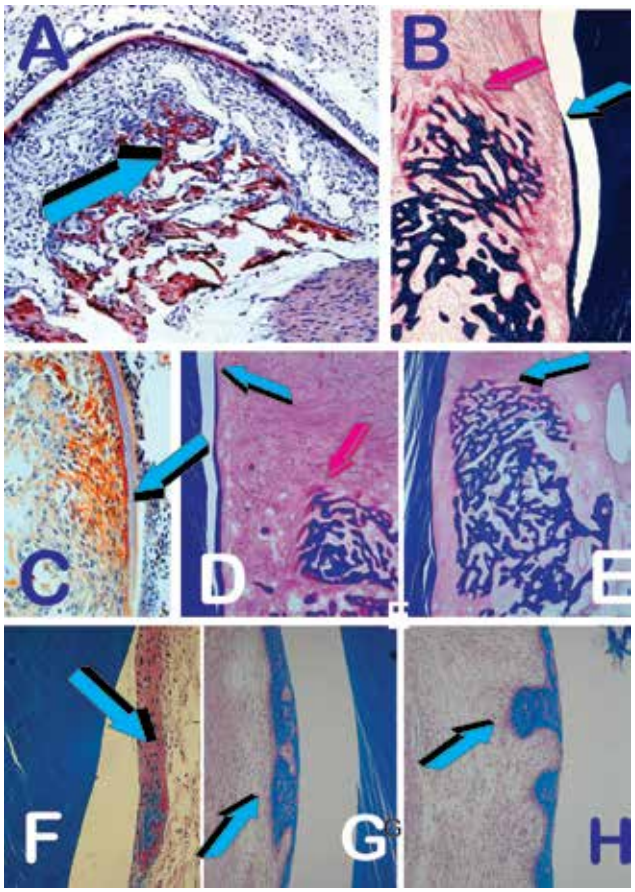


Figure 4. Structure/activity profile of isolated and cloned human bone morphogenetic proteins (hBMPs). [16,51,56,57] Our systematic studies in Class II furcation defects or bioreactors of the Chacma *baboon Papio ursinus* did show that single hBMPs induced selected periodontal regenerative traits when compared to other singly used hBMPs.[16,56,57] The structure/activity profile is based on the recapitulation of tissue development whereby proteins exploited in embryonic development are re-exploited in post-natal tissue induction and regeneration. [16,56,57] A. Immunolocalization of BMP-2 in the alveolar bone of a 16-day-old mouse pup (*blue* arrow.[43] Immunolocalization of BMP-2 is strictly localized in the developing alveolar during tooth morphogenesis. Lack of staining within the periodontal ligament space. [43] B. Tissue induction and morphogenesis in *Papio ursinus* showing that hBMP-2 when implanted in Class II furcation defects recapitulates embryonic tooth morphogenesis by regenerating alveolar bone covered by prominent osteoid seams (*magenta* arrow) with however limited cementogenesis (*blue* arrow). [56,57] C. Immunolocalization of osteogenic protein-1 (OP-1/BMP-7 during morphogenesis of the periodontal ligament fibers and the induction of cementogenesis (*blue* arrow). [56] D. *In vivo* in Class II furcation defects of *Papio ursinus* recombinant hOP-1 induces cementogenesis (*blue* arrow) and osteoid synthesis (*magenta* arrow) with limited however mineralized alveolar bone. E. Binary application of doses of the recombinant morphogens induce both cementogenesis together with prominent induction of mineralized bone covered by osteoid seams (*blue* arrow).[57] F,G,H. Digital images of Class furcation defects in *Papio ursinus* implanted with 125 µg hOP-1 and harvested on day 60 after implantation combined with xenogeneic bovine insoluble collagenous bone matrix. [52] Induction of cementoid matrix deposition with foci of mineralization (*blue* arrow F) along the root planed surface. G,H. Mineralized patterns of cementum deposition covered by thin layers of cementoid surfaced by cementoblasta (*blue* arrows). Undecalcified K-Plast embedded section stained free-floating with a modified Goldner' trichrome. [16,25,52]

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

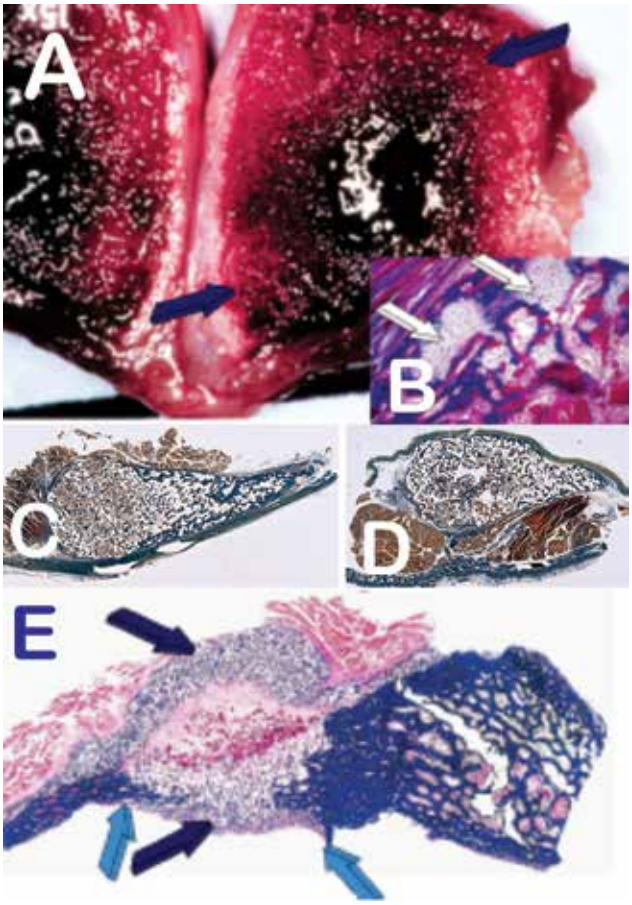


Figure 5. Synergistic induction of bone formation by combined binary application of 25 µg recombinant human osteogenic protein-1 (hOP-1) with 0.5 µg recombinant human transforming growth factor-β_1 (hTGF-β_1) at a ratio 20:1 hOP-1:hTGF-β_1 implanted in the *rectus abdominis* muscle of *Papio ursinus*.[20,46] hTGF-β_1 initiates the induction of bone formation at 5 µg per 100 mg of inactivated insoluble collagenous bone matrix.[46] A. The recombinant morphogen synergizes with hO-1 resulting in the induction of large corticalized heterotopic ossicles as early as 15 days after intramuscular *rectus abdominis* implantation (*dark blue* arrows) with mineralized trabeculae of newly formed bone with cartilage development (inset *white* arrows B). C,D. Synergistic induction of bone formation by binary application of 125 µg hOP-1 and 25 µg recombinant human transforming growth factor-β_3 (hTGF-β_3) adsorbed onto macroporous coral-derived bioreactors.[67] Bone preferentially formed at the periphery of the coral-derived constructs extending into the *temporalis* muscle. There is massive induction of newly formed bone outside the profile of the heterotopically implanted bioreactors. E. Calvarial tissue induction by molecular binary application of 100 µg recombinant human osteogenic protein-1 (hOP-1) with 25 µg platelet-derived porcine transforming growth-β_1 (pTGF-β_1).[20,68,69]

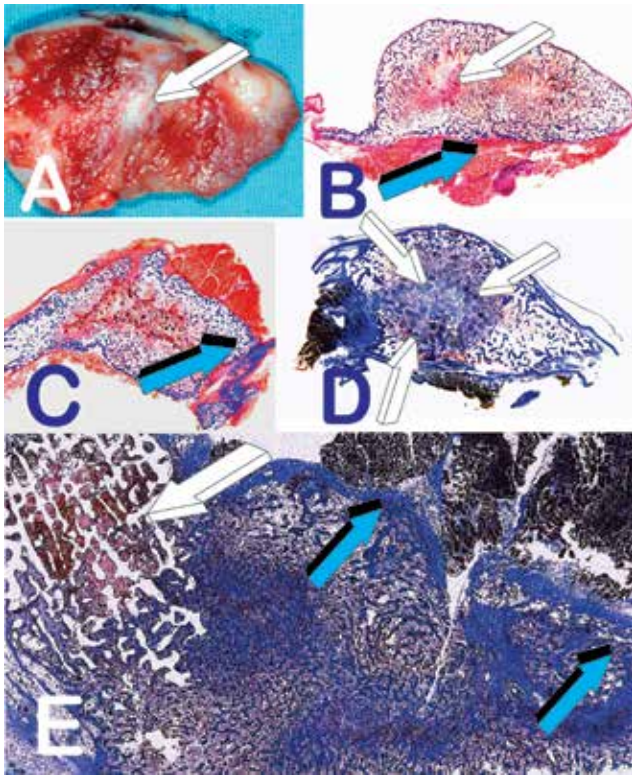


Figure 6. Induction of large corticalized ossicles upon heterotopic implantation of 125 µg recombinant human transforming growth factor-β_3 (hTGF-β_3) combined with inactive collagenous bone matrix as carrier in the *rectus abdominis* muscle of *Papio ursinus*. A,B. Generated ossicles were harvested on days 30 after intramuscular implantation [49] and processed for undecalcified histology after embedding in K Plast Resin. Sections, cut at 3 /4 µm were stained free-floating as described. [49] B,C. Low power images show corticalization of newly formed mineralized bone (*blue* arrows) within the *rectus abdominis* muscle. D. Biphasic macroporous hydroxyapatite/β-tricalcium phosphate preloaded with 25 µg hTGF-β_3 shows extensive induction of bone formation prominently exceeding the profile of the implanted macroporous hydroxyapatite/β-tricalcium phosphate bioreactor (*white* arrows). E. Coral-derived macroporous constructs (*white* arrow), preloaded with 250 µg hTGF-β_3 show pronounced induction of bone formation prominently exceeding the profile of the implanted bioreactor (*blue* arrows).[70,71] Newly formed bone extends for more than two cm from the profile of the coral-derived construct (*white* arrow E) and newly formed bone rippled and generated centimeters away from the pre-loaded hTGF-β_3 bioreactors. Tissue induction and morphogenesis are initiated by waves of continuously differentiating responding cells at the periphery of the implanted super-activated bioreactor, which also shows lack of bone differentiation within its preloaded macroporous spaces (*white* arrow E). Figure 6E epitomizes the tissue transfiguration *in vivo* by the hTGF-β_3 morphogen, transfiguring the *rectus abdominis* muscle into large corticalized ossicles transfiguring the available *rectus abdominis* myoblastic and pericyte cells into secreting osteoblasts.[20]

but molecularly different morphogenetic proteins. Binary applications were also applied in periodontal regenerative studies. [57,59,66] Our first heterotopic combination study yielded unprecedented results showing a synergistic interaction between recombinant human osteogenic protein-1 (hOP-1, also known as hBMP-7) and relatively low doses of hTGF-β_1 (Fig.5). [46,67]

We later provided mechanistic molecular data supporting the profound synergistic interactions between platelets-derived porcine transforming growth factor-β_1 (pTGF-β_1) and recombinant hOP-1. [68] Type IV collagen mRNA was highly expressed in synergistic tissues providing extracellular basement membrane' components for vascular invasion and capillary sprouting within the newly formed synergistic ossicles in the *rectus abdominis* muscle of *Papio ursinus*. [68,69]

Reconstitution of allogeneic insoluble collagenous bone matrix with 100 µg hOP-1 combined with 15 µg pTGF-β_1 and implanted in calvarial defects of *Papio ursinus* resulted in a substantial synergistic interaction of bone formation on day 30 displacing the *temporalis* muscle (Fig. 5e). [67,68,69]

Reconstituted coral-derived macroporous bioreactors with 125 µg hOP-1, 125 µg hTGF-β_3 and binary applications of 125 µg hOP-1 and 25 µg hTGF-β_3 in the *ratio* of 5:1 hOP-1:hTGF-β_3 were implanted in heterotopic intramuscular sites of the *rectus abdominis* muscle of *Papio ursinus*. Results showed prominent and substantial induction of bone formation extending far beyond the profile of the implanted super activated bioreactors (Figs. 5c,d). [67] Of interest, qRT-PCR showed prominent induction of TGF-β_3 mRNA with relatively low expression values of *OP-1* mRNA.[67,69]

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

The morphological hallmark of the synergistic induction of bone formation is the rapid differentiation of large osteoid seams enveloping haematopoietic bone marrow that forms by day 15 in newly generated ossicles in the *rectus abdominis* muscle of *Papio ursinus* (Figs. 5a,b).[46,67]

We also reported that synergistic binary application of hOP-1- and hTGF-β_1 in the *ratio* 20:1 respectively, initiate the heterotopic induction of rudimentary embryonic growth plates (Fig. 7a). This has indicated that the "*memory*" of developmental events in embryo is re-deployed postnatally by morphogen combinations (Fig. 7a). [46,69] Of interest, tissue induction and morphogenesis by TGF-β_3 applied singly in heterotopic *rectus abdominis* intramuscular sites also morphogenizes columnar chondrocytes as seen in the mammalian growth plate (Fig. 7b).

It is noteworthy that single applications of 125 or 250 µg hTGF-β_3 result in the rapid induction of heterotopic bone formation (Fig. 6). The induction of bone formation is superior to binary applications of recombinant hTGF-β_1 or hTGF-β_3 with hOP-1. [49,67,69] Mechanistically, the initiation of bone by the recombinant hTGF-β_3 invocates the rapid induction and expansion of the transformed mesenchymal tissue into large corticalized heterotopic ossicles with pronounced osteoblast-like cell differentiation at the periphery of the implanted reconstituted specimens with "*tissue transfiguration* in vivo" (Fig. 6e). [20,49]

The induction of bone forms beyond the geometric space of reconstituted carrier matrix, prominently expanding outside the profile of the macroporous delivery system, being either macroporous biphasic hydroxyapatite/β-tricalcium phosphate (HA/β-TCP) (Fig. 6d) or coral-derived macroporous bioreactors preloaded with 250 µg (hTGF-β_3 (Fig. 6g).

The image shown in Figure 6e shows significant and prominent osteogenesis predominantly surrounding the coral-derived macroporous bioreactor super activated by 250 µg hTGF-β_3 (Fig. 6e). [20,48,70,71] Of interest, a tenfold less dose of hTGF-β_3, i.e. 25 µg, initiates prominent induction of bone formation extending outside the profile of the heterotopically implanted super activated HA/β-TCP bioreactor (Figs. 6d white arrows. [70,71]

Molecularly, the rapid induction of bone formation by binary applications of hOP-1 and hTGF-β_3 or by hTGF-β_3 applied singly, resides in the up-regulation of selected genes involved in tissue induction and morphogenesis, i.e. *Osteocalcin*, *RUNX-2*, *OP-1*, TGF-β_1 and TGF-β_3 with however notably lack of *TGF*-β_2 up-regulation. [69] Of note, the induction of bone formation by the hTGF-β_3 isoform implanted singly is greater than ossicles generated by binary synergistic applications of hOP-1 with relatively low doses of either hTGF-β_1 or hTGF-β_3 (Fig. 2.6a). [46,69] Relatively high doses of the hTGF-β_3 morphogen (125 µg hTGF-β_3) initiate a developmental cascade of molecular and cellular events primarily characterized by the expression of multiple profiled bone morphogenetic proteins. [69] Together with significant chemotaxis, chemokinesis and cell migration of responding cells at the periphery of the hTGF-β_3-pre-treated bioreactors, the expressed and secreted BMPs induce rapid and extensive bone formation greater than the synergistic induction of bone formation. [69]

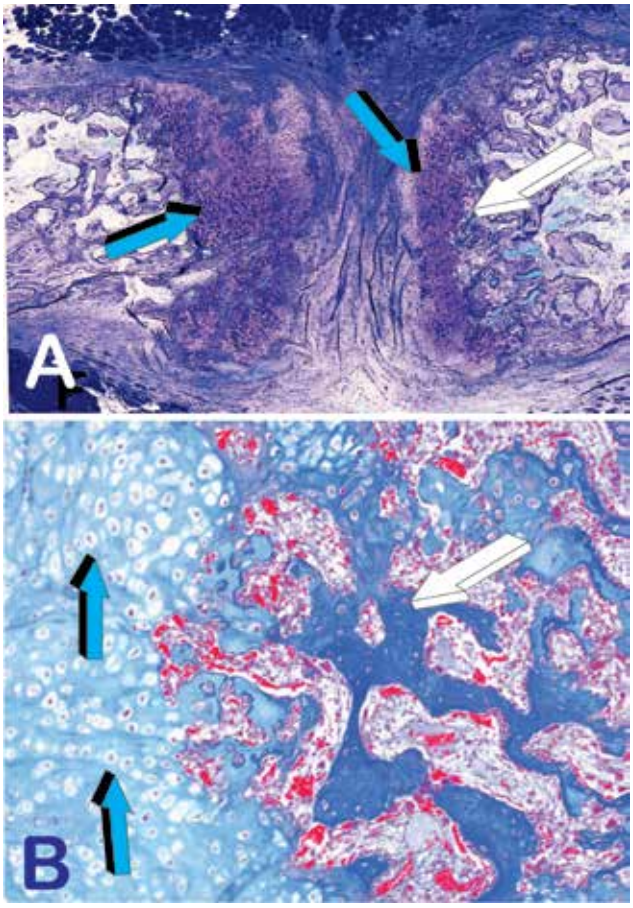


Figure 7. Intramuscular *rectus abdominis* induction of endochondral bone with cartilage anlages mimicking the induction of mammalian growth plates when binary application of hOP-1 are combined with relatively low doses of recombinant human transforming growth factor-β_1 (hTGF-β_1) A. and implanted with collagenous bone matrices in the *rectus abdominis* of *Papio ursinus*.[46] There is differentiation of chondroblastic cells (*blue* arrows) with subjacent trabeculae of mineralized bone (*white* arrow). There is recapitulation of embryonic development with the induction of growth plate within the *rectus abdominis* muscle with columns of chondroblastic cells reminiscent of the mammalian cartilaginous growth plate. [46] B. Heterotopic intramuscular implantation of 125 µg hTGF-β_3 rapidly induces mineralized bone also subjacent to cartilage induction with columns of hypertrophic chondrocytes highly reminiscent of the mammalian cartilaginous growth plate.

The reported data on the significant and pleiotropic biological activities of the hTGF-β_3 morphogen indicate that the TGF-β_3 gene masterminds' critical developmental events beyond bone and cartilage morphogenesis, ancestrally regulating skeletogenesis and the emergence of the craniofacial dentate masticatory apparatus, including the differentiation and initiation of cementogenesis. [25,60]

Synergistic molecular combinations were thus tested in Class II furcation defects of the Chacma baboon *Papio ursinus* (Fig. 2.4). [25,57,66] Our study that first attempted to address the structure-activity profile amongst BMPs family members did show that tissue morphogenesis induced by hOP-1 and hBMP-2 is qualitatively different when the morphogens are applied singly, hOP-1 inducing substantial cementogenesis (Figs. 4d,f,g,h). hBMP-2 treated defects showed limited induction of cementogenesis but a temporal enhancement of alveolar bone regeneration and remodeling (Fig. 4b). Although statistically not significant, the extent of cementogenesis by binary application showed pronounced induction of cementogenesis when compared to hBMP-2 treated specimens (Fig. 4e).

