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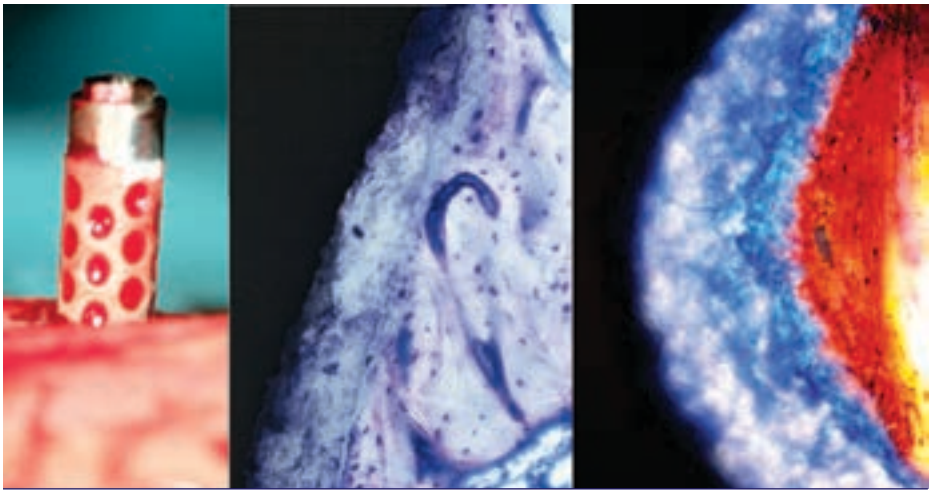
Dracula:
In 1897 Bram Stoker published his book Dracula... forever creating the horror of vampires! Strangely, the excessive fang like canines were not a feature of early Dracula films, but made their debut in the 1950's... now they are a regular and frightening dental characteristic of all media productions on the vampire.



Inductive surface' geometries: Beyond morphogens and stem cells
Hydroxyapatite-coated titanium implant with concavities across the substratum inserted into bone; tight osteointegration against the sintered crystalline hydroxyapatite; when intramuscularly implanted, the concavities spontaneously initiate the induction of bone formation: "The concavity: the shape of life"
For further details, see the article by Ripamonti and Duarte on page 421

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Our Front Cover for this Issue…

Teeth have on occasion been central to historical, social and humorous events. The **Front Cover** in 2019 will reflect some of these **Famous Teeth.**

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In 1897 Bram Stoker published his book Dracula... forever creating the horror of vampires! Strangely, the excessive fang like canines were not a feature of early Dracula films, but made their debut in the 1950's... now they are a regular and frightening dental characteristic of all media productions on the vampire.

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At, on and below the surface

SADJ September 2019, Vol. 74 No. 8 p415

WG Evans

Featuring at the Rugby World Cup are the variety of mouthguards… off-beat designs, some have left/right side different colours, some even appear to include images of frightening teeth… but the most critical factor is their constant presence... a conscientious use.

An outstanding example to every rugby player and enthusiast. In 2014, an Australian study reported that the prevalence of orofacial injury amongst rugby union players was 65%, and the most common injury was laceration to soft tissues in and around the mouth.[1]

Dental injuries occurred in 42% of the cases, with tooth loss being suffered in 35% of the instances. In New Zealand, referees have been granted the power of ensuring that every player must wear a mouthguard... and there has been a 43% reduction in dental injuries.[2]

South African World Cup players are wearing specially designed mouthguards which not only offer the desired protection but are also equipped with sensors which record the impact sustained when there is a clash of hard surfaces… heads with heads or heads with elbows or boot and heads… or indeed, heads against the hard surface of the field!

Any player who suffers such an impact and is then referred off the field for assessment may now be monitored and the extent of the impact determined, enabling better judgement on whether the player may returned to action, or more wisely, sent for further medical attention. These technological advances may make a significant contribution to the safety of players... and not only in rugby. An average impact has been measured at 20 to 30 equivalents of gravitational force (Gs) and a particularly hard impact at 40 Gs. Those impacts occur in Football, Hockey, Ice Hockey... the list continues.