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

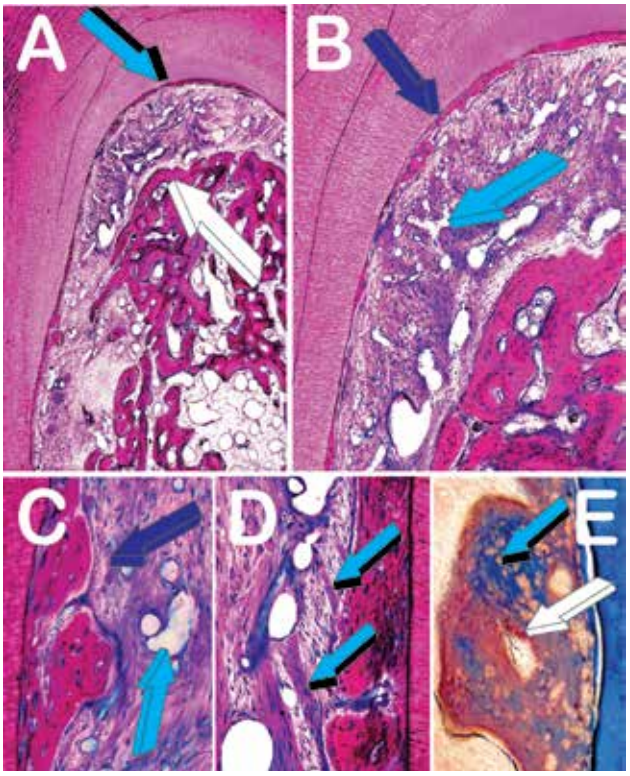


Figure 8. Series of digital microphotographs illustrating the vast pleiotropic activity of the mammalian transforming growth factor-β_3 regenerating the three essential components of the mammalian periodontal tissues: the alveolar bone (*white* arrow), the periodontal ligament system (*light blue* arrows) and the induction of cementogenesis with inserted periodontal ligament fibers (*dark blue* arrow). A,B. Low power images of Class II furcation defects or bioreactors prepared in mandibular molars of *Papio ursinus*.[60] Extensive induction of bone formation across the furcation defect with substantial induction of cementogenesis (*dark blue* arrow) facing a highly vascularized periodontal ligament space (*light blue* arrows. C,D,E. Induction of cementogenesis by 75 µg of hTGF-β_3 in Matrigel®Matrix resulting in thick layers of newly formed cementum (*dark blue* arrow C) always in close relationship with newly formed capillaries (*light blue* arrow C) and penetrating within the newly formed cementoid matrix (*white* arrow E). D. Periodontal ligament fibers connecting the newly deposited cemental matrices to the vascular microenvironments, providing collagenic basic structures for cell migration and riding the fibers from angiogenetic vessels to newly formed cementoid matrix along the planed root surface (*light blue* arrows D). E. High power view of newly deposited cementoid matrix along the planed root surface treated with 75 µg of hTGF-β_3 in Matrigel®Matrix.[60] There are foci of mineralization within the cementoid matrix (*light blue* arrow) and angiogenesis within the newly deposited cementoid matrix (*white* arrow E). Undecalcified sections processed, prepared and cut at 30 µm by the Exakt diamond saw cutting and grinding technique. [60]

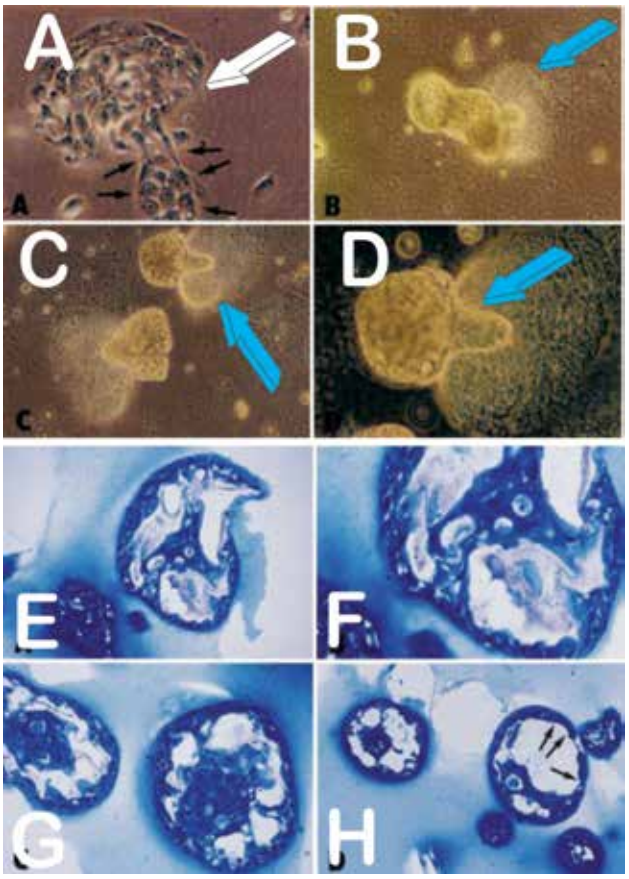


Figure 9. Fetal transitional epithelial cells harvested from bladders euthanized fetal Chacma baboons *Papio ursinus* were grown *in vitro* on Matrigel®Matrix.[74] A,B,C,D Digital images show the marked effect of extracellular matrix components of Matrigel®Matrix that resulted first in aggregation of transitional epithelial cells (*white* arrow A) with branching morphogenesis (*light blue* arrows). B,C,D. Transformation and induction of transitional epithelial cells with clusters of spheroidal organoids with branching morphogenesis (*light blue* arrows B,C,D). E,F,G,H. Explanted spheroidal organoids were processed and embedded into K-Plast resin, cut at 2 to 4 µm and stained with Toluidine blue.[74] Sectioning showed the organization of epithelial cells forming pseudo-cystic spaces lined by transformed transitional epithelial cells by extracellular matrix components of Matrigel®Matrix, namely type IV collagen and laminin.[74]

The demonstration of therapeutic mosaicism in periodontal tissue regeneration, as previously highlighted by immunolocalization studies during murine craniofacial and periodontal embryonic development,[43] will require extensive testing of *ratios* and doses of recombinant morphogen' combinations for optimal tissue engineering in clinical contexts.[57]

Synergistic molecular combinations of hOP-1 and hTGF-β_1 showed pronounced angiogenesis in the chick chorio-allantoic membrane (CAM)[69] when morphogens were applied at 20:1 *ratio* of hOP-1 and hTGF-β_1, respectively. Remarkably, the study showed that hOP-1 is *per se* angiogenic at doses of 100 and 1000 µg, comparable to the angiogenic activity of recombinant human basic fibroblast growth factor (hbFGF).[72]

Further studies showed that binary applications of hOP-1 and hTGF-β_3 in Matrigel®Matrix implanted in Class II furcation defects of *Papio ursinus* induced substantial periodontal tissue induction and regeneration.[66] The anatomy of the furcation defects however tempered the full morphogenetic drive of the synergistic binary applications that morphogenized large ossicles expanding toward the muco-periosteal flaps with the remarkable induction of cementogenesis along the planed root surfaces.[66]

A review of the literature shows the lack of biological studies aimed to define the structure-activity profiles of recombinant hBMPs; studies reporting synergistic interactions amongst members of the morphogenetic protein family are also lacking.[25] We have stated that the "*biological acceptance of the inductive activity of a single recombinant human morphogen about the natural milieu and equilibrium of a pleiotropic bone matrix endowed with several naturally derived proteins clustered within the extracellular matrix of bone has been the fundamental biological error of biotech companies developing recombinant BMPs*'.[25,56]

Clinician scientists were also far too eager to accept unconditionally the reported powerful biological activity of either hBMP-2 or hOP-1 and to test in various clinical

https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

settings single recombinant hBMPs, and later recalcitrant to even admit the failure of hBMPs' translation in clinical contexts. [70,71]

Biotechnology companies at the forefront of recombinant human inductive proteins marketed selected recombined BMPs as single proteins. Recombinant human proteins were packaged singly recombined with patented delivery systems, hBMP-2 by Genetic Institute, USA, and hOP-1 (also known as hBMP-7) by Stryker Biotech, USA.

Finally, and again despite substantial research experimentation on the formulation of delivery systems for recombinant human morphogens including hBMPs and hTGF-βs, the ideal carrier matrix for periodontal tissue induction is still not available. The use of hBMPs pre-combined with doses of allogeneic and/or xenogeneic insoluble collagenous bone matrix (ICBM) was used to deliver recombinant hOP-1 in Class II furcation defects of the Chacma baboon *Papio ursinus* (Fig. 4). [52,57,58] Binary synergistic combinations were also tested (Fig. 4e). [57] The use of allogeneic ICBM proved to deliver the biological activity of doses of naturally derived osteogenic BMPs fractions purified greater than 50.000-fold from crude bovine bone matrix extracts (Fig. 3).[51] Xenogeneic bovine ICBM was used to deliver the biological activity of gamma-irradiated hOP-1 in short (Figs. 4f.g.h) [52] and long-term experiments in the Chacma baboon *Papio ursinus*. [58]

The use of Matrigel®Matrix as delivery system for hTGF-β_3 doses has proven to be optimal for periodontal tissue induction when lyophilized doses of hTGF-β_3 in Matrigel®Matrix were implanted in Class II furcation defects of *Papio ursinus* (Fig, 8).[60] Research on osteogenic carriers needs to design therapeutic strategies based on cell biology of matrix-cell interactions for optimal outcome in the periodontal patient.[55]

The use of Matrigel®Matrix originally developed as a matrix substratum for *in vitro* research experiments [73] was later used to deliver recombinant hOP-1 in the subcutaneous bioassay in rodents.[74] Matrigel is a soluble extract of the Engelbreth-Holm-Swarm tumor which gels at room temperature to form a reconstituted basement membrane gel. [73] Matrigel promotes differentiation of a variety of cells. [73,75] Our studies evaluated the induction of organoids of transitional epithelial cells harvested from baboon fetal bladders and grown on Matrigel®Matrix (Fig. 9).[74]

Matrigel®Matrix contains, amongst other extracellular matrix components, laminin and type IV collagen, the essential constituents of capillary basement membranes. Both bind to BMPs [76,77] and TGF-β. [78] Importantly, not only osteogenic but also angiogenic morphogens are stored within basement membranes deposited into the subendothelial extracellular matrix.[79] The multiple binding and storage of morphogenetic and angiogenetic morphogens within the subendothelial basement membrane make the reconstituted basement membrane gel Matrigel®Matrix an ideal carrier based on cell biology of matrix cell interaction (Figs. 8; 9). [60,74]

2.4. BIOREACTORS FOR *DE NOVO* INITIATION OF CEMENTUM AND ALVEOLAR BONE ORGANOIDS

The binding of osteogenin, a bone morphogenetic protein purified to homogeneity from bovine and baboon bone matrices,[64] to type IV collagen, laminin, and transforming growth factor-β_1 [76,77,78] suggested to combine highly purified osteogenic fractions extracted from baboon bone matrices and recombinant human osteogenic protein-1 (hOP-1, also known as hBMP-7) with Matrigel®Matrix kept fluid on ice, to test the biological activity of purified osteogenic proteins when combined with Matrigel®Matrix implanted in heterotopic extraskeletal sites of the rodent bioassay. [74]

Recent time-study experiments combined morphological analyses to a time point molecular study of Class II furcation defects in *Papio ursinus* super activated by TGF-β_3 in Matrigel®Matrix. [60] The combined morphological and molecular analyses have indicated that relatively low doses of hTGF-β_3 (75 µg hTGF-β_3 in 600 µl Matrigel®Matrix) set into motion the *in vivo* development of multiple tissues and multicellular organoids within the implanted furcation bioreactors (Fig. 2.8).[60]

Ultimately, the surgical preparation of periodontal furcation defects in animal models including man is a complex surgical wound or bioreactor that after the implantation of morphogens either singly or in combinations initiates regenerative phenomena by gene expression pathways.[25,60]

Various tissues and cells with different embryological origins as well as the induction of angiogenesis from the severed periodontal ligament and alveolar bone spaces control the regenerative pathways of the newly established bioreactors. The most critical part of the bioreactor is a completely avascular rigid and mineralized dentin matrix layered with or without the avascular mineralized root cementum (Figs. 8a,b). The bioreactor of the furcation defect is thus a surgical micro-environment that may or may not promote cementogenesis along its avascular and mineralized root planed dentinal surfaces.

We did recently discuss whether differentiation of cementoblasts along the root planed dentinal surfaces occurs either at a considerable coronal distance from the apically positioned severed cementum after root planing, or from the apically positioned notch in the root surface, a *niche* of migrating responding cells we have defined as "*the only true regenerative microenvironment of the complex morphologies of the periodontal tissues*".[60]

Our series of histological analyses of undecalcified sections prepared by Reichert Jung sledge microtomes with tungsten carbide blades or by the Exakt diamond saw cutting and grinding technology shows that there is attachment, spreading and differentiation of cementoblasts coronally along the root surface (Figs. 8a,b *light blue arrows*). The differentiation of cementoblastic cells together with insertion of Sharpey's' fibers into the root surface is uniformly distributed along the length of the regenerating periodontal ligament space, extending to the furca of the defect (Figs. 2.8a,b *light blue arrows*).[25,56,60]

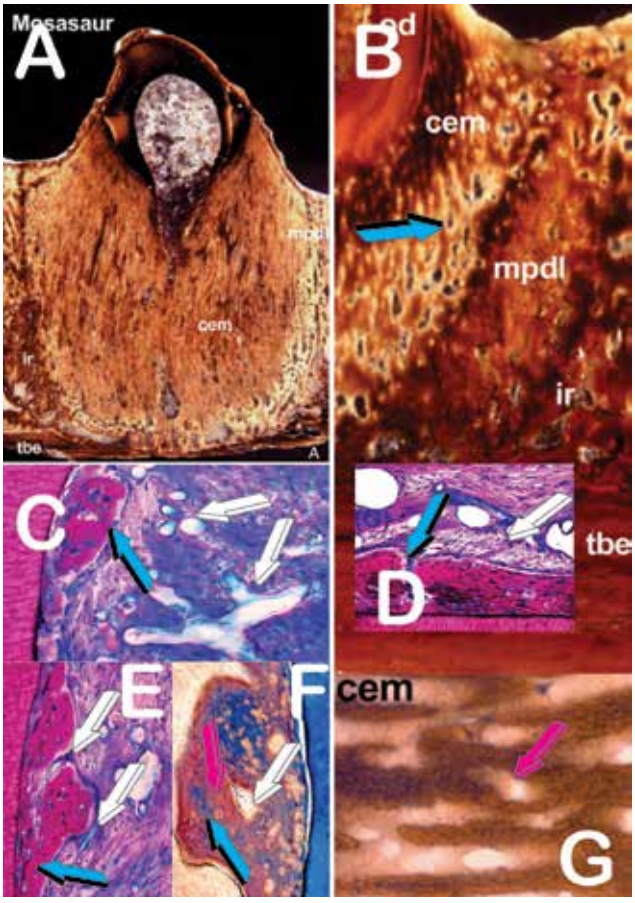
Critical contributions described the mechanical regulation of cell function by geometrically modulated substrata. The available data are critical to mechanistically understand the attachment, differentiation and spreading of cementoblastic cells on rigid substrata. [25]

Incisive research by Discher' laboratories has shown *in vitro* the role of micro pillars to affect subcellular nuclear geometry that further regulates stem cell differentiation and the induction of tissue patterning.[80] "*Stem cells feel the difference*" [80] when

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

cultured on different substrata' consistencies, i.e. between soft and hard substrata. [81,82] Molecular studies in *Cell* mechanistically reveals how "*Matrix elasticity directs stem cell lineage specification*". [83] Stem cells commit to specific phenotypes to tissue level elasticity. [80-83]

The above work on "*stem cells feeling the difference*" [80] is summarized by far reaching molecular and differentiating mechanistic insights, i.e. "*soft matrices that mimic brain are neurogenic*"; in contrast, "*comparatively rigid matrices that mimic collagenous bone prove osteogenic*". [83] The above statements are "*perhaps the most molecularly and intellectual fascinating aspect of biomimetism, biomimetic matrices and the induction of bone formation"*.[84]



In context of periodontal regeneration, the above data propose that hard mineralized and avascular matrix of root planed dentine proves to be cementogenic when in contact with mesenchymal stem cells either migrating from cellular *niches* within the dentinal notch or directly differentiating along the root surfaces, as recently proposed. [60] The dentine/cementum unit retains thus characteristics for the differentiation of selected phenotypes, also initiated by the exogenous application of osteogenic soluble molecular signals. [25,59]

Within the implanted furcation defects, *de novo* generated organoids form by multiple tissue induction of different tissues organized in intra-furcal organoids.[25,60]

Toward the root planed surfaces, there is the induction of substantial cementogenesis. Cementum is deposited firstly as cementoid matrix yet to be mineralized. [52,56,60] Cementoid tissue forms and extends into the periodontal ligament space with trabeculations underscoring the powerful role of the TGF-β_3 gene controlling cementogenesis in primates (Fig. 8). [26,56,60]

Matrigel®Matrix is an ideal combined soluble and insoluble signal that control the morphogenesis of organoids *in vitro* of transitional bladder cells when grown to confluence on Matrigel®Matrix substrata (Figs. 9a,b,c,d). [74] Newly generated organoids embedded in in K-Plast resin were cut at 2 to 3 µm using carbide tungsten knives mounted on Reichert Polycut sledge microtomes and stained with toluidine blue in 30% ethanol (Figs. 9e,f,g,h). [74]

Histological analyses show that organoids are formed by transitional fetal epithelial cells lining pseudo-cystic spaces organized by trabeculation of transitional epithelial cells generating the spheroidal organoids (Figs. 9e,f,g,h).

Analyses of undecalcified histological sections cut at 30 µm on the Exakt diamond saw, grinding and polishing system [60] suggested that trabeculations of newly formed cementoid matrices surrounding *foci* of mineralized cementum are recapitulating in extant primate species the induction of substantial cementogenesis as seen on undecalcified sections of dentate specimens of extinct mosasaur *Hallisaurus sternbergi* 168-165 Ma (Fig. 10). [85] Undecalcified sections of periodontal tissues of extinct mosasaur

Figure 10. Evolution and development of periodontal tissues including cementogenesis from extinct mosasaurs of the upper Jurassic Selnhofen deposits (168-165 Ma) A,B,G. to extant non-human primates *Papio ursinus* (C,D,E,F). A. The dentition of the mosasaur *Halisaurus sternbergi* shows extensive cementum formation (cem) with the construction of a trabecular-like pattern of cementum deposition. B. Detail of the trabecular-like pattern of cementum (*blue* arrow) extending from the orthodentin (od) to the mineralized periodontal ligament (mpdl). (g) High power view of trabeculated cementum in mosasaur *Halisaurus sternbergi* with capillaries invasion (*magenta* arrow) within the trabeculated cementum. Images courtesy of Xianghong Luan (Department of Oral Biology, University of Illinois, Chicago, IL, USA).[85] Cementogenesis in angiogenesis as documented by undecalcified section cut and polished to 30 µm by the Exakt diamond saw cutting and grinding technique.[25,60] C,D,E. Substantial cementum and cementoid deposition (*blue* arrows) along the root surfaces after bioreactors' implantation of 75 µg recombinant human transforming growth factor-β_3 (hTGF-β_3) in 300 µl Matrigel®Matrix. [60] There is vascular invasion within newly formed cementoid (*blue* arrow in C; *white* arrow in E. Cementoid matrix yet to mineralize (magenta arrow in E) surrounds mineralized cementum in blue (*blue* arrows in D and E). D. Highly developed thecodont attachment apparatus in *Papio ursinus* with substantial induction of cementogenesis (*blue* arrow) and vascular invasion (*white* arrows). F. High power view of newly formed mineralized cementum (*blue* arrow) with cementoid yet to mineralized matrix (*magenta* arrow) with capillary invasion within the cementoid matrix (*white* arrow). Figure 10 proposes that phylogenetically the development of cementogenesis initiated in extinct mosasaurs 168-165 Ma. Digital images of trabeculated cementum of mosasaurs indicated capillary vascular invasion (*magenta* arrow G) within the trabeculated cementum.[25] In extant primates *Papio ursinus*, 75 µl doses of hTGF-β_3 delivered to furcation bioreactors in 300 µl Matrigel®Matrix generate, across hundreds of millions of years, vascular invasion and capillary sprouting within newly induced cementum along the planed hTGF-β_3 treaded bioreactors, i.e., *cementogenesis in angiogenesis*.[25,56,60] The data highlighted by the iconographic plate propose that the primitiveness of the craniate masticatory craniofacial apparatus, [25,56] is controlled by the *TGF*-β_3 gene. The *TGF*-β_3 gene and gene product might have been responsible for the development and induction of cementogenesis in orders and species as diverse as mosasaurs and primates across millions of years of evolution. High doses of hTGF-β_3 in furcation bioreactors of *Papio ursinus* re-deploy the genetic memory of the primitiveness of the attachment apparatus in mosasaurs, re-initiating morphological constructs of cementogenesis in angiogenesis as originally developed and differentiated in the mosasaur *Halisaurus sternbergi* (Fig. 2.10g). The unique images shown in plate 10 further propose that angiogenesis, capillary sprouting, and the induction of prominent capillary invasion in the periodontal ligament space as well as into cementoid between inserted *bona fide* Sharpey's fibers is the essential mechanism of the induction of periodontal tissue regeneration, and of the induction of the alveolar bone. Panels D and E show the" *osteogenetic vessels*" of Trueta' definition [19] (*white* arrows) that construct cementogenesis in angiogenesis uniquely adapting and embracing (*white* arrows e) the newly formed cementoid matrices (C,D,E) regulating tissue morphogenesis in regeneration.[94,95]

https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

Hallisaurus sternbergi [84] show trabeculations of cementum with the possible presence of vascular canals and capillaries (Fig. 10g). [25,85]

Of interest, in extant primates *Papio ursinus*, newly formed cementoid and later mineralized cemental matrices are in a very intimate relationship with sprouting capillaries within the newly formed periodontal ligament space (Figs. 8; 10). Importantly, the newly formed cementum is vascularized, showing the presence of sprouting capillaries within the cemental matrix (Figs. 8; 10). [25,56,60]

The exquisite relationship between sprouting capillaries morphologically and thus molecularly touching the newly synthesized cemental matrix covered by cementoblasts indicates that the newly deposited cementoid synthesizes cemental extracellular matrix proteins that control angiogenesis within the periodontal ligament space (Fig. 10). [25,60] The role of cementum in the homeostasis of the periodontal ligament space is supported by the isolation of cemental proteins from cemental extracellular matrix and by the cloning of a new cemental protein, cementogenin. Cementogenin is secreted by cementoblasts and has a molecular weight of 18.5 kDa.[86]

The induction of a three-dimensional *in vivo* culture by combining the morphogenetic soluble signal of the recombinant hTGF-β_3 with the insoluble signals of the Matrigel®Matrix, collagen type IV and laminin with binding affinity to the TGF-β type 1, [78] morphogenizes the induction of newly formed cementum with capillary invasion within the yet to be mineralized cementoid matrix (Fig. 8).

Newly formed intra-furcal organoids induced by doses of hTGF-β_3 in Matrigel®Matrix show a periodontal ligament space supported by significant angiogenesis often in close contact with the newly formed cementoid matrix, together with the induction of a very vascularized alveolar bone (Figs. 8; 10). [25,56,60]

Of interest, the time study of the induction of periodontal tissue regeneration by the recombinant hTGF-β_3 in Matrigel®Matrix was morphologically and molecularly compared to heterotopic organoids generated by combining 250 µg doses of hTGF-β_3 to coral-derived bioreactors (Fig. 11). [60]

Super activated bioreactors with 250 µg hTGF-β_3 were implanted in heterotopic sites of the rectus abdominis muscle of the same animals implanted in Class II furcation defects with 75 µg doses of hTGF-β_3 in Matrigel®Matrix. [60] Heterotopic implantations of coral-derived macroporous bioreactors with or without 250 µg f hTGF-β_3 were implanted in the *rectus abdominis* muscle at the time of the periodontal surgical implantation, providing thus periodontal and heterotopic treated specimens harvested on day 60 for morphological and molecular analyses (Fig. 11). [60]

hTGF-β_3 super activated or untreated coral-derived macroporous bioreactors were intramuscularly implanted as positive controls to correlate the induction of bone formation in treated periodontal sites with *de novo* induction of bone in the *rectus abdominis* intramuscular sites. [60] These positive controls were additional to several treated and untreated coral-derived macroporous bioreactors implanted in the *rectus abdominis* muscle. [87,88,89]

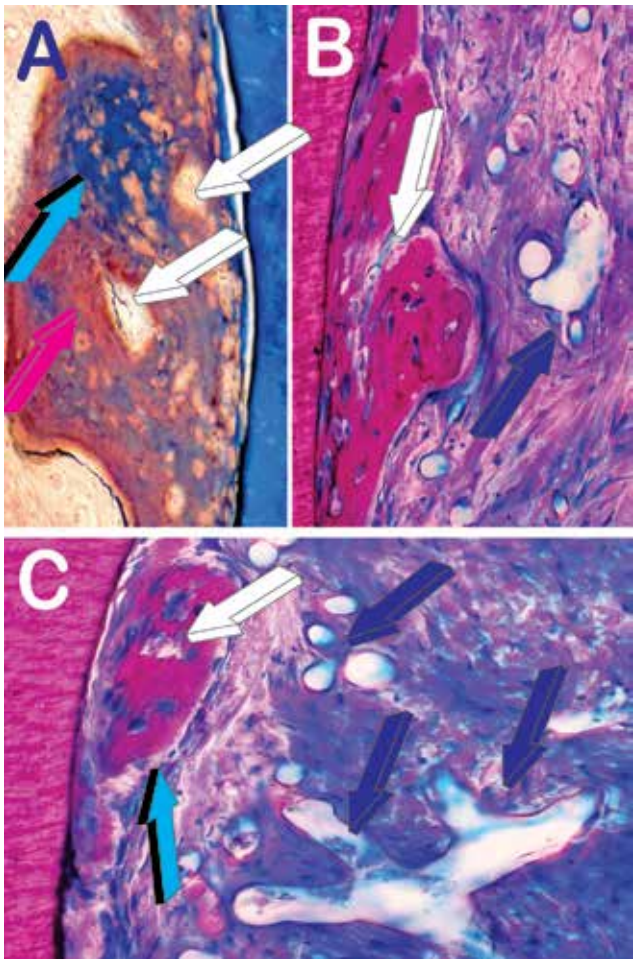


Figure 11. Cementogenesis and angiogenesis, "*the role of the vessels in osteogenesis*" [19] and the induction of cementogenesis in angiogenesis by 75 µg of hTGF-β_3 in Matrigel®Matrix. [60] A,B. Uniquely amongst our systematic studies in periodontal tissue induction and regeneration by the osteogenic proteins of the TGF-β supergene family, hTGF-β_3 in Matrigel®Matrix induces substantial cementogenesis characterized by contiguous prominent capillary sprouting and invasion not only surrounding the newly deposited cementoid matrix (*dark blue* arrows) but also invading the newly formed and deposited cementum, a biological construct we have defined as *cementogenesis in angiogenesis*. [25,56,60] C. Digital image summarizing the key ingredients of the hTGF-β_3 morphogen cell engineering the essential tissues regulating periodontal tissue induction and regeneration: cementoid deposition with *foci* of mineralization within the newly deposited cementoid matrix (*blue* arrow), and vascular invasion of the newly deposited cementoid matrix (*white* arrow). Undecalcified blocks were in Technovit, and sections cut using the Exakt 310 CP precision parallel control saw (Exakt Advanced Technologies GmbH). The Exakt AW 110 measuring and control system was used to grind and polish sections to 30 µm. Undecalcified sections were stained with methylene blue/basic fuchsin. [60]

sculpture of precisely organized multicellular structures of the bone bone-marrow organ. Of interest, the induction of bone can be initiated by several matrices of mammalian tissues including but not limited to demineralized bone matrices, dentin matrices, uroepithelium and a variety of calcium phosphate-based biomatrices either coral-derived biomimetic bioreactors or sintered crystalline hydroxyapatite macroporous constructs.[3]

Though the induction of bone is initiated by a variety of matrices as listed above, mechanistically the induction of bone is set into motion by the expression, synthesis and secretion of the bone morphogenetic proteins' genes products, ultimately the initiators of "*Bone: Formation by autoinduction*". [21]

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

The induction of bone formation by the hTGF-β_3 is a point in context. [86,87,88] The induction of bone formation initiates by expression of profiled *bone morphogenetic proteins* including *BMP-2* with subsequent induction of bone formation by the secreted BMPs gene products upon heterotopic implantation of hTGF-β_3. hTGF-β_3-treated bioreactors set into motion the expression of a variety of *BMPs* and TGF-β genes at different time points temporally and spatially regulating the induction of bone formation *via Noggin* expression. [89]

The classic view of a morphogen is that morphogenetic gradients specify gene expression in a distinct spatial order (Research article summary, [90] *Tissue Morphogenesis*). [90,91] The work of Yang et al. reported in *Science* presents an alternative pathway to tissue morphogenesis suggesting that morphogens beside modulation of individual cells induce their ultimate functional effects that enable the promotion of distinct supracellular phases that are capable of morphological transformation and organogenesis. [91]

Our research data on both the induction of large mineralized corticalized heterotopic ossicles and prominent cementogenesis together with the induction of alveolar bone regeneration in the non-human primate *Papio ursinus* have indicated that doses of recombinant hTGF-β_3 induce distinct supracellular phases that together with morphological transformation and organogenesis results in the generation of intramuscular mineralized bone organoids with prominent osteoid seams and bone marrow cavities. The generation of transformed periodontal bioreactors into organogenesis of alveolar bone attached to a highly vascularized periodontal ligament system is patterned by collagenic fibers attaching into substantial cementogenesis with capillary sprouting and angioblastic invasion. This results in cementogenesis in angiogenesis with *de novo* vascularized cementoid formation. [25,56,60]

Physiological expression of *BMPs* genes and gene products upon implantation of hTGF-β_3 may escape the antagonistic expression of Noggin, whereas direct implantation of large doses of hBMPs sets into motion the expression of Noggin tightly controlling the bone induction cascade in humans, as shown by limited effectiveness of hBMPs in clinical contexts. [92,93]

In his classic Editorial Comment "*The reality of a nebulous enigmatic myth*" [40] Marshall Urist states that pre-clinical and clinical research on the bone induction principle [30] "*are bound to dispel the myth and appreciate the reality of bone induction for the benefit of patients with crippling diseases of the bone and joints*". Fifty-seven years later the Bone Research Laboratory not in Los Angeles but in Johannesburg still strongly perceive "*The reality of a nebulous enigmatic myth*" when reading that several tens of milligrams of recombinant human BMPs are needed to induce an uninspiring bone volume in human patients.

The promise of therapeutic osteoinduction has been recognized during last Century research after pre-clinical and clinical studies. Human bone regeneration and human bone induction have proven to be an elusive target when compared to extraordinary results obtained in pre-clinical studies including non-human primate species. [16,17,92,93,94]

The induction of bone formation has dramatically shown that regenerative medicine in clinical context is on a different scale altogether when compared to animal models that may not adequately translate and reproduce morphogen-related therapeutic responses in *Homo sapiens*. Translation in clinical context of "*Tissue Induction*" [10] of "*Bone: Formation by autoinduction*" [21] has however failed, [87,91,92,93] and the promise of human bone induction remains a promise.

As a concluding comment perhaps, it is worth ending *verbatim* with a statement of a rather controversial manuscript that stated that "*the limited morphogens' activity in human patients when compared to different pre-clinical models including the non-human primate* Papio ursinus *may not indicate the failure of the bone induction principle*[30] *in humans but simply the mere fact that both TGFF-βs and BMPs are developmentally and biologically not Nature proposal for regeneration of skeletal defects in human patients*". [92]

The above work has highlighted a biological problem rather than a biotechnology problem, i.e. recombinant human morphogens, recombination techniques, doses of recombinant proteins, delivery systems, age of human patients and the like. Both recombinant hBMPs and hTGF-β_3 proteins do indue bone in heterotopic sites of animal models, and the induction of bone formation recapitulates embryonic development. As stated, "*In evolutionary molecular biology contexts however, the pleiotropic activity of both proteins' family and the induction of bone formation in heterotopic sites are developmental, and thus not suitable to induce bone when recombinant morphogens are singly implanted in orthotopic skeletal defects, the latter lacking the developmental biological platform*". [92]

To end, we have thoroughly discussed the biological significant of the heterotopic bioassays that since the last two Centuries have stated to cellular and molecular biologists, and tissue reconstructionists alike, that if a protein and/or any extracellular matrix or matrices initiate the induction of bone in extraskeletal sites, where there is no bone, such protein and/or matrix is *per se* osteoinductive and as such, it can be used to translate the "*bone induction principle*" [30] into human skeletal defects. The proteins induce bone where there is no bone *via* developmental phases of embryonic development, and as such, however, fail the induction of bone formation when applied to human skeletal defects. [92]

The developments and use of the heterotopic bioassays to test unequivocally the remarkable prerogative of certain proteins and/or matrices to initiate the biological landscape of the induction of bone formation has been essential for the purification, isolation and cloning of several proteins bestowed with the unique capacity or prerogative of initiating the induction of bone formation where there is no bone. It has however sidetracked the clinical translation of the "*bone induction principle*"[30] since it was based on a pleiotropic developmental function without however the capacity of clinical translation into orthotopic bony sites, biologically lacking developmental phenomena.

ACKNOWLEDMENTS

The continuous and focused research experimentation on the induction of bone formation spanned for 30 years, from March 1994 to the end of March 2024 when upon a debatable yet not-negotiable request by the Deanery of the

https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

Faculty of Health Sciences, the Bone Research Laboratory ceased to exist, after more than 30 years of important critical research findings in non-human and human primates.

The author of this manuscript still wishes to thank the University of the Witwatersrand, Johannesburg for running the research laboratories from the late eighties at the Dental Research Institute, and from March 1994 at the then inaugurated the Bone Research laboratory, until March this year 2024 and now to the refurbished laboratories of the School of Clinical Medicine, Internal Medicine, jointly with the Laboratories of Molecular and Cellular Biology headed by Raquel Duarte and her team. Together with the Bone Research Laboratory, the molecular biology team resolved molecularly the spontaneous induction of bone formation by macroporous calcium phosphate-based bioreactors, the apparent redundancy of the induction of bone formation by the mammalian transforming growth factor-β isoforms, and the synergistic induction of bone formation, partially touched upon by this manuscript. This contribution to periodontal "*Tissue Induction*" could not have been possible without the dedication, discipline and expertise of Laura Roden (née Yeates who excelled in purification of naturally derived bone morphogenetic proteins' fractions from baboon bone matrices), Barbara van den Heever and Ruqayya Parak who excelled in cutting undecalcified sections using Reichert' sledge heavy duty microtomes mounted with carbide-tungsten knives and the Exakt diamond saw cutting and grinding technique. Digital images of their sections are now spread in more than 250 publications and in 3 CRC Press Volumes on the induction of bone formation in non-human primates.

A final grateful thank to Alberto and his team of managers at the Superspar at the Islands Broederstroom for continuous support for IT connection and printing of several manuscripts during the preparation of this review.