The development of the special mouthguards has been conducted in the United Kingdom, with collaboration between Sports and Wellbeing Analytics, Keytree and Swansea University and initial testing was carried out by the Welsh team, the Ospreys.[3]

It may seem a massive jump from the turmoil of rugby to the tranquil pages of this issue... but lets look at the major content, a comprehensive survey of the research work aimed at unravelling some of the intricacies of the growth of bone. The investigations have revealed an intriguing situation… namely that the shape of surfaces … the nano or minute… shape may have an influence on stimulating the process of osteogenesis.

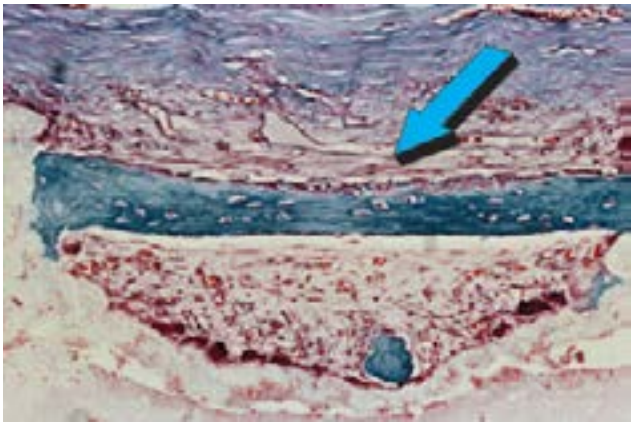

Figure 14 C. (From: Article 1 - Inductive surface' geometries: Beyond morphogens and stem cells)
Collagenous condensations (dark blue arrows) stretching across a concavity of a hydroxyapatite-coated titanium implant 5 days after implantation in the *rectus abdominis* muscle of the non-human primate *Papio ursinus*. The scanning electron macroscopy (SEM) image depicts tractional patterning forces of fibroblastic/myofibroblastic-like cells secreting collagen fibres whilst moving from the edges of the concavities across the concavities of the hydroxyapatite-coated substratum.
(**C**) Transformation of collagenous condensation across concavities into bone matrix 90 days after heterotopic implantation in the *rectus abdominis* muscle of the non-human primate *Papio ursinus*.
(**C**) Decalcified sections cut at 6 µm stained with toluidine blue in 30% ethanol. Original magnification (**B**,**C**) x37.

A plane surface appears to have no or little influence but miniscule variations of the surface result in cellular modifications and cellular activities.

These relevations may have long reaching influences, for they may point the way towards our ability to stimulate or to initiate bone growth... as for example a mandibular fracture sustained in rugby! There remains much to be done... for the results are those determined in animal studies... and have not proven repeatable in humans... as yet!

Are we simply scratching the surface of what may be a momentous discovery... the ability to call at will on bone... and possibly other tissues? Whatever the future, it is most warranted that we look beneath the surface and consider the paper in depth, bringing us up to date with seminal research efforts.

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3. http:/businesstech.co.za/news/enterprise/341853/this-hi-tech-mouthguard-aims-tackle-concussion-risks-in-rugby.

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The new word of mouth - social media in dentistry

SADJ September 2019, Vol. 74 No. 8 p416 - p417

KC Makhubele

We have all heard by now about the Fourth Industrial Revolution (4IR) and sometimes called 4.0. Recently the term has been popularised by the President of the Republic of South Africa, President Cyril Ramaphosa.

Whether for good or bad, we are on the path of a technological revolution that is radically shaping the way in which we relate to the world and to one another. What is 4IR?

The 4IR is characterised by technologies that include robotics, the Internet of things (IOT), artificial intelligence (AI) and big data. While in a manufacturing context these technologies are shaping the 'factories of the future' (a Web of interconnected machines creating products that are pre-programmed, while all the time uploading process data, completely without the involvement of any humans), these same technologies are also impacting most other industries and dentistry is not immune.

That is the big picture. On a smaller scale is the use of social media in dentistry, particularly for Marketing and Public relations purposes. There is no doubt that for decades, the dental community has relied almost exclusively on reputation and word of mouth marketing to help build their practices.