REFERENCES

1. Romer AS. The "Ancient History" of bone. Ann NY Acad Sci 1963; 109: 168-176.
2. Ripamonti U, Roden L, van den Heever B. Sharks, shark cartilages and shark teeth: A collaborative Africa-USA study to attempt to induce "*Bone: formation by autoinduction" in] cartilaginous fishes*. South Afr Dent J. 2018; 73: 11-21.
3. Ripamonti U. The Induction of Bone Formation and the Osteogenic Proteins of the Transforming Growth Factor-β Supergene family. *Pleiotropism and Redundancy*. In: U. Ripamonti (ed.) *The Geometric Induction of Bone Formation* CRC Press, Taylor & Francis Group. Boca Raton FL, USA, 2021, Chapter 3, 51-68.
4. Lim AW. The emerging era of cell engineering: Harnessing the modularity of cells to program complex biological functions. Science 2022, 378: 848-852.
5. Massagué J. The transforming growth factor-beta family. Annu Rev Cell Biol. 1990:6:597-641.doi: 10.1146/annurev.cb.06.110190.003121.
6. Kingsley DM. The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms Genes Dev. 1994 8(2):133-46. doi: 10.1101/gad.8.2.133.
7. Feng XH, Derynck R. Specificity and versatility in TGF-beta signaling through Smads. Ann Rev Cell Develop Biology 2005; 21: 659-93. http://dx.doi.org/10.1146/annurev.cellbio.21.022404.142018
8. Pakyari M, Farrokhi A, Maharlooei MK, Ghahary A. Critical Role of Transforming Growth Factor Beta in Different Phases of Wound Healing. Advances in Wound Care Volume 2, 5, 2013 © 2013, Mary Ann Liebert, Inc. https://doi.org/10.1089/wound.2012.0406
9. Richardson L, Wilcockson SG, Guglielmi L. Hill C. Context-dependent TGFβ family signalling in cell fate regulation. Nature Rev Mol Cell Biol. 2023, 24: 876–94 https://doi.org/10.1038/s41580-023-00638-3
10. Levander G. Tissue induction. Nature 1945: 155: 148-49.
11. Ripamonti U. Biomimetic functionalized surfaces and the induction of bone formation. Invited Expert Opinion Tissue Engineering 2017; 23: 1197-1209.
12. Langer R, Brem H, Kalterman K, Klein M, Folkm an J. Isolations of a cartilage factor that inhibits tumor neovascularization. Science 1976; 193, 4247: 70-72, DOI: 10.1126/science.93585
13. Lee A, Langer R. Shark cartilage contains inhibitors of tumour angiogenesis. Science 1983; 16;221(4616):1185-7. doi: 10.1126/science.6193581.
14. Moses MA, Sudhalter J, Langer R. Identification of an inhibitor of neovascularization from cartilage. Science 1990. 15;248(4961):1408-10. DOI: 10.1126/science.1694043
15. Moses MA, Langer R. Inhibitors of angiogenesis. Biotechnology 1991; 9(7): 630-34, doi: 10.1038/nbt0791-630
16. Ripamonti U. Soluble osteogenic molecular signals and the induction of bone formation. Biomaterials 2006; 27: 807-822.
17. Ripamonti U, Ferretti C, Heliotis M. Soluble and insoluble signals and the induction of bone formation: Molecular therapeutics recapitulating development. J Anat. 2006; 209: 447-468.
18. Ripamonti U, Heliotis M, Ferretti C. Bone morphogenetic proteins and the induction of bone formation: From laboratory to patients. Oral Maxillofac Surg Clin North Am. 2007; 19: 575-589.
19. Trueta, J. The role of the vessels in osteogenesis. J Bone Joint Surg. 1963, 45B, 402-18
20. Ripamonti U. Regenerative Medicine, the Induction of Bone Formation, Bone Tissue Engineering, and the Osteogenic Proteins of the Transforming Growth Factor-β Supergene Family. In: Ripamonti U (ed.) CRC Press Taylor & Francis, Boca Raton USA, *Induction of Bone Formation in Primates. The Transforming Growth Factor-beta3*; 2016, Chapter 2, 15-46.
21. Urist MR. Bone: formation by autoinduction. Science 1965 150(3698):893-899 doi: 10.1126/science.150.3698.893.
22. Turing AM. The Chemical Basis of Morphogenesis. Turing Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, Vol. 237, No. 641. (Aug. 14, 1952), pp. 37-72.
23. Ripamonti U, Ramoshebi LN, Patton J, Matsaba T, Teare J, Renton L. Soluble signals and insoluble substrata: Novel molecular cues instructing the induction of bone. In: EJ Massaro and JM Rogers (Eds.), Chapter 15, The Skeleton. Humana Press, 2004; pp 217-227.
24. Sampath TK, Rashka KE, Doctor JS, Tucker RF, Hoffman FM. Drosophila transforming growth factor beta superfamily proteins induce endochondral bone formation in mammals. Proc Natl Acad Sci. USA 1993; 90(13), 6004-6008 https://doi.org/10.1073/pnas.90.13.6004
25. Ripamonti U. Developmental patterns of periodontal tissue regeneration. Developmental diversities of tooth morphogenesis do also map capacity of periodontal tissue regeneration? J Periodont Res. 2019; 54: 10-26; doi: 10.1111/jre.12596
26. Pääbo S. The Human condition—A molecular approach. Cell 2014; 157:216-26. doi:https://doi.org/10.1016/j.cell.2013.12.036
27. Ripamonti U, Duarte R, Ferretti C, Reddi AH. Osteogenic competence and potency of the Bone induction principle: Inductive substrates that initiate "*Bone: Formation by autoinduction*". J Craniofac Surg. 2022; 33(3): 971-984.
28. Levander G. A study of bone regeneration. Surg Gynec Obst. 1938; 67(6): 705-14.
29. Reddi AH, Huggins CB. Biochemical sequences in the transformation of normal fibroblasts in adolescent rats. Proc Natl Acad Sci U S A. 1972, 69(6):1601-05. doi: 10.1073/pnas.69.6.1601.
30. Urist MR, Silverman BF, Büring K, Dubuc FL, Rosemberg JM. The bone induction principle Clin Orthop Relat Res. 1967 53: 243-83.
31. Sampath, T. K.; Reddi, A. H. Dissociative extraction and reconstitution of extracellular matrix components involved in local bone differentiation. Proc Natl Acad Sci. USA 1981; 78:7599-7603.
32. Sampath, T. K.; Reddi, A. H. Homology of bone-inductive proteins from human,= monkey, bovine, and rat extracellular matrix. Proc Natl Acad Sci. USA 1983, *80,* 6591-95.
33. Ripamonti U, Reddi AH. Bone morphogenetic proteins: Applications in plastic and reconstructive surgery. Adv Plast Reconst Surg. 1995; Vol. 11: 47-65.
34. Urist MR, Strates BS. Bone morphogenetic protein. J Dent Res. 1971; 50: 1392-1406.
35. Urist MR, Hou YK, Brownell AG, Hohl WM, Buyske J, Lietze A, Tempst P, Hunkapiller M, DeLange RJ. Purification of bovine bone morphogenetic protein by hydroxyapatite chromatography. Proc Natl Acad Sci. U S A 1984; 81(2):371-5. doi: 10.1073/pnas.81.2.371.
36. Sampath TK, Muthukumaran N, Reddi AH. Isolation of ostogenin, an extracellular matrix-associated, bone-inductive protein, by heparin affinity chromatography. Proc Natl Acad Sci. USA 1987; 84(20):7109-13. doi: 10.1073/pnas.84.20.7109.
37. Wang EA, Rosen V, Cordes P, Hewick RM, Kriz MJ, Luxenberg DP, Sibley BS, Wozney JM. Purification and characterization of other distinct bone-inducing factors. Proc Nat Acad Sci USA, 1988, 85 (24): 9484-9488, *https://doi.org/10.1073/pnas.85.24.9484*
38. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA. Novel regulators of bone formation: molecular clones and activities. Science. 1988; 42(4885):1528-34. doi: 10.1126/science.3201241.PMID: 3201241
39. Celeste AJ, Iannazzi JA, Taylor RC, Hewick RM, Rosen V, Wang EA, Wozney JM Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone. Proc Natl Acad Sci, U S A. 1990 Dec;87(24):9843-7. doi: 10.1073/pnas.87.24.9843.
40. Urist MR. The reality of a nebulous, enigmatic myth. Clin Orthop Rel Res. 1968; 59:3-6. 49.
41. Åberg T, Wozney J, Thesleff I. Expression patterns of bone morphogenetic proteins (*Bmps*) in the developing mouse tooth suggest roles in morphogenesis and cell differentiation. Dev Dyn. 1997; 210:383–3. https://doi.org/10.1002/(SICI)1097-0177(199712)210:4<383::AID-AJA3>3.0.CO;2-C
42. Ripamonti U, Duneas N. Tissue morphogenesis and regeneration by bone morphogenetic proteins. Plast Reconst Surg. 1998; 101: 227-239.
43. Thomadakis G, Crooks J, Rueger D, Ripamonti U. Immunolocalization of bone morphogenetic protein-2, -3 and osteogenic protein-1 during murine tooth morphogenesis and other craniofacial structures. Eur J Oral Sci. 1999; 107: 368-377.
44. Reddi AH. Role of morphogenetic proteins in skeletal tissue engineering and regeneration. Nat Biotechnol 1988; 16(3): 247-52. doi: 10.1038/nbt0398-247.
45. Ripamonti U. Soluble, insoluble and geometric signals sculpt the architecture of mineralized issues. J Cell Mol Med. 2004; 8: 169-180.
46. Ripamonti U, Duneas N, van den Heever B, Bosch C, Crooks J. Recombinant transforming growth factor-β_1 induces endochondral bone in the baboon and synergizes with recombinant osteogenic protein-1 (bone morphogenetic protein-7) to initiate rapid bone formation. J Bone Miner Res. 1997; 12: 1584-1595.
47. Ripamonti U, Crooks J, Matsaba T, Tasker J. Induction of endochondral bone formation by recombinant human transforming growth factor-β_2 in the baboon (*Papio ursinus*). Growth Factors 2000; 17: 269-285.
48. Ripamonti U. Osteogenic Proteins of the Transforming Growth Factor-ß Superfamily. In: HL Henry and AW Norman (Eds.), Encyclopedia of Hormones. Academic Press, 2003, pp 80-86.
49. Ripamonti U, Ramoshebi LN, Teare J, Renton L, Ferretti C. The induction of endochondral bone formation by transforming growth factor-β_3: Experimental studies in the non-human primate *Papio ursinus*. J Cell Mol Med. 2008; 12: 1029-1048.
50. Ripamonti U. Bone induction by recombinant human osteogenic protein-1 (hOP-1, BMP-7) in the primate *Papio ursinus* with expression of mRNA of gene products of the TGF-β superfamily. J Cell Mol Med. 2005; 9: 911-928.

www.sada.co.za / SADJ Vol. 80 No.4
https://doi.org/10.17159/sadj.v80i04.22714
The SADJ is licensed under Creative Commons Licence CC-BY-NC-4.0.

51. Ripamonti U, Heliotis M, van den Heever B, Reddi AH. Bone morphogenetic proteins induce periodontal regeneration in the baboon *(Papio ursinus).* J Periodont Res. 1994; 29: 439-445.
52. Ripamonti U, Heliotis M, Sampath TK, Rueger D. Induction of cementogenesis by recombinant human osteogenic protein-1 (hOP-1/BMP-7) in the baboon *(Papio ursinus).* Archives of Oral Biology 1996; 41: 121-126.
54. Ripamonti U, Petit J-C. Bone morphogenetic proteins, cementogenesis, myoblastic stem cells and the induction of periodontal tissue regeneration. Cyt Grow Fact Rev. 2009; 20: 489-499.
55. Ripamonti U, Reddi AH. Periodontal regeneration: Potential role of bone morphogenetic proteins. J Periodont Res. 1994; 29: 225-235.
56. Ripamonti U. The induction of bone formation: From bone morphogenetic proteins to the transforming growth factor-β3 protein – Redundancy, pleiotropy, and the induction of cementogenesis. The South Afr Dent J. 2021; 76(6): 331-356.
57. Ripamonti U, Crooks J, Petit J-C, Rueger D. Periodontal tissue regeneration by combined applications of recombinant human osteogenic protein-1 and bone morphogenetic protein-2. A pilot study in Chacma baboons (*Papio ursinus*). Eur J Oral Sci. 2001; 109: 241-248.
58. Ripamonti U, Crooks J, Teare J, Petit J-C, Rueger DC. Periodontal tissue regeneration by recombinant human osteogenic protein-1 in periodontally-induced furcation defects of the primate *Papio ursinus*. S Afr J Sci. 2002; 98: 361-368.
59. Ripamonti U. Re-defining the induction of periodontal tissue regeneration in primates by the osteogenic proteins of the transforming growth factor-β supergene family. J Periodont Res. 2016; 51: 699-715.
60. Ripamonti U, Parak R, Klar RM, Dickens C, Dix-Peek T, Duarte R. Cementogenesis and osteogenesis in periodontal tissue regeneration by recombinant human transforming growth factor-β_3: a pilot study in *Papio ursinus*. J Clin Periodont. 2017; 44: 83-95.
61. Teare J, Ramoshebi LN, Ripamonti U. Periodontal tissue regeneration by recombinant human transforming growth factor-β_3 in *Papio ursinus*. J Periodont Res. 2008; 43: 1-8.
62. Ripamonti U, Parak R, Petit J-C. Induction of cementogenesis and periodontal ligament regeneration by recombinant human transforming growth factor-β_3 in Matrigel with *rectus abdominis* responding cells. J Periodont Res. 2009; 44: 141-152.
63. Ripamonti U, Ma S, Cunningham N, Yeates L, Reddi AH. Initiation of bone regeneration in adult baboons by osteogenin, a bone morphogenetic protein. Matrix 1992; 12: 369-380.
64. Coura GS, Garcez RC, Mendes de Agular CBN, Alvarez-Silva M, Magini RS, Trentin AG. Human periodontal ligament: a niche of neuronal crest stem cells. J Periodont Res 2008; 43: 331-36.
65. *Han* J, *Menicanin* D, *Gronthos* S, *Bartold* PM. Stem cells, tissue engineering and periodontal regeneration. Aust Dent J 2014; 59 Suppl 1:117-30. doi: 10.1111/adj.12100. Epub 2013 Sep 23.
66. Teare JA, Petit J-C, Ripamonti U. Synergistic induction of periodontal tissue regeneration by binary application of hTGF-β_3 and hOP-1 in Class II furcation defects of *Papio ursinus*. J Periodont Res. 2011 Dec 6. doi: 10.1111/j.1600-0765.2011.01438.x. [Epub ahead of print]; 2012; 47: 336-344.
67. Ripamonti U, Klar RM, Renton LF, Ferretti C. Synergistic induction of bone formation by hOP- 1, hTGF-β_3 and inhibition by zoledronate in macroporous coral-derived hydroxyapatites. *Biomaterials* 2010; 31: 6400-6410.
68. Duneas N, Crooks J, Ripamonti U. Transforming growth factor-β_1: Induction of bone morphogenetic protein genes expression during endochondral bone formation in the baboon, and synergistic interaction with osteogenic protein-1 (BMP-7). Growth Factors 1998; 15: 259-277.
69. Ripamonti U, Parak R, Klar RM, Dickens C, Dix-Peek T, Duarte R. The synergistic induction of bone formation by the osteogenic proteins of the TGF-β supergene family. Biomaterials 2016; 104: 279-296. Doi: 10.1016/j.biomaterials.2016.07.018.
70. Ripamonti U, Teare J, Ferretti C. A macroscopic bioreactor super activated by the Recombinnat human transforming growth factor-β_3. Frontiers in Physiology *www.frontiersin.org* 2012;3:1-18.
71. Ripamonti U, Duarte R, Ferretti C. Re-evaluating the induction of bone formation in primates. Biomaterials 2014; 35: 9407-9422.
72. Ramoshebi LN, Ripamonti U. Osteogenic protein-1, a bone morphogenetic protein, induces angiogenesis in the chick chorioallantoic membrane and synergize with basic fibroblast growth factor and transforming growth factor-β_1. Anat Rec. 2000; 259: 97-107.
73. Kleinman HK, McGarvey ML, Hassell JR, Star VL, Cannon FB, Laurie GW, Martin GR. Basement membrane complexes with biological activities. *Biochemistry* 1986; 25(2): 312-318 DOI: 10.1021/bi00350a005
74. Ripamonti U, van den Heever B, Heliotis M, Dal Mas I, Hahnle U, Biscardi A. Local delivery of bone morphogenetic proteins using a reconstituted basement membrane gel: Tissue engineering with Matrigel. S Afr J Sci. 2002; 98: 429-433.
75. Vukicevic S, Luyten FP, Kleinman HK, Reddi AH. Differentiation of canalicular cell processes in bone cells by basement membrane matrix components: Regulation by discrete domains of laminin. Cell 1990, 63, 437-445.
76. Paralkar VM, Nandedkar AK, Pointer RH, Kleinman HK, Reddi AH. Interaction of osteogenin, a heparin binding bone morphogenetic protein, with type IV collagen. J Biol Chem. 1990 5;265(28):17281-4.
77. Vukicevic S, Latin V, Chen P, Batorsky R, Reddi AH, Sampath TK. Localization of osteogenic protein-1 (bone morphogenetic protein-7) during human embryonic development: high affinity binding to basement membranes. Biochem Biophys Res Commun. 1994; 198(2):693-700. doi: 10.1006/bbrc.1994.1100.
78. Paralkar VM, Vukicevic S, Reddi AH. Transforming growth factor beta type 1 binds to collagen IV of basement membrane matrix: implications for development. Dev Biol. 1991;143(2):303-8. doi: 10.1016/0012-1606(91)90081-d.
79. Folkman J, Klagsburn M, Sasse J, Wadzinsky M, Ingber D, Vlodavsky I. A heparin-binding protein – basic fibroblast growth factor – is stored within basement membrane. Am J Path. 1988; 130(2), 393-400 -ncbi.nim.nih.gov
80. Buxboim A, Discher DE. Stem Cells Feel the Difference. Nature Methods 2010, *7* (5), 695-697.
81. Discher DE, Janmey P, Wang Y-L. Tissue Cells Feel and Respond to the Stiffness of Their Substrate. Science 2005, 310, 1139-43.
82. Fu J. Wang, Y-K, Yang MT. Desai RA, Yu X, Liu Z, Chen CS, Mechanical Regulation of Cell Function with Geometrically Modulated Elastomeric Substrates. Nature Methods 2010, doi:10.1033/NMETH.1487.
83. Engler AJ, Sen S, Sweeney L, Discher DE. Matrix Elasticity Directs Stem Cell Lineage Specification. Cell 2006; 126: 677–89. doi10.1016/j.cell.2006.06.044.
84. Ripamonti U. Biomimetism, biomimetic matrices and the induction of bone formation. *J Cell Mol Med.* 2009; 13 9B: 2953-2972.
85. Luan X, Walker C, Dangaria S, Iti Y, Druzinsky R, Jarosius K, Lesot H, Rieppel O. The mosasaur tooth attachment apparatus as paradigm for the evolution of the gnathostome periodontium. Evol & Develop 2009; https://doi.org/10.1111/j.1525-142X.2009.00327.x
86. Jiménez-Durán K, López-Letay S, Zeichner-David M, Higinio Arzate H. Cloning, expression and biological activity of cementogenin (CMGN): A novel protein from human cementum. *Manuscript ID:* 1009419, 2022, *www.frontiersin.org*
87. Klar M R, Duarte R, Dix-Peek T, Ripamonti U. The induction of bone formation by the recombinant human transforming growth factor-β_3. Biomaterials 2014; 35: 2773-2788 *http://dx.doi.org/10.1016/j.biomaterials.2013.12.062*
88. Ripamonti U, Dix-Peek T, Parak R, Milner B, Duarte R. Profiling bone morphogenetic Proteins and transforming growth factor-βs by hTGF-β_3 pre-treated coral-derived macroporous constructs: The power of one. Biomaterials 2015; 49: 90-102.
89. Research Article Summary, Tissue Morphogenesis, Editorial Science 382, 902, 2023.
90. Yang S. Palmquist KH, Nathan L, Pfeifer CR, Schultheiss PJ, Sharma A, Kam LC, Miller PW, Shyer AE, Rodrigues AR. Morphogens enable interacting supracellular phases that generate organ architecture. Science 2023; 382, 902. https://doi: 10.1126/science.adg5579
91. Ripamonti U. Global morphogenesis regulating tissue architecture and organogenesis. Biomaterials Advances 2025; 172 214262, *https://doi.org/10.1016/j.bioadv.2025.214262*
92. Ripamonti U, Tsiridis E, Ferretti C, Kerawala CJ, Mantalaris A, Heliotis M. Perspectives in regenerative medicine and tissue engineering of bone. J Oral Maxillofac Surg. 2010; doi:10.1016/j.bjoms.
93. Ferretti C, Ripamonti U. Special Editorial: The *conundrum* of human osteoinduction: Is the bone induction principle failing clinical translation? J Craniofac Surg. 2021; 32: 1287-1289; doi:10.1097/SCS0000000000007429.
94. Ramasamy SK, Kusumbe AP, Adams RH. Regulation of tissue morphogenesis by endothelial cell-derived signals. Trends in Cell Biol. 2015; 25(3): 148-57. https:/doi: 10.1016/j.tcb.2014.11.007.Epub 2014 Dec 17.
95. Gomez-Salinero JM, Rafii S. Endothelial cell adaptation in regeneration. Science 2018;362(419): 1116-11.https://doi: 10.1126/science.aar4800.

CPD questionnaire on page 226

The Continuing Professional Development (CPD) section provides for twenty general questions and five ethics questions. The section provides members with a valuable source of CPD points whilst also achieving the objective of CPD, to assure continuing education. The importance of continuing professional development should not be underestimated, it is a career-long obligation for practicing professionals.

CURAPROX
HYDROSONIC PRO

INCREDIBLY GENTLE, BETTER RESULTS

HELP YOUR PATIENTS MAINTAIN HEALTHY, ATTRACTIVE TEETH AND GUMS THANKS TO INNOVATIVE SWISS BRUSH HEAD TECHNOLOGY.

WWW.CURAPROX.CO.ZA

SWISS PREMIUM ORAL CARE

www.sada.co.za / SADJ Vol. 80 No.4

Management of Dental Caries in an HIV-infected Child: A Case Report.

SADJ MAY 2025, Vol. 80 No.4 P214-P217

P Gwengu[1], BK Bunn[2], S Mudau[3]

ABSTRACT

Background

HIV/AIDS remains prevalent in Sub-Saharan Africa with South Africa maintaining the highest number of daily infections and the largest population of individuals living with HIV. Dental caries as an oral disease itself, is highly prevalent in the same epidemiological region. Contemporary literature documents an increased prevalence and severity of dental caries in association with HIV- infection. This case report describes the clinical presentation of dental caries in an HIV-infected child whilst outlining the management undertaken.

Case Report

A 5-year-old male patient was diagnosed with Human Immunodeficiency Virus (HIV) infection shortly after birth. The patient was initiated on Highly Active Antiretroviral Therapy (HAART) in 2017. HIV was acquired in this case because of vertical transmission from mother-to-child. Clinical examination showed dental caries on all primary molars as well as the interproximal surfaces of the anterior teeth.

Discussion

Oral disease is a common manifestation of HIV within the paediatric patient group. The oral manifestations of HIV need to be documented to underscore the significance of these in Paediatric Dentistry. The detailed management of dental disease should be highlighted to compare treatment with that undertaken in HIV-negative patients.

Conclusion

Increased research related to HIV-status and caries risk is essential for understanding the management principles required in treating these patients. Furthermore, the synergistic relationship between dental caries and lowered immunity should be highlighted.

Authors' information:

1. P Gwengu: DipOH, BDS, MpH, MDent (Comm Dent). Department of Operative Dentistry, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, South Africa. ORCID: 0000-0002-1429-2396
2. BK Bunn: BDS, FCPath (SA) Oral, MDent (Oral Pathology). Department of Operative Dentistry, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, South Africa. ORCID: 0000-0001-5699-4997
3. S Mudau: BDS. Department of Operative Dentistry, School of Oral Health Sciences, Sefako Makgatho Health Sciences University.

Corresponding Author:
Name: BK Bunn
Address: Department of Operative Dentistry, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, South Africa.
Email: belindabunn@gmail.com
Cell: 082-708 5868

Author contributions

P Gwengu – conceptualisation, writing and editing
BK Bunn – writing, editing and submission
S Mudau – clinician who retrieved case, writing and editing

Keywords

HAART, HIV, ART, oral manifestations, dental caries, Paediatric Dentistry.