The profession has to learn some form of social media, whether it's Facebooking, Twittering LinkedIn, blogging, video marketing, Youtubing, paid social ads or even podcasts. The reason? That's where the dentistry-specific community is – your patients, colleagues, suppliers and other professionals with whom we seek to collaborate. I can assure you that they are listening, watching and if you are not there, its not you they will remember when they need dental services.

Therefore, if your business doesn't have an online presence, it may be practically invisible to the majority of potential patients in your community.

Social Media is the new word of mouth

While television, radio, and print media still own a lion's share of marketing dollars, word-of-mouth referrals are still the most effective and trustworthy way to acquire new patients. In today's increasingly digital world, that word-of-mouth referral is more likely than ever to come from a social media platform. Have a look at some staggering information, based on various separate studies, about social media advertising:

- 75.3% of people admitted that they purchased a product or service because they saw it on some form of a social media platform.
- 61% percent are likely to trust information posted by providers;
- 42% look for other consumer's reviews of health-related providers, products, services, and experiences;
- 41% are likely to share with providers *via* social media
- 32% review family and friends' experiences;
- 29% look for other patients' experiences with the same disease or condition; and
- 24% watch health-related videos or images posted by patients

Khomi C Makhubele: Chief Executive Officer, SADA

Social Media can help or hurt your practice and your professional career

Now for every opportunity, one has to accept that there are risks which we need to navigate. Social media is meant as a public forum, and it needs to be treated like one or else it may hurt your professional career and or practice. Health Practitioners need to take special care about how they engage in social media.

Their actions might reflect their ability to inspire trust in a general public that requires dependable, quality care and or breach ethical and professional conduct.

Whilst we are at it, let's define advertising for our own purposes: According to the Health Professions Council of South Africa (HPCSA), in relation to any health establishment, orthodox medicine, complementary medicine, medical device, scheduled substance or health-related product or service, "advertising" refers to any written, pictorial, visual or other descriptive matter or verbal statement or reference in respect thereof that appears in a newspaper, magazine, pamphlet, website, social media space or other publications; or it is distributed to members of the public or is brought to the attention of members of the public in any manner.

The intention is to promote the sale of the orthodox medicine, complementary medicine, medical device, scheduled substance or health-related product, or to direct the public to any particular health establishment or health-related service.

Currently there are no clear regulations on the use of social media by health practitioners, but there are key ethical and professional guidelines which one can follow to avoid falling foul of the law.

I intend delivering giving key lectures on Social Media Marketing for the Oral Health Professional in the very near future. Hopefully I shall be able to impart some knowledge that can help you in your professional career and practice.

KC Makhubule,
Chief Executive Officer,
The Dental Association of South Africa.

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CONGRESS AND EXHIBITION

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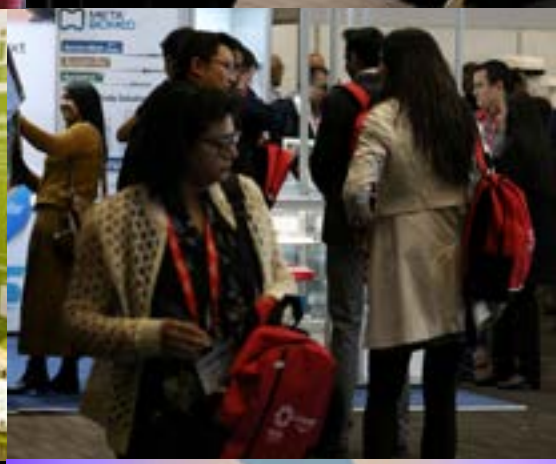
- An excellent **exhibition area**
- Well attended **workshops**
- Phenomenal **guest speakers**
- Exciting **social functions**

A random display of the excited **SADA Dental & Oral Health Congress and Exhibition** that was held at the **Inkosi Albert Luthuli International Convention Centre** in Durban from the **30th of August to the 1st of September 2018**





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Inductive surface geometries: Beyond morphogens and stem cells

SADJ September 2019, Vol. 74 No. 8 p421 - p444

U Ripamonti[1], R Duarte[2]

ABSTRACT

The requirement of biomimetic matrices for bone tissue engineering is now to construct functionalized surfaces with bioactive nanotopographical geometric cues. Functionalized surfaces prime stem cells to set into motion gene expression, synthesis and embedding of gene products within the functionalized surfaces.

Embedded proteins initiate the spontaneous induction of bone formation without the exogenous application of the soluble osteogenic molecular signals of the transforming growth factor-β (TGF-β) supergene family.

Concavities cut by osteoclastogenesis along the surfaces of calcium phosphate-based biomimetic matrices are the driving morphogenetic cues that initiate the induction of bone formation biomimetizing the remodelling cycle of the cortico-cancellous bone. Geometric recognition activates mechano-transducer(s) that generate cytoskeletal motors that deforms the nuclear geometry, ultimately regulating stem cell differentiation.

Osteoclastogenesis drives nano-patterned geometric topographies releasing Ca^{++} that induce angiogenesis and cellular differentiation. Expressed and secreted bone morphogenetic proteins (BMPs) are embedded into the implanted substrata initiating the induction of bone formation as a secondary response.

Cell attachment to geometrically modified titania' surfaces is controlled by Rho-associated kinase (ROCK) and focal adhesion kinase (FAK) up regulating the transcription factor RUNX2 controlling osteoblasts differentiation and BMPs expression. Geometry is the unifying biological theme of "finding meaning in complexity", a theme that sets morphogenetic inductive cues *via* nanotopographical surface modifications.

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2. **R Duarte:** Secondary author - 20%

INTRODUCTION AND DEFINITIONS:
Osteoinduction and osteoinductive biomaterials

The creative concept of this re-defining review on functionalized and osteoinductive surfaces of biomaterials arose during the preparation of a dissertation which focused on biomimetic matrices that drive the induction of bone formation.[1]

The associated revision of several papers warranted a review of a multitude of digital images and histological sections which had been generated on the phenomenon of the spontaneous and intrinsic osteoinductive activity of certain calcium phosphate-based macroporous bio-mimetic matrices.[2,3]

This revision and re-evaluation has led to the desire to focus on those functionalized surfaces which provide inductive microenvironments, set by the geometric configuration of a variety of treated or untreated surfaces of biomimetic matrices.

Our extensive revision together with the re-evaluation of seminal contributions on the induction of tissue morphogenesis by geometrically modified surfaces, emphasized the Leading Edge Editorial in Cell "*Pulling It All Together*".[4]

The extraordinary research on molecular cellular pathways and "*seemingly infinite biological networks*" has suggested a novel theme in future molecular and cellular studies: "*Finding Meaning in Complexity*".[4]

Similarly, since the last Century, a plethora of manuscripts has reached the pages of several Journals, reporting on the power of surface topography controlling cellular differentiation and gene expression mechanisms. Hence, this review wishes to present the unifying biological theme of geometry in leading the science of surfaces to construct biomimetic matrices that *per se*, in their own right, initiate the spontaneous induction of bone formation.

Geometry is indeed the unifying theme of "Finding Meaning in Complexity", a theme that brings together geometric cues that functionalize biomimetic surfaces.[4]

This review cannot proceed without unfolding the extraordinary uniqueness of the induction of bone formation so lucidly presented by MR Urist in Science as "*Bone: formation by autoinduction*"[5] and by AH Reddi in the Proceedings National Academy of Science as "*Biochemical sequences in the transformation of normal*

fibroblasts in adolescent rats".[6] Both contributions describe the powerful "osteogenic activity" inherent within allogeneic, demineralized bone matrices, raising the fact that morphogens, defined by Turing as "*forms generating substances*"[7], must exist within the implanted matrices.