Introduction

The World Health Organisation (WHO) defines HIV as a viral infection which targets the immune system, weakening its defence against other forms of infectious disease. The virus destroys the function of immune cells which is measured by the CD4 T-cell count. In 2020, the WHO documented that an estimated 680 000 (480 000 to 1.0 million) people died because of HIV-associated disease with an estimated 1.5 million (1 to 2 million) newly acquired HIV- infections. Furthermore, it was estimated that by the end of 2020, there were 37.7 million (30.2 to 45.1 million) people living with HIV and over two thirds of these (25.4 million) reside in WHO African regions.[1]

The elimination of mother-to-child (vertical) transmission is a global public health goal and priority. Studies have shown that antiretroviral intervention can assist to reduce the risk of vertical transmission with a documented 15 to 30% risk reduction during pregnancy and labour, less than 2% risk reduction with non-breast feeding and less than 5% risk reduction with breastfeeding.[1, 2]

Although HIV-infection is associated with well-known pathologies, the need remains for comprehensive comparative studies to determine the association between HIV and early childhood caries. Contemporary studies from several African countries as well as Thailand, have shown a distinct increase in both the prevalence and severity of dental caries in both children and adult patients living with HIV-infection.[3-8] HIV-infection is characterised by impairment of functional immunity thereby facilitating the development of opportunistic infections particularly in the oral cavity. About 30 to 80% of HIV- infected individuals present with one or more oral disease/manifestations. In spite of the initiation of HAART which has reduced the incidence of oral manifestations, an increase in dental caries has been reported within the HIV-positive population.[2] Even if there is initiation of HAART in South Africa , the country is still faced with many individuals who do not disclose their HIV status. This may lead to no uptake of HAART by those HIV- infected individuals, thus it invariably relates well with some of the cited reasons for non-disclosure of serostatus amongst pregnant women which were documented in a study conducted in Tshwane, Pretoria – South Africa in 2007. Some of the reasons cited by these pregnant women were that their biggest fear was discrimination and abandonment.[9] South Africa has the largest combination antiretroviral therapy programme resulting in survival benefits, but the number of infections is high in key populations such as young females. Advances in the use of antiretroviral therapy for HIV in the prevention of transmission of HIV has brought hope and optimism to

control the spread of HIV-infection.[10] There is a high burden of HIV-infections in Southern Africa with the prevalence of HIV-positivity among 15 to 49-year-old's in KwaZulu Natal at 27,6%; being three times higher than in the Western Cape Province at 9,2%.[10] A recent study undertaken in Cape Town, revealed a high prevalence of dental caries (78,8%) in a group of HIV-positive children between the ages of 2 and 12-years.[7]

Case Report

A 5-year-old male patient accompanied by his mother, presented for a dental consultation at the Paedodontics Clinic of the Oral and Dental Hospital at Sefako Makgatho Health Sciences University. The main complaint of the patient was "painful, rotten teeth". The patient's mother explained that several black spots were initially observed on the teeth when the patient was about 4-years old, although these did not prompt any form of intervention as the affected teeth were going to be shed. The mother confirmed that the child had been diagnosed with HIV in his first year of life at which time antiretroviral therapy was initiated. The mother expressed great discomfort to mention the term "HIV" and stated that this information has never been disclosed to the son.

Clinical extra-oral examination showed bilateral submandibular lymphadenopathy with pain on the left. In addition, eczema was noted on the facial skin and the patient's lips were markedly cracked whilst he displayed short stature for his age. Intra-oral examination showed a draining fistula in association with tooth 75 (Figure 1). There was carious involvement of teeth 51, 52, 53, 61, 63, 65, 74, 84 and 85 (Figure 2).

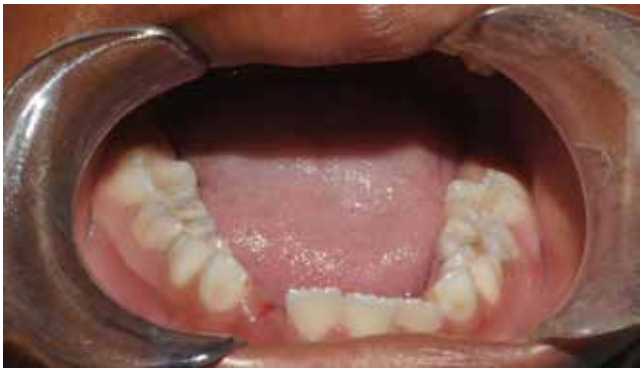


Figure 1: Clinical intra-oral photograph of the mandibular dentition in which a fistula and gingival abscess can be seen on the buccal gingiva in association with the carious 75.

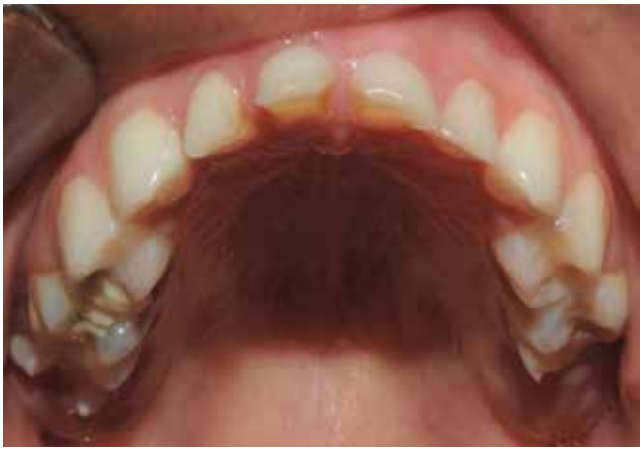


Figure 2: Intra-oral photograph of the maxillary dentition. Gross carious involvement of the molar teeth is depicted as well as interproximal decay of the incisor teeth.

Informed consent was obtained from the mother to continue with treatment. The child was noted to be both negative and withdrawn and was slightly uncooperative. Periapical and panoramic radiographs were taken to assess the extent of carious defects and to assist with treatment planning (Figures 3 to 5).

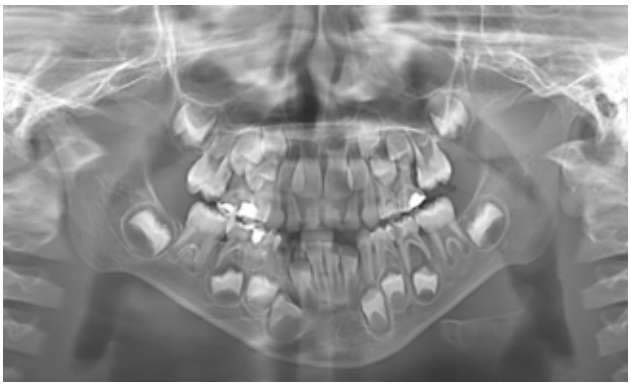


Figure 3: Panoramic radiograph obtained for diagnostic and treatment planning purposes. Carious involvement of the deciduous dentition is conspicuous.

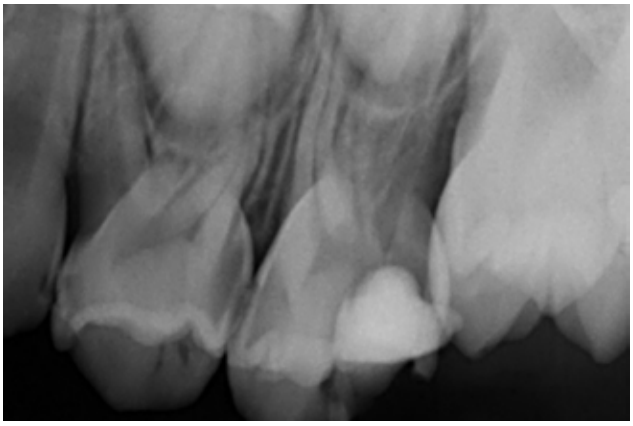


Figure 4: Periapical radiograph of the upper right deciduous dentition highlighting interproximal caries in which the Atraumatic Restorative Technique (ART) has been used.

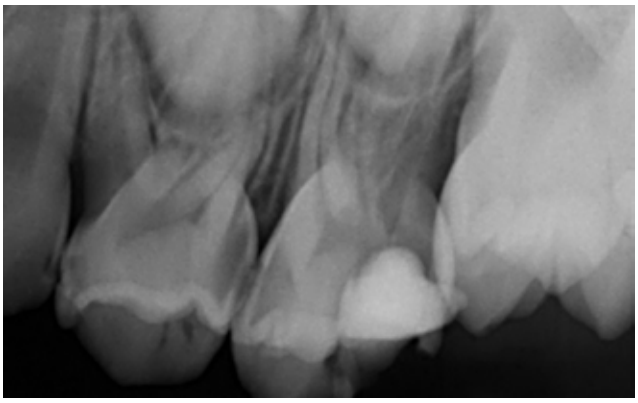


Figure 5: Periapical radiograph of upper left maxillary dentition showing interproximal decay with encroachment of the pulp of tooth 64. The tooth was previously treated by means of Atraumatic Restorative Technique (ART).

After discussing the treatment plan options, it was agreed that an emergency extraction of tooth 75 be performed (Figure 6). The preventive phase was subsequently commenced and comprised of a scaling and polishing, demonstration of oral hygiene maintenance, placement of fissure sealants on teeth 16, 26, 36 and 46 using 3M™ Clinpro ™ sealant as well as a

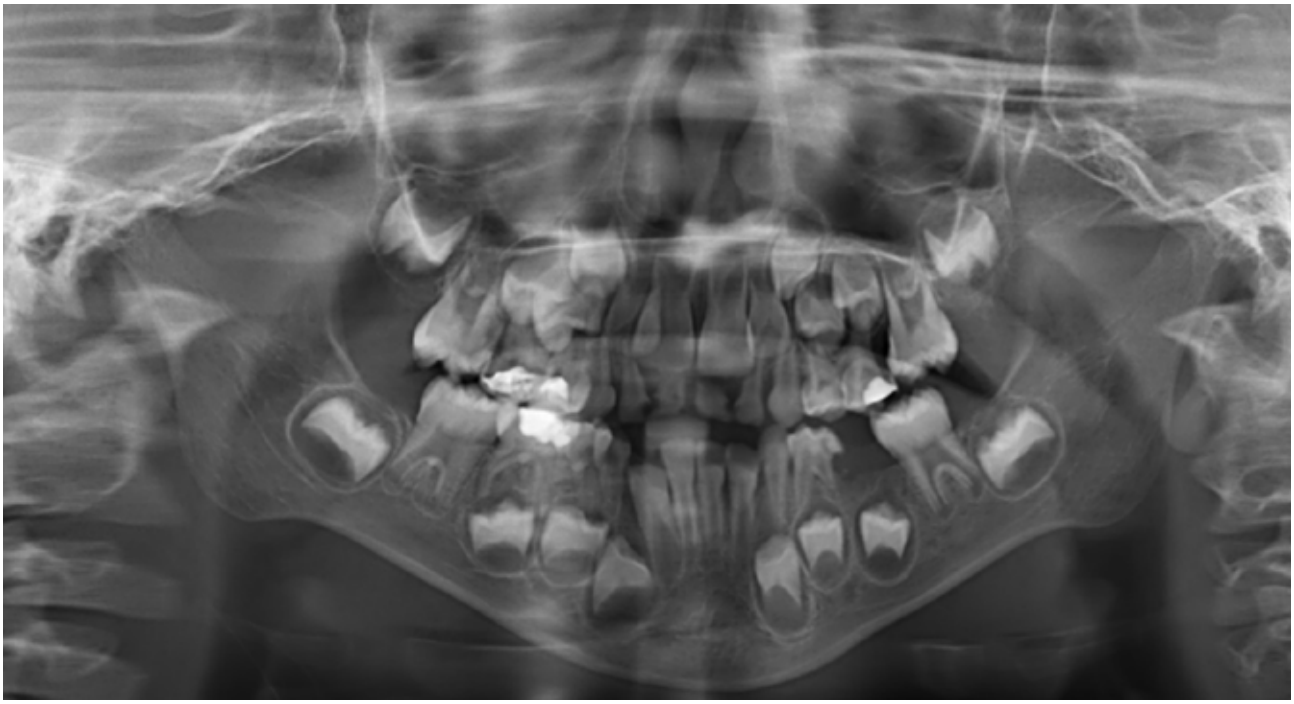


Figure 6: Panoramic radiograph following emergency management including extraction of tooth 75.

diet review. The corrective phase included pulpotomies on tooth 55 and tooth 85 using Ultradent Astringedent™ -15.5% Ferric Sulfate to stop bleeding followed by the application of pure Dentsply Kalzinol- Zinc Oxide Eugenol Cement Powder and normal saline as a medicament. Restorations using 3M™ Ketac™ Universal Glass Ionomer were done on teeth 55, 84 and 85.

Unfortunately, the patient's mother, who is the primary caregiver, was unavailable for subsequent visits where the placement of stainless-steel crowns and space maintainers were planned. This was to be followed by the maintenance phase to be conducted at 3-monthly and then 6-monthly intervals.

DISCUSSION:

The number of paediatric patients affected by HIV remains high in developing countries with vertical transmission from mother-to-child being the major contributor .[11] Oral disease remains one of the common manifestations of HIV in paediatric patients. Oral lesions are due to dysregulation of oral microbiota which facilitates opportunistic infections in a subset of patients who still have immature immunity. South Africa has a high burden of HIV-infection and there is a need to examine the relationship between caries and HIV-infection particularly in the epidemiological regions where infection rate is high.[2, 6]

The prevalence of dental caries is much higher in HIV-infected children than in those who are HIV-negative.[3-8] It is thus imperative that oral health education be prioritised together with regular screening and prevention.[11] A study performed in children with HIV/AIDS since 1992 demonstrated a range of problems regarding dental caries and to date it is notable that there are subsequent studies that confirmed these observations . The study by Howell and others showed that medication such as Zidovudine and Nystatin for candidiasis had high sucrose levels which promotes the development of dental caries. Another side effect of these medications is decreased saliva flow which also contributes to the prevalence of dental caries in these patients.[12] Another study in 1996 by Madigan and others in which caries prevalence was assessed between HIV-infected children and their uninfected siblings in the same environment confirmed a greater risk for dental decay particularly involving the primary dentition in those who were HIV-positive.[13] A different study conducted in the United States in 2011 confirmed the findings of sucrose and its effects on saliva production and further confirmed an association between the incidence of dental decay and HIV-infected patients who have not yet started taking HAART The association decreases in patients who have undergone HAART-initiation.[14] A study performed in 2004 firmly established a significantly higher prevalence of dental caries in HIV-infected children which correlated with the severity of HIV disease. This study identified high levels of salivary IgA which lead to the hypothesis that the sucrose content of medication, a high caloric diet used to compensate for weight loss in combination with immune deficiency and decreased saliva flow all promote the development of dental caries.[15] Indeed different studies from different parts of the world have also reported that there was high caries experience amongst HIV-infected children[15,16,17] . In our South African context, a study conducted by Mohamed and others in 2020 stated that children as the vulnerable group continue to suffer the impact of the HIV/AIDS pandemic and they may have an increased caries experience compared with their healthy peers.[7]

It has been shown that HIV-infected children and adults have a significantly higher caries experience with involvement of both the deciduous and permanent dentitions. Furthermore, the presence of caries in HIV-positive children is often associated with hypoplasia of the deciduous dentition, gingival inflammation and lower CD4 T-cell counts.[3] A study conducted amongst children with HIV infection in Tygerberg Hospital's Paediatric Infectious Disease Clinic in Cape Town ,South Africa highlighted the oral health status among children living with HIV, with regard to their dental caries and the majority of which were untreated . [7] Amongst several factors that were important it was evident that the increased

prevalence of dental decay in children with HIV was due to a lack of awareness regarding oral health issues as well as inadequate access to oral health services. [7]

The impact of dental caries on the quality of life and daily functioning in both children with HIV and their accompanying caregivers is of great public concern. Caries affects mastication, growth and development. Furthermore, the pain and frequent dental visits which are required, may affect those who are of school going age and work attendance by the accompanying parents /guardian/ caregivers. A carious dentition may have a long-term psychological effect on patients which may be compounded by the stigma associated with HIV-status. The increasing prevalence of dental caries in developing countries is largely because of increased consumption of sugary foods, poor oral hygiene, xerostomia, as well as an altered oral microbiome in conjunction with low levels of awareness of the problem in health care workers.[5,7,18]

A Ugandan study conducted in patients attending an HIV Care Clinic, revealed general xerostomia within in all HIV-positive patients. This was shown to be because of HIV infiltration of the lymphoid rich salivary glands where it infects the follicular dendritic cells during apparent dormancy whilst it replicates and may result in increased numbers of CD8 lymphocytes. This is what results in generalised lymphadenopathy throughout the body. Many of these patients develop cystic lymphoid hyperplasia of the salivary glands characterised by lymphoepithelial cyst formation. Involvement of the salivary parenchyma results in decreased salivary flow which increases the risk for caries development. The same study showed that female gender and longer duration of antiretroviral treatment are independent risk factors for caries development. Chronic antiretroviral therapy may cause alterations in the normal microbial flora of the oral cavity thus facilitating opportunistic infections which is exacerbated by xerostomia. Moreover, HIV-positive patients are at risk for malnutrition which is worsened by dental caries especially in children and the elderly.[5]

The oral status of HIV-infected children who attended a Paediatric Infectious Diseases Clinic in Cape Town, South Africa was investigated.[7] Sixty-six HIV-positive children aged two to twelve years were recruited for investigation. It was shown that the prevalence of dental caries was exceedingly high (78,8%). More notably, it was determined that an unmet treatment need of 90,4% was recorded among participants. HIV-infected children in the Western Cape often receive their antiretroviral treatment at facilities like Tygerberg Hospital's Paediatric Infectious Diseases Clinic. [7] These findings suggest a need for intensified collaborative care between Paediatric Clinics and Paediatric Dental Clinics.[7] Doctors and nurses are usually the first health care workers to have contact with HIV-infected children. Increasing awareness of the high prevalence of dental caries in these patients is essential to provide holistic patient care.[7]

CONCLUSION:

It is thus apparent that additional research into the association between HIV-infection and dental caries is required. The literary findings sugg est a relationship exists between HIV-infection and dental caries which requires an understanding of how lowered immunity may synergistically affect multiple factors predisposing to the development of dental caries. There is a need for effective oral health interventions in HIV-positive patients. Thus, the awareness of the high prevalence of dental caries in association with HIV emphasizes the need for strategic public oral health care policies which highlight preventative care and include comprehensive care programmes to holistically meet the needs of these patients.

REFERENCES:

1. Organization WH. HIV and AIDS. Available at: https://www.who.int/news-room/fact-sheets/detail/hiv-aids. Accessed 17 October 2024.
2. Coker M, El-Kamary SS, Enwonwu C, et al. Perinatal HIV Infection and Exposure and Their Association With Dental Caries in Nigerian Children. *Pediatr Infect Dis J.* Jan 2018;37(1):59-65.
3. Akhigbe P, Chukwumah NM, Folayan MO, et al. Age-specific associations with dental caries in HIV-infected, exposed but uninfected and HIV-unexposed uninfected children in Nigeria. *BMC Oral Health.* Sep 27 2022;22(1):429.
4. Murererehe J, Malele-Kolisa Y, Niragire F, Yengopal V. Prevalence of dental caries and associated risk factors among People Living with HIV/AIDS and HIV uninfected adults at an HIV clinic in Kigali, Rwanda. *PLoS One.* 2023;18(4):e0276245.
5. Kalanzi D, Mayanja-Kizza H, Nakanjako D, Mwesigwa CL, Ssenyonga R, Amaechi BT. Prevalence and factors associated with dental caries in patients attending an HIV care clinic in Uganda: a cross sectional study. *BMC Oral Health.* Jul 19 2019;19(1):159.
6. Coker MO, Akhigbe P, Osagie E, et al. Dental caries and its association with the oral microbiomes and HIV in young children-Nigeria (DOMHaIN): a cohort study. *BMC Oral Health.* Dec 4 2021;21(1):620.
7. Mohamed N, Mathiba OP, Mulder R. Oral status of HIV-infected children aged 12 years or younger who attended a Paediatric Infectious Diseases Clinic in Cape Town. *Clin Exp Dent Res.* Feb 2020;6(1):75-81.
8. Kikuchi K, Yasuoka J, Tuot S, et al. Dental caries in association with viral load in children living with HIV in Phnom Penh, Cambodia: a cross-sectional study. *BMC Oral Health.* Mar 25 2021;21(1):159.
9. isser MJ, Neufeld S, de Villiers A, Makin JD, Forsyth BW. To tell or not to tell: South African women's disclosure of HIV status during pregnancy. *AIDS Care.* Oct 2008;20(9):1138-1145.
10. Delva W, Abdool Karim Q. The HIV epidemic in Southern Africa - Is an AIDS-free generation possible? *Curr HIV/AIDS Rep.* Jun 2014;11(2):99-108.
11. Lauritano D, Moreo G, Oberti L, et al. Oral Manifestations in HIV-Positive Children: A Systematic Review. *Pathogens.* Jan 31 2020;9(2).
12. Howell RB, Jandinski J, Palumbo P, Shey Z, Houpt M. Dental caries in HIV-infected children. *Pediatr Dent.* Nov-Dec 1992;14(6):370-371.
13. Madigan A, Murray PA, Houpt M, Catalanotto F, Feuerman M. Caries experience and cariogenic markers in HIV-positive children and their siblings. *Pediatr Dent.* Mar-Apr 1996;18(2):129-136.
14. Rwenyonyi CM, Kutesa A, Muwazi L, Okullo I, Kasangaki A, Kekitinwa A. Oral Manifestations in HIV/AIDS-Infected Children. *Eur J Dent.* Jul 2011;5(3):291-298.
15. Castro GF, Souza IP, Lopes S, Stashenko P, Teles RP. Salivary IgA to cariogenic bacteria in HIV-positive children and its correlation with caries prevalence and levels of cariogenic microorganisms. *Oral Microbiol Immunol.* Oct 2004;19(5):281-288.
16. Nabbanja, J. , Gitta, S. , Peterson, S. , & Rwenyonyi, C. M. (2013). Orofacial manifestations in HIV positive children attending Mildmay Clinic in Uganda. Odontology, 100, 116–120.
17. Yengopal, V. , Kolisa, Y. , Thekiso, M. D. , & Molefe, M. P. (2016). The child and adolescent with HIV in resource poor countries. Oral Diseases, 22, 25–34.
18. Teshome A, Muche A, Girma B. Prevalence of Dental Caries and Associated Factors in East Africa, 2000-2020: Systematic Review and Meta-Analysis. *Front Public Health.* 2021;9:645091.

CPD questionnaire on page 226

The Continuing Professional Development (CPD) section provides for twenty general questions and five ethics questions. The section provides members with a valuable source of CPD points whilst also achieving the objective of CPD, to assure continuing education. The importance of continuing professional development should not be underestimated, it is a career-long obligation for practicing professionals.

www.sada.co.za / SADJ Vol. 80 No.4

What's new for the clinician – summaries of recently published papers (May 2025)

SADJ MAY 2025, Vol. 80 No.4 P218-221

Edited and compiled by Prof V Yengopal, Faculty of Dentistry, University of the Western Cape

1. ARE DENTAL PROFESSIONALS AWARE OF THE DISCOVERY OF NEWLY IDENTIFIED SALIVARY GLANDS?

In October 2020, several journals and news sites reported the discovery of an organ in the throat—the tubarial salivary gland (TSG).[1] The gland was named for its anatomic proximity to the torus tubarius, a cartilaginous elevation near the opening of the eustachian tube.

Valstar and associates[1] noticed an unknown structure bilaterally in the posterior nasopharynx with ligand uptake like that of the major salivary glands. The discovery was made while screening prostate carcinoma patients in the Netherlands Cancer Institute using radiolabelled ligands to prostatic-specific membrane antigen (PSMA) and subsequent positron-emission tomography/computed tomography scan. The gross features were further characterized in human cadaver dissection with the usage of histochemistry and immunohistochemistry (PSMA, alpha-amylase) and taking prostate (for PSMA) and parotid/pancreas (for amylase) samples as controls. In live subjects, magnetic resonance imaging also confirmed the presence of tubarial glands (see Fig 1)

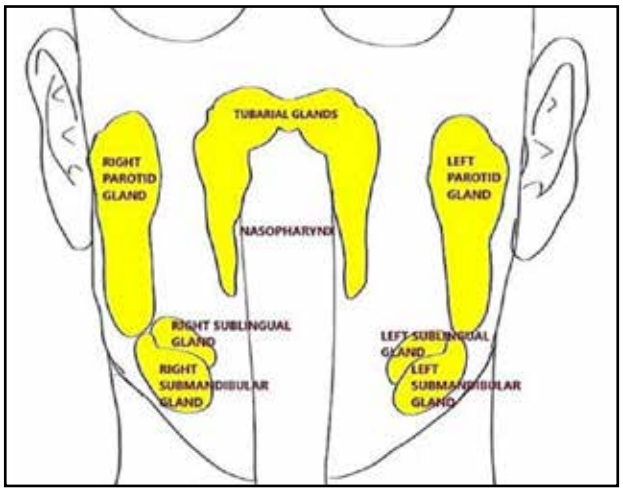


Figure 1: Schematic representation to show site of Tubarial Glands

The tubarial glands are considered similar to the sublingual salivary glands due to the absence of amylase secretion, the presence of multiple drainage ducts, their lack of a capsule, and the similarity in the types and frequencies of salivary gland tumours found in both the nasopharynx and sublingual salivary glands[1]. However, there is ongoing debate as to whether the tubarial glands are more like sublingual glands or palatal glands.

Tuberial glands are located in a difficult to access anatomical position below the base of the skull and are best assessed by nasal endoscopy. Conventional imaging methods, such as ultrasound and CT, have never been able to identify and interpret this submucosal structure as a salivary gland. However, in recent years, multiparametric magnetic resonance (MR) imaging has enabled clearer detection of these glands. Some authors believe that the Tubarial Gland is a new organ. This discovery has drawn significant attention from the scientific community but has also raised questions and sparked debates. One of the main points of discussion is whether the tubarial glands should be classified as major or minor salivary glands or considered a separate organ. To classify a structure as an organ, it must have a distinct shape and structure, consist of multiple tissue types, and perform specific functions.

Some authors suggest that it might be more appropriate to interpret the tubarial glands as a macroscopic part of the entire salivary gland organ system rather than as a separate organ. However, some authors disagree with this perspective. They argue that classifying the tubarial glands as salivary glands is incorrect because these glands open into a region associated with the respiratory system rather than the digestive system. Other authors have also highlighted that these glands are "taxonomically distinct from salivary glands" due to their anatomical positioning within the respiratory tract, the presence of respiratory epithelium in the overlying mucosa, and the absence of the enzyme salivary amylase. They also emphasize that for the tubarial glands to be considered a new organ, distinct blood flow, lymphatic drainage, and neural connections in the surrounding tissues must be identified.

Pringle et al (2024)[2] summarized their recommendation against classifying tubarial glands as salivary glands in six key points:

- Tubarial Glands do not contribute to oral fluid: Tubarial glands should not be considered salivary glands because they do not secrete fluids into the oral cavity. They might be classified as ectopic salivary glands only if the fluid they secrete is shown to have a composition similar to that of saliva.

- Histopathological characteristics resemble minor glands: Histological analysis suggests that tubarial glands are a conglomerate of minor glands, not a major salivary gland. Their duct openings resemble those of minor glands like the labial glands, rather than major salivary glands.

- Absence of salivary enzyme markers: Unlike typical salivary glands, tubarial glands do not secrete amylase, a hallmark enzyme of salivary secretion. Additional salivary markers, such as agglutinin, lactoferrin, and lysozyme, could help clarify whether these glands should be considered true salivary glands.

- Uncertain stimuli for secretion: The exact stimuli that trigger secretion from tubarial glands remain unknown. Salivary glands typically respond to chewing, gustatory,

or olfactory stimuli, but it is unclear whether these factors influence the tubarial glands.

- Lack of secretions characterization: It is important to analyze the secretions from the tubarial glands to better understand their function and composition. This could be achieved through methods used to collect secretions from minor salivary glands, such as endoscopic examination of the nasopharynx.

- Need for further research to determine function: More research is needed to determine the precise role and function of the secretions produced by the tubarial glands. A detailed analysis of their secretions and response to various stimuli will provide more insight into whether these glands serve functions comparable to salivary glands or have a distinct role.

Concluding Remarks

More recently, researchers have reported that, regardless of how the tubarial tissue is classified, the presence of mucous acinar cells in the nasopharynx remains a consistent observation. They demonstrated the importance of this tubarial tissue for oral intake, noting that damage to the tubarial glands is linked to dysphagia. Therefore, the clinical significance of tubarial tissue, particularly its role in conditions such as dry mouth due to radiation damage or autoimmune diseases like Sjogren's syndrome, should be recognized.

Implications for dental professionals

The tubarial gland secretes mucin, which keeps the mucosal surface moist, thus preventing heat-induced desiccation caused by the breathing of air. The physiological function of tubarial glands is believed to be the lubrication and moistening of the nasopharynx and oropharynx. It is well-known that high-dose radiotherapy applied to the salivary glands during the treatment of head and neck cancer or brain metastases can lead to loss of function, xerostomia, and dysphagia. Affected patients experience difficulty in food intake, digestion, speech, and an increased risk of caries and oral infections. Therefore, the discovery of tubarial glands is particularly relevant to all professionals concerned with oral health.

REFERENCE

1. Valstar MH, de Bakker BS, Steenbakkers RJ, de Jong KH, Smit LA, Nulent TJ, van Es RJ, Hofland I, de Keizer B, Jasperse B, Balm AJ. The tubarial salivary glands: A potential new organ at risk for radiotherapy. Radiotherapy and Oncology. 2021 Jan 1;154:292-8.
2. Pringle S, Bikker FJ, Vogel W, de Bakker BS, Hofland I, van der Vegt B, Bootsma H, Kroese FG, Vissink A, Valstar M. Away from definition and back to the clinic–As response to Kumar et al,'Evidence is not sufficient to declare the tubal gland conglomerates as salivary'. Radiotherapy and Oncology. 2024 Jan 1;190

2. HEALTH COMPLAINTS BEFORE AND AT ONE AND FIVE YEARS AFTER REMOVAL OF DENTAL AMALGAM RESTORATIONS

The Minamata Convention on Mercury (MCM) is a binding multilateral agreement that aims to protect human health and the environment from anthropogenic mercury releases[1]. The MCM focuses on banning new mercury mines, phasing out existing mines, phasing out and phasing down mercury use in products and processes, controlling environmental emissions, and regulating the informal sector of artisanal and small-scale gold mining[2]. South Africa joined the Minamata Convention on Mercury on April 29, 2019, and is thus obligated to adhere to the measures aimed at reducing environmental releases of mercury[3].

In response to the MCM, the World Health Organization (WHO) released an oral health briefing on preventing and treating dental caries with mercury-free products and minimal intervention[4]. In addition, resin-based composites were, for the first time, included in the WHO's 23rd edition of the Essential Medicines List (EML) released in 2023[5]. The current EML also contains Fluoride toothpaste, gel, mouth rinse and varnish, Silver Diamine Fluoride, and Glass Ionomer Cement, which provided an impetus for the availability, affordability, and accessibility of essential interventions in the management and prevention of dental caries while aligning with the goals of MCM.

In the efforts to domesticate the MCM in South Africa, the Department of Forestry, Fisheries, and the Environment developed national regulations for the management of mercury in South Africa. The regulations are expected to become effective on the 1st of April 2025, and have implications for oral health services as they address the waste management practices for dental amalgam[6]. In the regulations, the best management practices for dental amalgam use (BMPs), including the installation of amalgam separators in the clinics using dental amalgam, will become mandatory and contravention litigable.

Notably, the regulations do not address provisions aimed at protecting the developing neural from the effect of mercury resulting from the placement of dental amalgam, such as restricting the use of dental amalgam in children under 15 years of age and protecting pregnant and breastfeeding mothers.

The faculty of Dentistry, UWC hosted a Symposium on An Update on Dental Amalgam in South African Dental Training Institutions on March 27, 2025. From the feedback received, all of the dental schools do not use amalgam as a restorative material anymore and are well on the way of phasing out the use of amalgam in the training environment. There is also an acknowledgement that all dental schools should comply with the requirements of placing amalgam separators on some chairs on the clinical training platform where they may be a need to treatment patients who need their old amalgams to be removed and replaced with safer options.

Patients with health complaints attributed to their amalgam fillings have in general several symptoms in common with patients with medically unexplained physical symptoms (MUPS). The similarity of the health complaints in these two patient groups allows comparison over time. In 2012 the Norwegian Directorate of Health initiated an experimental treatment project including patients with subjective health complaints attributed to dental amalgam. This project was called the Bergen Amalgam Trial and was designed as a prospective cohort study with three non-equivalent groups: Amalgam cohort, patients with medically unexplained physical symptoms (MUPS) cohort, and Healthy cohort. The target population was the Amalgam cohort (patients with MUPS attributed to dental amalgam restorations) who had all amalgam restorations removed and replaced with other dental restorative materials. The MUPS cohort and the Healthy cohort were used as comparison groups. The primary outcome of the project was general health complaints index (GHC-index) 1 year after removal of all amalgam fillings was completed, which was significantly decreased ($p < 0.001$) in the Amalgam cohort. Patients in the comparison cohorts did not have their amalgam fillings removed and there was no significant change in the general health complaints index (GHC-index) at follow-up in these cohorts.

www.sada.co.za / SADJ Vol. 80 No.3

Sinha et al (2024)[1] used the amalgam cohort from the Bergen trial to present a descriptive analysis characterizing changes of specific health complaints after removal of all amalgam restorations over time periods of 1 and 5 years.

Materials and methods

Participants in the Amalgam cohort form the Bergen Trail were recruited to participate in this study.

At baseline, the first questionnaire (Q1) was sent to all three cohorts [healthy cohort; amalgam cohort and MUPS cohort) after written informed consent was obtained. This was followed by removal of amalgam restorations in the Amalgam cohort. A follow-up questionnaire (Q2) was sent by mail to the patients in the Amalgam cohort 1 year after removal of the last amalgam restoration. Q2 was sent to the comparison groups 2 years after the completion of the baseline questionnaire. Four years after completion of Q2, the second follow-up questionnaire (Q3) was distributed to the cohorts.

The primary outcome of the Bergen Amalgam Trial was changes in the general health complaints index (GHC-index) at 12-month follow-up after amalgam removal was completed.

The secondary outcome was to assess the health complaints attributed to dental amalgam fillings using the Munich Amalgam Scale (MAS). MAS is a questionnaire-based tool used to assess health complaints attributed to dental amalgam fillings and consists of 50 items, each scored on a scale from 0 to 3, where 0 means "not at all" and 3 means "very much" for the intensity of each symptom. The scale is used primarily in research on medically unexplained physical symptoms (MUPS) linked to amalgam fillings, to measure changes in symptom intensity before and after removal of amalgam restorations

Results

A total of 59 patients with health complaints attributed to dental amalgam sent an application for participation in the project. After consideration of inclusion and exclusion criteria, 37 participants were included in the Amalgam cohort. A total of 32 participants had all amalgam fillings removed and responded to Q2.

Fifty-two patients were recruited to the MUPS cohort and signed an informed consent. Among these, 44 participants responded to the baseline questionnaire. In total, 33 patients fulfilled the MUPS cohort criteria, and of these 28 responded to both Q1 and Q2.

The third cohort consisted of healthy volunteers and was mainly recruited from dental practice. Among 28 participants who signed an informed consent, 25 responded to Q1 and six of them did not respond to Q2. Thus, 19 were available for the analysis of change scores between baseline and Q2. The second follow-up questionnaire (Q3) was completed by 22 participants in the Amalgam cohort, 22 participants in the MUPS cohort, and by 12 participants in the Healthy cohort.

Amalgam cohort: Local and general health complaints

Symptom reduction

In the Amalgam cohort the mean intensity of each of all 23 items was reduced at the first follow-up (Q2) compared with the baseline (Q1). Fourteen of the symptoms were significantly reduced. At the second follow-up (Q3), reduction in mean intensity was observed for all but one item compared with baseline (Q1)

Consistent significant changes

Five local symptoms were significantly decreased from baseline at both follow-ups: 'intraoral burning sensation', 'intraoral pain/ tenderness', 'taste disturbance', 'dry mouth', and 'facial stiffness/paraesthesia. Seven of the GHC were consistently changed: 'pain from muscles and joints', 'cardiovascular symptoms', 'fatigue', 'dizziness' 'memory problems', 'difficulty to concentrate', and 'anxiety/ depression'

Amalgam cohort: Munich Amalgam Scale

Patients in the Amalgam cohort also responded to the Munich Amalgam Scale. The highest Effect sizes were found for 'metallic taste' (0.93), 'stress at the job' (0.83), 'skin rash' (0.57), 'sensitivity to cold and wind' (0.54), 'worries – restlessness' (0.53), and 'irritability' (0.52); and significant reduction of intensity of symptoms was shown. Moderate Effect sizes (around 0.4) were found for items 'feeling of walking next to oneself', 'arrythmia', 'diarrhoea', 'increased urge to toilet', 'indecision', 'abdominal pain', 'lack of concentration', 'fluctuating mood', 'anxiety', and 'burning sensation in tongue', all being statistically significant

Explorative analyses of the Amalgam cohort including age and gender in the mixed effects model indicated significant effects from both age and gender for the item 'pain from muscles and joints'. Women had, at an average, a higher overall intensity of 'pain from muscles and joints' (mean 1.8; 95% confidence interval [CI] from 0.4 to 3.4, $p = 0.016$). An increase of 10 years of age was associated with lower intensity of 'pain from muscles and joints' (mean 1.3; 95% CI from 0.2 to 2.3; $p = 0.014$). Significant effects from age, but not for gender, were observed for the symptoms 'fatigue', 'intraoral stiffness/paraesthesia', and 'pain from temporomandibular joints'. An increase of 10 years of age was associated with a lower 'fatigue' symptom intensity (1.5; 95% CI from 0.4 to 2.6; $p = 0.008$), lower 'intraoral stiffness/ paraesthesia' (0.6; 95% CI from 0.2 to 1.0; $p = 0.005$), and lower 'pain from temporomandibular joints' (1.3; 95% CI from 0.4 to 2.2; $p = 0.005$).

Conclusions

This study supports the hypothesis that reduction of intensity of symptoms, which could be caused by mercury, occurs after removal of amalgam restorations in patients with medically unexplained physical symptoms (MUPS) attributed to amalgam.

Implications for practice

There remains controversy on the effects of amalgam on patient's heath mainly due to the poor quality of the published studies but environmental concerns have taken precedence and there is a worldwide move to phase down or phase out the use of amalgam in dentistry.

REFERENCE

1. Sinha N, Hamre HJ, Musial F, Werner EL, Björkman L. Health complaints before and at one and five years after removal of dental amalgam restorations–data from a prospective cohort study in Norway. Acta Odontologica Scandinavica. 2024 May 3;83:40260.

The future is made today. The future is made by you.

Your research could transform the future and advance patient safety practices.

Join the growing community of researchers backed by The MPS Foundation – the not-for-profit research initiative shaping the future of patient safety and healthcare professional wellbeing.

Since 2022 we have supported over 50 projects with funding between £2,500 and £199,000.

Submit your Expression of Interest for a research grant today at thempsfoundation.org

Closing date
2 May 2025 at 17.00 BST

THE MPS FOUNDATION

The MPS Foundation is a trading name and division of The Medical Protection Society Limited (MPS). MPS is a company limited by guarantee. Registered in England No. 00036142 at Level 19, The Shard, 32 London Bridge Street, London, SE1 9SG. VAT number 524 251475. MPS is not an insurance company. All the benefits of membership of MPS are discretionary as set out in the Memorandum and Articles of Association.
For information on MPS's use of your personal data and your rights, please see our Privacy Notice on the website.

2412206203 01/25

www.sada.co.za / SADJ Vol. 80 No.4

Listening: The most valuable service you won't charge for!