In addition, their work proposes the development of morphogenetic gradients as being ultimately responsible for the induction of bone formation.[7-12] Developmentally, morphogens are released from a localized group of embryonic cells or "*organizing centers*"[11-13]which induce cellular differentiation and morphogenesis.[10]

Research in the last century has shown that morphogens are at the very crux of tissue induction and morphogenesis in preclinical and clinical contexts.[14-18]

Whether morphogens' gradients self-organize specific morphogenetic patterns according to different diffusion properties or gradients[7] or "influence each other in triggering the spontaneous emergence of stable, long-range patterns of morphogen activity"[19], only "Morpheus unbound"[19] can initiate the cascade of pattern formation and the induction of tissue morphogenesis.[19,20]

This review wishes to re-discover critical references on the role of geometry on tissue transformation and on the induction of bone formation.[21-24] Such references and images set the theme of this communication that wishes to convey the proposal that it is surface geometry, at the macroscopic and microscopic, nano-topographic, levels, that establishes the molecular and cellular parameters for the differentiation of various microenvironments that initiate the ripple-like cascade of tissue induction and morphogenesis.[25-27]

The induction of tissue morphogenesis, and in this context, the induction of bone formation, is thus not the sole prerogative of the soluble osteogenic molecular signals. It is the result of a connubium of the supramolecular assembly of the soluble molecular signals together with the insoluble signal or substratum that ultimately controls and modulates the powerful inductive activity of the morphogenetic soluble signals.[20-23,28-30]

Osteoinductive biomaterials

How can we define an "*osteoinductive biomaterial*"? The term induction, firstly used by Spemann,[13] was later re-deployed by Levander, Moss, Urist and Reddi.[17]

The latter also introduced the term "*transformability*" and "*transformation*" of "*normal fibroblasts in adolescent rats*".[6] These transformative events may be set into motion by morphogenetic gradients available to responding cells after implantation of acid/alcohol extracted biological matrices including diaphyseal bone matrices.[16-18,27]

A classic paper that highlighted the emerging concept of "*bioactivity*" was published by Hulbert et al.[31], recognizing the critical role of porosity in biomaterials' design and incorporation.[31]

This contribution showed that macroporous hydroxyapatites constructed with an interconnecting porous network allowed for osteo-conduction and that macroporous hydroxyapatites with pores sizes greater than 150 µm were seen to host the formation of osteonic remodeling bone.[31]

The paper further highlighted that bone growth into the ceramic structure "*was analogous to the infiltration of a cancellous bone structure by new compact Haversian bone*", thus suggesting biomimetism of the invading trabecular bone structure within the implanted calcium phosphate-based matrix.[31]

An inductive biomaterial is thus a biomaterial that together with (or in combination with) soluble osteogenic molecular signals initiates the ripple-like cascade of tissue induction and bone morphogenesis.[16-18]

The acid test in defining an osteoinductive biomaterial is to demonstrate its osteoinductivity in heterotopic extra skeletal sites, either intramuscularly[5] or subcutaneously.[6] This avoids the ambiguities of the orthotopic site, where bone formation occurs from the viable bone at the interfaces of treated defects. [5,6,16-18]

The classic work of Levander, Moss, Huggins, Urist, and Reddi[27,29,30] culminated in the biological understanding that intact bone and dentin demineralized matrices are endowed with the striking prerogative of the *de novo* initiating endochondral bone formation when implanted in heterotopic intramuscular or subcutaneous sites of rodents and lagomorphs[5,6,16-18,27-30]

The question now arises whether a biomaterial without the exogenous application of osteogenic soluble molecular signals can, in its own right, initiate the cascade of bone differentiation by induction, even when implanted in heterotopic extra skeletal sites where there is no bone? Reporting *verbatim* the introductory statements of a seminal contribution to Nature[32], Gustav Levander asks whether tissue regeneration "*develops merely from pre-existing cells, which grow out from the original cell-units, or may also some other material be imagined as participating in the occurrence of regeneration?*"

Levander further states that "*it has simply been taken for granted that when bone formation is obtained, this must have emanated from transferred bone cells*".[32] Using non-vital alcohol extracts of bone tissue implanted intramuscularly in animal models, Levander reported that newly formed bone initiated by induction "*within the fully developed organism under the influence of an extractable substance*".[32]

Levander concludes that "*the circumstance that a tissue is able to affect another in a specifically differentiating direction I have termed induction, a term borrowed by Spemann and his school at the turn of the Century*".[13,32]