SADJ MAY 2025, Vol. 80 No.4 P218-219

LM Sykes[1]; HT van den Bergh[2]; P Nethononda[3], H Bernitz[4]

"Knowledge Speaks, but Wisdom Listens"

Jimi Hendricks

ABSTRACT

Dental practitioners cannot charge additional fees for the time spent "listening" to their patients, however, the non-financial cost benefits of this time spent may be of a far greater value than is generally realised. Consciously paying attention to a patient and engaging in meaningful communication with them play a valuable part in providing patient-centred care and fostering a positive healthcare experience. Time constraints and billing pressures are a reality in today's clinical practice. The art of listening to patients aligns with both ethical care and sustainable practice management. It's about balancing financial **viability with compassionate patient care**.

Introduction

Dental practitioners are not allowed to charge additional fees for the time spent "listening" to their patients. The fees they charge are for the services they provide, such as the consultation, examination and treatment planning, and the subsequent performance of clinical procedures. Time spent listening or talking to a patient falls under the general scope of the consultation visit, and is included in the overall fee for that appointment (*see Code 8101 and 8104 description).[1] This brings up the question of whether we, as individuals, health care providers, or society, truly listen to one another, rather than merely observing at a surface level and forming opinions based on a superficial, and often subjective impression refered to as cognitive bias. While a practitioner cannot charge for added listening-time, the non-financial cost benefits may be of a far greater value. Listening and communicating well helps build trust, ensures accurate diagnoses, and leads to better outcomes.[2] When patients feel heard, they are more likely to share important details about their symptoms, concerns, and medical history, which can lead to a more complete and accurate diagnosis.[3]

Authors' information:

1. Leanne M Sykes BSc, BDS, MDent, Dip Research Ethics (IRENSA), Dip Forensic Odont, Dip ESMEA, FCD (Pros) Head of Department of Prosthodontics, University of Pretoria. Leanne.sykes@up.ac.za Orcid: https://orcid.org/0000-0002-2002-6238
2. Herman T van den Bergh BChD, MSc. (Dent) Registrar
3. Portia Nethononda BSc, BDS, PGDip (Clinical), MChD (Pros) Senior Lecturer Prosthodontics Department, University of Pretoria. Herman van den Bergh. Orcid: https://orcid.org/0000-0002-8571-818X. Portia Nethononda. Orcid: https://orcid.org/0009-0004-1978-0702
4. Herman Bernit BChD, MSc, Dip Odont, PhD Extraordinary Professor, Department of Maxillo-facial Pathology, University of Pretoria.

Corresponding author:
Name: Leanne Sykes
Email: Leanne.sykes@up.ac.za

Author Contribution:
Leanne Sykes – Primary author – 70%
Herman van den Bergh – 10%
Portia Nethononda – 10%
Herman Bernitz – 10%

It also promotes a sense of respect and empathy, making patients feel valued and more likely to follow treatment plans. Overall, listening is key to providing patient-centred care and fostering a positive healthcare experience.[4] The following case highlights the need for clinicians to spend time listening to their patients and sets the scene for the subsequent ethical discussion.

Case scenario

A patient presented to a new dentist seeking treatment. Before even taking the time to listen to her main complaint, establish her desires, or examine her mouth, the clinician began to discuss her "ugly front teeth", and the need to have all six anterior maxillary teeth crowned to give her a "beautiful smile" (Figure 1). The patient was rather disconcerted, as she had never been too worried about her smile, and was seeking relief from the severe pain she had been experiencing in the 4th quadrant. The dentist then decided it might be prudent to take a radiograph and investigate further. An orthopantomogram (Figure 2) revealed several dental problems, the most obvious being the large carious lesion on the 46. The patient was told that this tooth could be restored if she was willing to undergo a root canal therapy and pay for a seventh crown. If not it could be extracted immediately while the follow-up visits were being scheduled.

Figure 1. Patient's presenting oral appearance

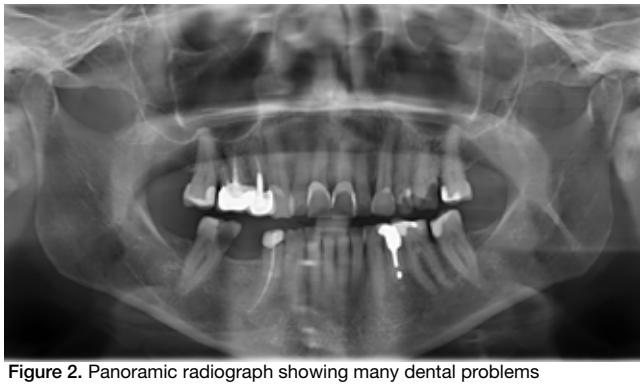


Figure 2. Panoramic radiograph showing many dental problems

At this stage, the patient became anxious and psychologically distressed. Firstly, because she felt the clinician had not taken the time to talk to her, or listen to her presenting problem. She had sought treatment because she was in pain, yet this had not even been acknowledged. Furthermore, she had never considered her smile unattractive and was now feeling self-conscious and embarrassed at the thought that other people may have been commenting on her looks without her knowledge for many years. She felt so unnerved and upset, that she cut the visit short and left the rooms without having her main complaint, the pain, addressed.

Hearing versus Listening

Hearing can be defined as a passive physiological process of perceiving sound through the ears by a physical process of detecting sound waves and recognizing that noise is present. No active or conscious effort is required, as it happens automatically if the auditory system is functioning. Merely hearing something does not necessarily lead to understanding or retaining information without active input and engagement.

Listening is that active process of paying attention to, understanding, and interpreting the sounds or words that have been heard. It involves conscious effort to decode the unspoken messages, and construct meaning from what is being communicated. This requires mental focus, attention, interest, comprehension, engagement, and an emotional connection in order to understand and respond with empathy or action based on the information.[2,4]

The key distinction between the two is that hearing is passive and physiological, while listening is active, intentional, and cognitive. Effective communication requires listening, not just hearing, and involves attention, empathy, patience, openness, and comprehension, making it a critical skill in personal and professional communication.[5]

Key Elements of Listening and Communicating

Receiving: The physical act of hearing the sound or observing the nonverbal cues of the speaker.

Understanding: Making sense of the words, tone, and context of what is being communicated.

Evaluating: Judging the content, intent, and relevance of the message.

Responding: Providing verbal or nonverbal feedback to show comprehension and engagement.

Remembering: Retaining the information shared for future reference or action.

The phrase “I see you, but do I hear you?” is a powerful reflection on the distinction between acknowledgment and understanding in communication. It highlights the idea that truly hearing someone goes beyond the spoken word. It includes acknowledging their presence, noticing or observing their behaviour, engaging with them to try form a connection, and responding in a manner that shows you genuinely value what they have said. The seeing is about recognition, while the hearing relates to the connection and comprehension. This question also challenges whether we, as individuals or society, truly listen to one another, rather than merely observing at a surface level.

The Importance of Listening in a Healthcare setting

A. Patient related factors

During the initial consultation, a practitioner must spend time engaging with their patients and listening attentively to their presenting complaints. This involves actively listening and hearing not just the words, but also the emotions and context behind what the patient is saying. It’s not just about checking off symptoms, but about picking up on the nuances, concerns, and any underlying fears that might affect their well-being or their willingness to follow through with treatment. This in turn will help the healthcare provider understand the full picture of the patient’s physical, emotional, and social state of health. Being genuinely present and attentive also builds trust, which can be especially important if patients are feeling vulnerable or scared about their condition, as-yet-unknown diagnosis and possible treatment options. At the same time listening to patients can be seen as an ethical and legal requirement as it shows the following:[4,6,7]

1. Respect for Autonomy:
Listening shows respect for a patient’s right to make decisions about their own health and treatment. It ensures they can express their values, concerns, and preferences, which are crucial in shared decision-making.

2. Building Trust and Loyalty:
Active listening builds a foundation of trust between patients and caregivers. It is also crucial that they are honest when communicating with their patients, and this will encourage honesty, adherence to treatment, and better overall outcomes in return. They will also be more likely to return for all their future treatment needs, and recommend the practice to others

3. Empathy and Compassion:
Listening is an act of compassion that acknowledges the patient’s feelings and experiences. It helps create a space where patients feel seen and understood, reducing feelings of isolation or fear. Furthermore, many patients feel anxious or vulnerable in dental consulting rooms. When you take the time to listen, it helps reduce those feelings and creates a safe environment for them.

4. Improved Quality of Care:
Patients often provide key details about their symptoms, history, or lifestyle that can guide better diagnosis and treatment. Ignoring them risks missing crucial information.

5. Advocacy and Equity:
Listening ensures that marginalized or vulnerable populations have their voices heard, preventing disparities in care and promoting ethical principles of justice and fairness.

6. Non-Maleficence:
Ignoring or dismissing a patient’s concerns can lead to harm, whether through misdiagnosis, inappropriate treatment, or emotional distress. Listening actively minimizes such risks.

7. Informed Consent:
Engaging with patients ensures they understand their options, risks, and benefits, empowering them to make informed choices about their care.

In essence, listening is not only a cornerstone of ethical practice but also a critical tool for effective, patient-centred care.

B. Practitioner related factors

While it is not permissible to charge fees for extra time spent listening to a patient, it is still absolutely worth the time in the long run. It can lead to:[2,4,8]

1. Improved Patient Outcomes

Listening can lead to a better understanding of symptoms, concerns, and underlying issues. Accurate diagnosis and treatment decisions are more likely, reducing follow-ups or complications that could take more time later.

2. Relationship Building

A strong patient-provider relationship fosters loyalty and satisfaction. Patients who feel heard are more likely to return, and to adhere to treatment plans, benefiting long-term success. They are also more likely to recommend you to family and friends, which is valuable for growing a practice and reputation.

3. Personal Professional Fulfilment

Listening is part of the human connection that makes healthcare meaningful. Taking the time to listen can provide personal satisfaction and remind a clinician why they chose this field.

4. Legal and Ethical Safeguards

Patients who feel unheard are more likely to pursue legal action if something goes wrong. Many reported cases of litigation relate to poor communication and overcharging as opposed to bad treatment. Listening carefully reduces misunderstandings and helps ensure clarity in care.

5. Patient Satisfaction

A patient who feels heard is far more likely to leave with positive feelings and return for future visits when necessary. Even if the actual time spent with a patient is short, quality listening can make them feel like they received personalized, focused attention.

6. Efficient Communication

While listening may take more time initially, it can help prevent confusion and repeated questions, and reduce the number of unnecessary follow-up calls or visits. Thus investing time early can save time later.

Strategies to aid effective listening

It is clear that effective listening plays an important role in any dental consultation, and can impact on the treatment outcome. Patients who feel heard become more open to suggestions, more compliant with treatment, and more satisfied with the healthcare practitioner. At the same time, healthcare practitioners who were considered good listeners and communicators also tended to experience less malpractice litigation.[9]

Listening as a skill set is not well understood. Merely hearing sound does not guarantee that an individual is actively focusing on what is being said or processing the meaning from it. While dental training may teach communication skills, the aspect of developing good listening skills is often overlooked as it is seen as common sense or falls under the banner of empathy and as such, does not get the attention it deserves.[9]

Authors, Barker and Watson (2000) suggested that before any meaningful listening can occur, practitioners need to identify what type of listener they are, and then be taught skills that tap into their individual profiles. They proposed that there are four main listener types: (1) people-oriented listeners; (2) action-oriented listeners; (3) content-oriented listeners, and (4) time-oriented listeners. They noted that healthcare practitioners can have a combination of these profiles, which impacts how they should be taught the various listening skills.[10] They then proposed different listening skill sets that can be taught to healthcare practitioners, which include: (1) discriminative listening, that focuses on interpreting the message based on how the words are articulated and not on the words themselves, (2) critical listening, where one not only hears what is being said but also comprehends, analyses and evaluates what is being said to form an opinion, (3) empathic listening also known as reflective listening wherein the listener tries to understand the patient's point of view, (4) therapeutic listening also referred to as diagnostic listening undertaken by qualified health personnel, and (5) comprehensive listening which is a deeper level of active listening characterized by internalizing the spoken words and engaging the speaker to fully understand what they mean.[10]

Many listening exercises can be taught and learned to help practitioners develop effective listening skills, but these often require trained personnel to teach them. However, as a starting point clinicians can consciously try to eliminate distraction, be more focused on the person who is speaking (their patients), avoid thinking of an immediate answer, show respect to patients' opinions and desires, and pay attention to voice changes as well as their more subtle body language during the conversation.[11]

CONCLUSION

Ultimately, while billing pressures are a reality, listening to patients aligns with both ethical care and sustainable practice management. It's about balancing financial viability with compassionate patient care.

REFERENCES

1. Government gazette – Dental Codes. Accessed at: file:///C:/Users/u28681942/Documents/ethics%20and%20human%20rights/dental%20codes%20and%20description.pdf; Accessed on:28-01-2025
2. Jagosh J, Donald Boudreau J, Steinert Y, MacDonald ME, Ingram L. The importance of physician listening from the patients' perspective: Enhancing diagnosis, healing, and the doctor–patient relationship. Patient Educ. Couns. 2011; 85(3):369-74. doi:https://doi.org/10.1016/j.pec.2011.01.028
3. Lang F, Floyd MR, Beine KL. Clues to patients' explanations and concerns about their illnesses: A call for active listening. Arch. Fam. Med. 2000; 9(3):222.
4. Epstein RM, Beach MC. "I don't need your pills, I need your attention:" Steps toward deep listening in medical encounters. Current Opinion in Psychology. 2023; 53:101685. doi:https://doi.org/10.1016/j.copsyc.2023.101685
5. Worthington DL. Modelling and measuring cognitive components of listening. The sourcebook of listening research: Methodology and measures. 2017:70-96.
6. Pietrzykowski T, Smilowska K. The reality of informed consent: empirical studies on patient comprehension-systematic review. Trials. 2021; 22(1):57. doi:10.1186/s13063-020-04969-w
7. Heston TF, Pahang JA. Moral injury and the four pillars of bioethics. F1000Res. 2019; 8:1193. doi:10.12688/f1000research.19754.4
8. Robertson K. Active listening: more than just paying attention. Aust. Fam. Physician. 2005; 34(12):1053-5.
9. Shand-McIntosh et al. (2011). Listening Education. Issue 2, (Vol. 3): pp 5- 15.
10. Johnston MK, Weaver JB, Watson KB, at al Listening Styles: Biological or Psychological Differences? January 2000. International Journal of Listening 14(1):32-46; DOI:10.1080/10904018.2000.10499034
11. Brittin, M. (2005). Keys to improving your listening skills. Family Practice Management, 12, 68

**Code 8101 refers to a Full Mouth Examination, charting, and treatment planning. It states that no further examination fees shall be chargeable until the treatment plan resulting from this consultation is completed with the exception of code 8102. This includes the issuing of a prescription where only medication is prescribed. Code 8104 refers to a consultation for a specific problem and not to a full mouth examination, charting, and treatment planning. Except in those cases where the fee is determined "by arrangement", the fee for the rendering of a service that is not listed in this schedule shall be based on the fee in respect of a comparable service that is listed therein and Rule 002 must be indicated together with the tariff code.*

G-ænial™ A'CHORD

Advanced universal composite with unishade simplicity

SCAN HERE
for more information
about G-ænial® A'CHORD

Simplified unishades
Five core shades that replace all 16 classic VITA shades with ease

Beautiful aesthetics
Natural fluorescence and high gloss level and gloss retention

Revolutionary technology
Proprietary filler and coating, high stain and wear resistance and excellent radiopacity

Additional shades for extreme challenges
Cervical, opaque, enamel and bleach shades available as well

Optimal handling
Easy to manipulate with an instrument or a brush

Smile for the World
Since 1921
100 years of Quality in Dental

GC UNITED KINGDOM Ltd.
Liesl Stoffberg
Mobile: +27 76 904 9055
liesl.stoffbergmelonas@gc.dental
https://uk.gceurope.com
Regions: Coastal Territory including Cape Town and KwaZulu-Natal

GC UNITED KINGDOM Ltd.
Heleen de Bruin
Mobile: +27 82 493 1314
heleen.debruin@gc.dental
https://uk.gceurope.com
Regions: Inland Territory including Gauteng, Mpumalanga, Limpopo, North West, and the Free State

CPD questionnaire

Tissue induction

1. **Which statement is CORRECT. The transforming growth factor-β3 (TGF-β3) induces bone formation in primates primarily through which one of the following mechanisms?**
   - A. By forming direct mineral deposits onto existing collagen fibers
   - B. By converting muscle cells directly into osteoblasts
   - C. By priming mesenchymal cells to express bone morphogenetic proteins (BMPs), initiating bone formation
   - D. By increasing calcium uptake in skeletal tissues
   - E. By removing inhibitory molecules from periodontal tissues

2. **Which statement is CORRECT. The term "bone: formation by autoinduction" refers to which one of the following?**
   - A. The manual implantation of pre-formed bone grafts to initiate bone regeneration
   - B. The direct transformation of cementoblasts into osteoblasts in situ
   - C. A mechanical process of bone formation without molecular mediators
   - D. The capacity of specific proteins to initiate bone formation where there is no bone, through developmental processes
   - E. The stimulation of periosteal cells by physical pressure to form bone

Radiation protection and compliance with radiation safety standards by dental professionals and radiographers in rural Limpopo Province, South Africa: a cross-sectional study

3. **Select the CORRECT answer. What is the maximum permissible dose of ionizing radiation exposure for radiation workers such as dental health professionals in South Africa?**
   - A. 20 mSv per year
   - B. 50 mSv per year
   - C. 10 mSv per year
   - D. 100 mSv over a lifetime

4. **Choose the CORRECT option. Which of the following patients would require special considerations for radiation exposure and protection in dental radiography?**
   - A. Adults males with no underlying medical conditions
   - B. Pregnant patients during the first trimester
   - C. Elderly patients with osteoporosis and history of falls
   - D. Paediatric patients undergoing surgery

5. **Which statement is CORRECT. What is the ALARA principle in radiation compliance and safety?**
   - A. Appropriate Limitation and Radiation Adjustment
   - B. As Little As Radiation Allows
   - C. As Low As Reasonably Achievable
   - D. A Limited Amount of Radiographic Allowance

6. **Choose the CORRECT regulation. Which of the following regulations is most relevant for radiation safety in dental practice?**
   - A. WHO Framework Convention on Tobacco Control
   - B. International Commission on Radiological Protection (ICRP) recommendations.
   - C. The Kyoto Protocol.
   - D. OSHA Guidelines on Workplace Radiation Safety and Protocol.

7. **Select the CORRECT answer. Which of the following is the most effective method to minimize radiation exposure to dental health professionals?**
   - A. Standing much closer to the x-ray machine during operation
   - B. Wearing thick latex gloves while operating the x-ray machine
   - C. Using a wall or lead-lined barrier for shielding
   - D. Reducing the duration of the x-ray exposure for each patient.

The effects of two forms of commercially available denture adhesives on the growth of Candida albicans in vitro

8. **Which option is CORRECT. What is a potential consequence of using denture adhesives that promote Candida albicans growth?**
   - A. Increased risk of developing denture stomatitis
   - B. Reduced adhesion of the dentures
   - C. Improved antimicrobial activity in the oral cavity
   - D. Lower rates of oral plaque formation growth?

9. **Which of the following options is CORRECT. What role does the pH of denture adhesives play in the growth of Candida albicans?**
   - A. A higher pH may promote Candida albicans growth
   - B. A lower pH may inhibit Candida albicans growth
   - C. pH has no significant impact on fungal growth
   - D. pH only affects bacterial, not fungal, growth

10. **Select the CORRECT statement. What are the effects of denture adhesives on biofilm formation?**
    - A. The adhesive will prevent biofilm formation completely
    - B. The adhesive may either promote or inhibit biofilm formation depending on its chemical composition
    - C. Denture adhesives have no effect on biofilm formation
    - D. Denture adhesives will always increase the biofilm formation of Candida albicans

11. **Select the CORRECT answer. The following factor/s may influence the growth of Candida albicans?**
    - A. The temperature of the experiment
    - B. The layer thickness of the denture adhesive applied
    - C. The concentration of active ingredients in the denture adhesive
    - D. All of the above

Management of dental caries in an HIV-infected child: a case report

12. Which statement is CORRECT. Vertical transmission of HIV refers to which one of the following?
A. Transmission through sexual contact
B. Transmission through use of intravenous needles
C. Transmission through blood transfusion
D. Transmission from mother-to-child
E. Transmission through infected fluids including saliva

13. Choose the CORRECT option. Oral manifestations of HIV include which one of the following?
A. Linear gingival erythema
B. Black hairy tongue
C. Snail-track ulcers
D. Vesicles of HSV-1
E. Tetracycline discoloration of teeth

14. Which option is CORRECT. The clinical severity and progression of HIV can be measured by which one of the following?
A. White cell count
B. CRP levels
C. CD8 T-cell count
D. CD4 T-cell count
E. Elevated ESR

15. Select the CORRECT answer. Progression of HIV infection to AIDS is the result of which of the following?
A. Re-infection with HIV
B. Overwhelming TB infection
C. Recurrent Aphthous ulcers
D. Non-compliance of HAART
E. HPV infection in younger patients

Evidence-based Dentistry: What's new for the clinician – summaries of recently published papers

16. Select the CORRECT answer. In the Sihna et study, the data used for the study can best be described as
A. Primary data
B. Secondary data
C. Tertiary Data
D. Health data

17. Which option is CORRECT. How many cohorts were used to collect data from in the Sinha et al study?
A. One
B. Two
C. Three
D. Four

18. Choose the CORRECT answer. In the Sinha et al study, how many participants were recruited to the MUPS cohort?
A. 28
B. 33
C. 44
D. 52

19. Choose the CORRECT answer. In the Sinha et al study, how many participants completed the questionnaire (Q1) in the healthy cohort group?
A. 28
B. 25
C. 19
D. 6

20. Which answer is CORRECT. In the Sinha et al study, how many participants were included in the amalgam cohort?
A. 5
B. 32
C. 37
D. 59

Ethics: Listening: The most valuable procedure you won't charge for

21. Choose the CORRECT option. Effective listening involves
A. Actively paying attention to a patient's spoken word
B. Attempting to understand and interpret a patient's desires
C. Carrying out work that the patient has specifically requested
D. Only a) and b) above
E. All of the above

22. Select the CORRECT option. Which of the following are essential for effective communication?
A. Accurate record keeping
B. Not charging for extra time spent listening
C. Feeling empathy towards a patient's complaint
D. All of the above
E. Only b) and c) above

23. Which of the following is CORRECT. Respect for autonomy refers to:
A. Acknowledging a patient's feelings
B. Preventing disparities in healthcare provision
C. Building trust between a patient and their doctor
D. Allowing patient's to make decisions about their own treatment
E. All of the above

24. Which answer is CORRECT. According to Barker and Watson, critical listening involves:
A. Interpreting the message behind the words
B. Analysing what is being said
C. Not forming an opinion about what is being said
D. Understanding a patient's point of view
E. Internalizing the spoken words

25. Select the CORRECT answer. Good listening skills also include:
A. Showing respect for patients
B. Observing body language
C. Eliminating surrounding distractions
D. All of the above
E. Only b) and c) above

www.sada.co.za / SADJ Vol. 80 No.1

Instructions to authors

THE SOUTH AFRICAN DENTAL JOURNAL
SADJ
SADA THE SOUTH AFRICAN DENTAL ASSOCIATION

Thank you for considering the submission of your work to the Journal for possible publication. We welcome papers which may be Original Research, Clinical Review, Case Reports, Clinical Communications, Letters or Notes.

The South African Dental Journal (SADJ) is a peer reviewed, Open Access Journal, published by the South African Dental Association (SADA). The Journal primarily carries research articles which reflect oral and general health issues in Africa but also publishes papers covering the widest consideration of all health sciences. In addition, items of specific relevance to members of the Association find dissemination through the Journal. The Journal is published ten times each year in electronic format. Hard copy is available by arrangement.

We shall be obliged if your submission is prepared respecting all the details listed in these Instructions. This facilitates our process and ensures more rapid responses to you. Please use and submit the Checklist for Authors suplied on page 645 for confirmation. Thank you.

Submission Enquiries

The Editorial Assistant Mr Dumi Ngoepe
Email addresses: sadj@sada.co.za/editor@sada.co.za
For submission instructions kindly refer to the "Submission instructions" page.

Language

All articles must be submitted in English. Spelling should be in accord with the Shorter Oxford English Dictionary.
All articles must be submitted in English. Spelling should be in accord with the Shorter Oxford English Dictionary.

Clinical Research

Articles should adhere to the protocols of the Helsinki Declaration: (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical research-involving-hu-man-subjects/.

Clinical Trials

Clinical trials should conform to the Consort Statement (Consolidated Statements of Reporting Trials) and Reviews to the PRISMA checklist (Preferred Reporting Items for Systematic Reviews and Meta Analyses) (http://www.equator-network.org).

Authors

Authors should meet the criteria for authorship as in the documents of the International Committee of Medical Journal Editors (ICMJE):

1. Substantial contributions to the conception or design of the work or the acquisition, analysis or interpretation of data for the work, AND
2. Drafting the work or revising it critically for important intellec-tual content, AND
3. Final approval of the version to be published, AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (www.icmje.org).
5. The front page of the manuscript should list the title of the article, the author's(s') name(s), and their qualification(s), affiliations and positions held, telephone and fax numbers and address(es), including Email address(es), if available. It is especially important that details of the Corresponding Author should be clearly stated.
6. Please submit on the front page a list of up to eight Keywords.
7. In the case of multiple authors, the role played and the respective contribution made by each should be recorded. For example: "Principal Researcher- 40%, Writing Article- 30%, Tissue Analysis- 20%, Microscopic Examination- 10%", etc.
8. A recent requirement is that authors should be registered with ORCID. This is a number registering you as an Open Researcher and Contributor. Go to the ORCID website home page at https://orcid.org/ and follow the Three Easy Steps indicated in green. Please submit the ORCID number with your author details.

Title

To be kept as brief, clear and unambiguous as possible.

Abstract

The abstract shall consist of not more than 200 words.
For research articles, the summary should be structured under the following headings: Introduction, Aims and Objectives, Design, Methods, Results and Conclusions. Do not include references in the Abstract.

Text

- Articles should be clear and concise.
- Text should be typed in Times New Roman font, size 11; double-spaced with a 3 cm. margin on the sides, top and bottom. Each page must be clearly numbered.
- Please include electronic numbering of lines throughout the document.
- Tables should be clearly identified, using Roman numerals ie. Table I, Table II etc.
- Authors are requested to note and adhere to the current style of the Journal particularly with respect to paragraph settings and headings.

Length of the article

In general, papers should be between 4000 and 5000 words, although this is flexible. The Editor reserves the right to edit the length of an article in conjunction with the author(s) and SADJ reserves the right to charge for excess/additional pages. The first four pages of original research papers published in the SADJ will be free of charge after which a charge of R500 per page or part thereof wil l be levied.

Illustrations/graphics/photographs

- Illustrations/graphics/photographs must be appropriate to the content of the manuscript.
- Digital images with a DPI of at least 300 should be supplied. Photocopies and pdf. files of photographs are not acceptable.
- **Please note:** Figures should be included in the text and sent separately in jpg. format.
- The Figure numbers must be in Arabic numerals and clearly identified for each illustration, graphic or photograph. Please remember to record Figure numbers in the text.
- **Permission:** Where any text, tables or illustrations are used

from previously published work, permission must first be obtained from the holder of copyright and a copy of the agreement must be submitted with the article. Suitable acknowledgement must be recorded in the article.

Continuing Professional Development
Please supply 4-5 Multiple-choice Questions (MCQ's) with 4 or 5 options per question related to your article. Questions must have only one correct answer, and indicate this correct answer clearly.

References

- References should be set out in the Vancouver style and only approved abbreviations of journal titles should be used (consult the List of Journals Indexed in Index Medicus for these details at: http://www.nlm.nih.gov/tsd/serials/lji.html).
- References should be inserted seriatim in the text using superscript numbers and should be listed at the end of the article in numerical order.
- A reference in the text should appear as indicated: "...as the results of a previous study showed.23"
- Where there are several papers referenced, the superscript numbers would appear as: "...previous studies have shown. [3,5,7,9-12,14]"
- Do not list the references alphabetically.
- It is the author's responsibility to verify each reference from its original source. Please note that an article may be rejected if the referencing is inaccurate.
- Names and initials of all authors should be given unless there are more than six, in which case the first three names should be given, followed by *et al.,*. First and last page numbers should be given. Where it is applicable the page numbers should be abbreviated by omitting redundant numbers eg. pages 456 to 478 is recorded as 456-78, and 456 to 459 as 456-9, but 398 to 401 is recorded as 398-401.
- Notice that volume numbers are not given in bold, authors are not linked by 'and' or '&', and the year of publication appears after the name of the journal. No item should appear in italics except for foreign terms, eg *in vivo.*

Journal references should appear thus:

Smith NC, Haines A. The role of the dentist in public health promotion. Br Dent J. 1983; 298: 249-51.

Book references should be set out as follows:

Terblanche N. Principles of Periodontology, 4th ed. London: Butterworth, 1985: 96-101.

Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA, Smith RT, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974: 457-72.

Manuscripts accepted but not yet published may be included as references followed by the words 'in press'.

'Unpublished observations' and 'personal communications' may be cited in the text but not in the reference list.

Declaration

All sources of funding, possible financial interest/s or incentives in products or services mentioned in the article must be disclosed. Authors are kindly requested to read and sign the attached declaration on page 450.

No articles that have been published previously, or that are currently being considered for publication elsewhere, will be accepted. Authors are kindly requested to verify that their article complies with this condition.

Ethics

Where relevant, authors should indicate whether their research has been approved by the Ethics Committee of their Institution or by other research ethics committees.

Conflict of interest

Authors must disclose their involvement with any company either owned by them or from which they have received a grant or remuneration or with which they have an association, and must declare any other personal interest they may have which would constitute a Conflict of Interest. These may include per- sonal relationships, academic competition, or intellectual beliefs. Should there be no applicable Conflicts of Interest this should also be so stated. The statement will be included at the end of the text.

Copyright
The South African Dental Journal is a peer reviewed, Open Access Journal, adhering to the Budapest Open Access Initiative: "By 'open access' to this literature, we mean its free availability on the public internet, permitting any users to read, download, copy, distribute, print, search, or link to the full texts of these articles, crawl them for indexing, pass them as data to software, or use them for any other lawful purpose, without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. The only constraint on reproduction and distribution, and the only role for copyright in this domain, should be to give authors control over the integrity of their work and the right to be properly acknowledged and cited."
https://access.okfn.org/definition/index.html

The Managing Editor reserves the right to decline articles, photographs or illustrations where products or services are mentioned that are not appropriate.

Submission

The paper should be submitted in one file including all Tables and Figures and their accompanying Legends Figures should also be submitted separately file in JPEG. format.

Please submit the paper in electronic format in Word along with separate Figures in JPEG. format to: sadj@sada.co.za and to neil.wood@smu.co.za, accompanied by a covering letter and the Declaration on page 450 signed by the author(s).

Galley proofs

No galley proofs will be provided please ensure that all submssions are correct after the review revision submission.

Editorial Policy

Authors may also wish to refer to the Editorial Policy of the SADJ available on the SADA website.

Enquiries
Enquiries regarding Journal matters can be directed to Mr Dumi Ngoepe, Editorial Assistant, at SADA headquarters on: Tel: +27 (0)11 484 5288
Email: sadj@sada.co.za

www.sada.co.za / SADJ Vol. 80 No.1

Submission Instructions

THE SOUTH AFRICAN DENTAL JOURNAL
SADJ
SADA
THE SOUTH AFRICAN DENTAL ASSOCIATION

To submit a new paper to SADJ, do as follows:

1 If this is your first time using this platform, please Register by completing the online form (once off):

Link:
https://journals.assaf.org.za/index.php/sadj/user/register

2 Once registered, make sure you are still logged in (Login every time you want to submit a new paper):

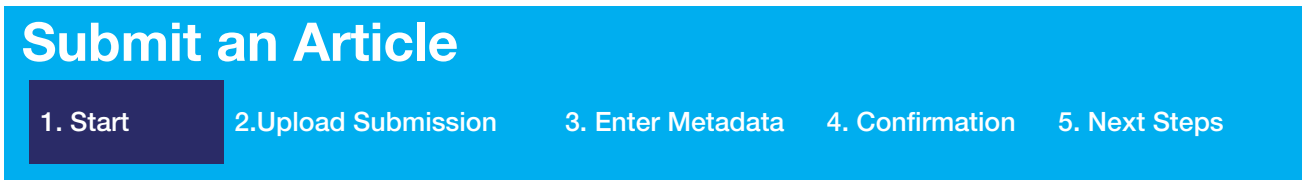
Link:
https://journals.assaf.org.za/index.php/sadj/login

3 Click on Make a new submission. on the righthand side of the page. Please ensure that the article is anonymized/No names on the submitted article

4 Familiarize yourself with the Author Guidelines.

Then click on Make a new submission.

5 Follow the 5 steps as part of the wizard, until the last screen. You will receive a confirmation email once successfully submitted, and the Journal Manager/Editor will be notified.



If you are struggling on how to load/submit kindly email Dumi Ngoepe at SADA on sadj@sada.co.za for assistance.

NOTE: Incomplete submissions will be returned to the authors and will not be considered for peer-review until complete

Declaration by Author/s

THE SOUTH AFRICAN DENTAL JOURNAL
SADJ
SADA
THE SOUTH AFRICAN DENTAL ASSOCIATION

TITLE

.....

.....

.....

AUTHOR/S

.....

www.sada.co.za / SADJ Vol. 80 No.1 AUTHOR GUIDELINES < 231

.....

I/We, the undersigned confirm and declare that:

1 This manuscript is my/our original work and I am/we are the owner/s of this manuscript and possess rights of copyright.

2 I/we confirm that this manuscript has not been published previously and it is not currently considered for publication elsewhere. Has this article been submitted to any other journal and if so, has it been rejected?

YES ☐ NO ☐

3 For no consideration or royalty, I/we hereby assign, transfer and make over to SADA all the rights of copyright, which have or will come into existence in relation to this manuscript.

4 I/we waive in favour of SADA or any successors in title any moral rights which may be vested in me/us.

5 The manuscript does not constitute an infringement of any copyright and I/we indemnify SADA against all loss or damage, from any cause arising which SADA may sustain as a result of having been granted copy-rights to the manuscript.

6 The research has been approved by the Ethics Committee of my/our institution/s or the Ethics Commit-tee/s of other accredited research facilities.

7 I/we have disclosed in my/our Acknowledgments all sources of funding, possible financial interest/s or incen-tives in products or services mentioned in the paper.

..... Initial(s) and Surname	..... Signature	..... Date
..... Initial(s) and Surname	..... Signature	..... Date
..... Initial(s) and Surname	..... Signature	..... Date
..... Initial(s) and Surname	..... Signature	..... Date
..... Initial(s) and Surname	..... Signature	..... Date
..... Initial(s) and Surname	..... Signature	..... Date
..... Initial(s) and Surname	..... Signature	..... Date

www.sada.co.za / SADJ Vol. 80 No.1

Author's Checklist

1	Have you read the Instructions to Authors?
2	Are you submitting electronically?
3	Have you provided all author information including first names, affiliations, qualifications, positions held, Department and Institution, ORCID number, contact details?
4	Is the first author under the age of 35 on submission of the article?
5	Have you provided all details of the Communicating Author?
6	Have you submitted questions for the CPD section? (four or five multiple choice, one correct answer)?
7	Have you submitted details of the contribution of each author... can be percentage or descriptive... or both?
8	Is the first author under the age of 35 on submission of the article?
9	Have you provided all details of the Communicating Author?
10	Have you submitted questions for the CPD section? (four or five multiple choice, one correct answer)?
11	Are the references quoted according to Journal policy, both in the text and in the list of references?
12	Have all authors signed the Letter of Submission?

BRILLIANT
BRUSH HEADS

CURAPROX

HYDROSONIC PRO Keeps your mouth in shape and your body healthy.

Download Curaprox app now

Available on the

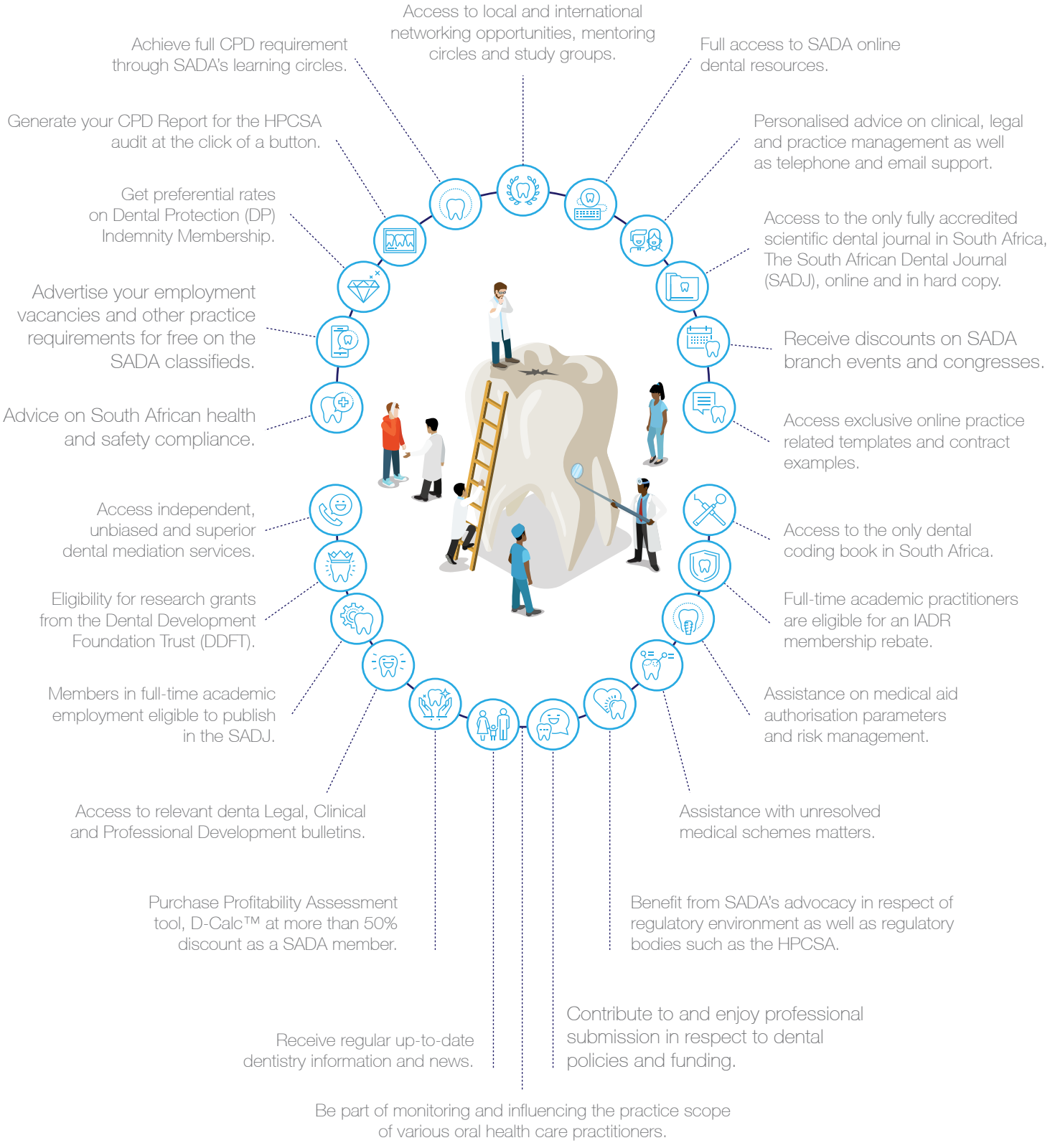
GET IT ON

SWISS PREMIUM

BENEFITS OF JOINING SADA

SADA supports its members throughout their time in the profession - from their time as students in the field, straight through their professional careers, and into retirement.

Our members benefit from the below advantages, amongst others.



SADA
The South African Dental Association **(SADA)** NPC

visit our website at www.sada.co.za for more information.