Such "an extractable substance" marks the beginning of the understanding of morphogenetic substances or morphogens that set the stage of tissue induction and morphogenesis.[5-7,14-18] The concluding statements

of the Levander communication in Nature are wrought to a powerful biological platform that seals the tissue-engineering paradigm by stating, "regeneration of tissue is, in other words, a repetition of embryonal development".[32]

The same "extractable substance" is active both during the embryonal differentiation and during post-fetal growth.[32] On this great truth is deeply rooted the dream of the tissue engineering paradigm: morphogens exploited in embryonic development can be re-deployed to mechanistically initiate post-natal tissue induction.[14-18,27,30]

Bone and dentine, as applies to all mineralized matrices, are in the solid state, yet have compartmentalized soluble signals interacting with responding cells. In an incisive mini review in Cell,[33] Reddi states that bone "*is in both a soluble and a solid state that is regulated by signals in solutions interacting with the extracellular matrix*", and that "*a beautiful example of the interface of signals and extracellular matrix is the human skeleton*"[33] and further that the "*skeleton is a giant molecular machine*" [AH Reddi, personal communication 2014].

Identification of putative morphogens within the bone matrix was further complicated by the fact that small quantities of proteins are bound to both the organic and inorganic components of the extracellular matrix of bone.[16-18,34] Solubilization of putative osteogenic soluble molecular signals was classically achieved by the chaotropic extraction of the intact demineralized extracellular matrix of bone (A chaotropic agent is a molecule in water solution that can disrupt hydrogen bondings).[16-18,35,36]

The chaotropic extraction did result in the isolation of soluble molecular signals and an insoluble signal, or substratum, the insoluble collagenous bone matrix (ICBM) obtained after the dissociative extraction of the extra cellular matrix of bone.[16-18,34,35]

The chaotropic dissociative extraction of 4M guanidinium hydrochloride (Gdn-HCl) or 6 M Urea unlocked the problem of the "bone matrix in the solid state".[33] The solubilized proteins indicated that the extracellular matrix of bone is a large repository of morphogenetic signals.[14-18]

Solubilized signals or proteins were thus purified to homogeneity by newly designed chromatographic procedures and cloned.[15-18;37-39] The expression of the recombinant human morphogenetic proteins (BMPs) showed homology with several other inductive and differentiating morphogens of the transforming growth factor-β (TGF-β) supergene family.[15-18;37-39]

The grand experimentation of Reddi's group defined the operational reconstitution of the purified soluble osteogenetic molecular signals with an insoluble signal or substratum,[33-37] acting as a carrier to deliver the biological activity of the purified osteogenic proteins.

The inactive and insoluble collagenous bone matrix after chaotropic dissociative extraction when "*reconstituted*"[35] with the solubilized osteogenic molecular signals, restored the bone induction cascade,[35] propelling the "*bone induction principle*"[40] in clinical contexts.[16-18]

The systematic experimentation of Reddi and co-workers on the "bone induction principle"[40] established the critical role of the carrier substratum as a delivery system for the biological activity of the extracted proteins.[14-18,36,37]

Since the early nineties, we have reported a novel and exciting concept of tissue engineering and regenerative medicine: the induction of bone formation upon the implantation of intrinsically osteoinductive biomaterials.

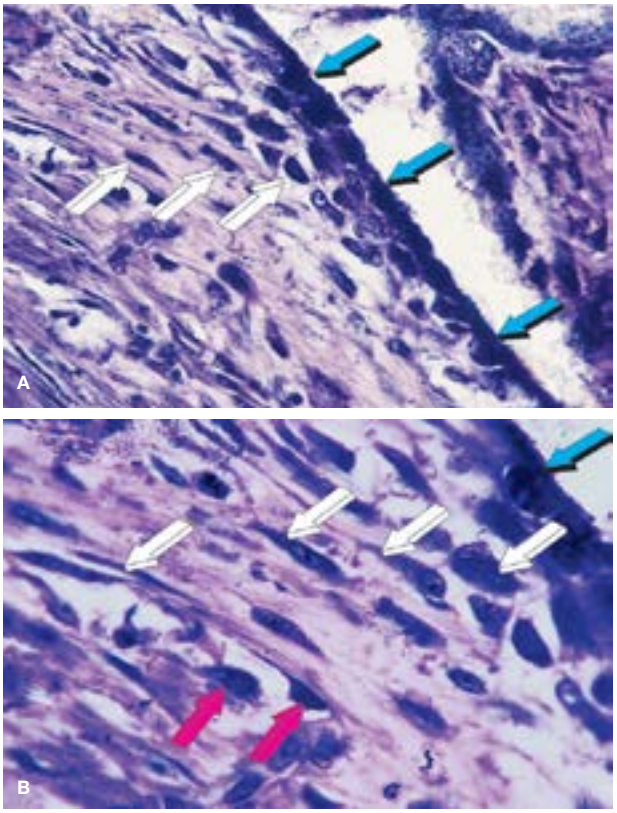

Figure 1. Coral-derived constructs[2,3,41] were implanted in the *rectus abdominis* muscle of adult male Chacma baboons *Papio ursinus* and generated tissues harvested on day 30, 60 and 90 for morphological and histomorphometrical analyses including alkaline phosphatase staining and immunolocalization studies for laminin, type I and IV collagens. Undecalcified specimen blocks were embedded in Historesin (LKB Bromma Sweden) as described.[41]
A. Undecalcified section cut on day 60: differentiation of cellular elements within the invaded macroporous spaces at the hydroxyapatite interface directly onto the hydroxyapatite substratum (light blue arrows). Differentiating cells, interpreted as osteoblasts, are directly differentiating against the hydroxyapatite surface. Differentiating cells are continuously provided by migrating osteoprogenitors from the vascular compartment (white arrows).
B. Nucleation and differentiation of osteoblasts at the hydroxyapatite interface (light blue arrow). The differentiating or "transformation microenvironment" at the hydroxyapatite interface is continuously fed by responding migrating osteoprogenitors cells (white arrows) that migrate from the vascular compartment towards the osteogenic compartment of the transformation microenvironment.
Note the different **status** of cellular differentiation as seen morphologically according to the relative migration across morphogenetic gradients established by the transforming microenvironments at the substratum' interface. Large cells with hypertrophic nuclei (magenta arrows) differentiate attached to the basement membranes of the invading capillaries providing thus, as per Trueta' definition[54], osteogenetic vessels for the constant supply of responding cells migrating from the vascular to the osteogenic compartment of the transformation microenvironment. Undecalcified sections cut at **6 µm** (Polycut-S, Reichert, Heidelberg, Germany) stained free-floating with toluidine blue in 30% ethanol.
Original magnification: (A) x125; (B) x 175.

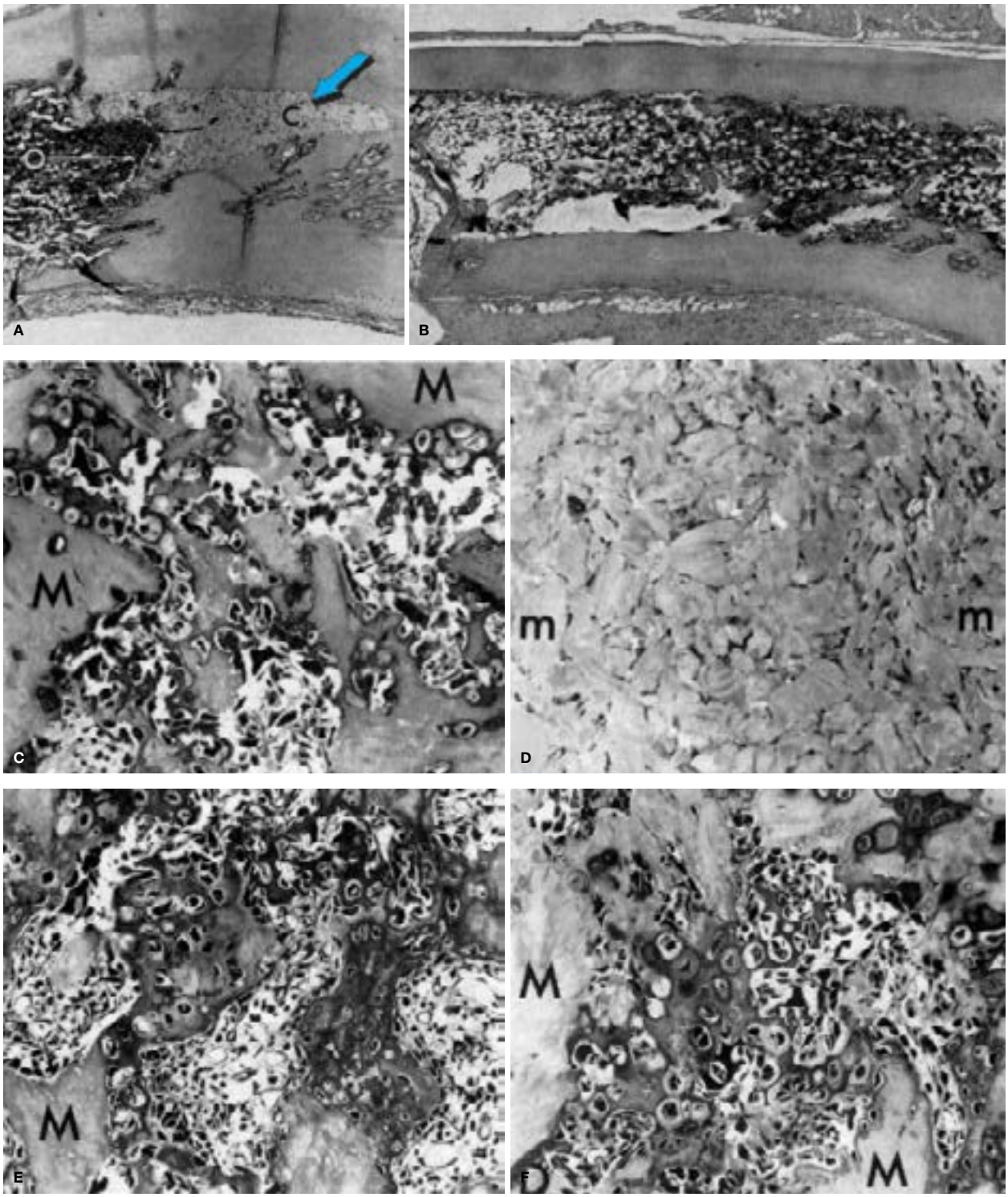

Figure 2. Tissue induction and morphogenesis by geometrically different inductive substrata: the effect of substratum geometry on the induction of endochondral bone formation. Courtesy of AH Reddi, Influence of Geometry of Transplanted Tooth and Bone on Transformation of Fibroblasts. *Proceedings of the Society for Experimental Biology and Medicine*: 143: 634-7, 1973.[21]

A. Demineralized whole incisor harvested on day 28 after heterotopic implantation in the subcutaneous space of recipient rodents.[21] Bone formation by induction with the development of an ossicle (O) with bone marrow. The root apex is populated by newly formed cartilage (C) (light blue arrow).

B. Open demineralized tooth incisor (after apex resection) shows the induction of bone with marrow formation across the pulp chamber but lack of cartilage formation. As reported,[21] the "temporal sequence of fibroblast-chondroblast-osteoblast transformation was profoundly influenced by the geometry of the transformant." Undecalcified sections, original magnification (A) x 2.7; (B) x 1.7. C.D.E.F. The effect of geometry of demineralized bone matrix (DBM), the inductor, on tissue induction and differentiation upon implantation in the subcutaneous space of the rat.[23] Courtesy of AH Reddi "The Journal of Cell Biology", Importance of geometry of the extracellular matrix in endochondral bone differentiation. 98: 2192-7, 1984.[23]

C. Heterotopic induction of bone formation by coarse demineralized matrix (M) (light blue arrows) particle size **74-420 µm**: vascular invasion, chondrolysis and induction of bone formation.

D. Fine matrix (m) (light blue arrows): complete lack of bone differentiation.

E. Reconstitution of coarse collagenous inactive residue matrix (M) with fine matrix Gdn HCl extracts: restoration of the bone induction activity with vascular invasion.

F. Reconstitution of coarse matrix (M) (light blue arrows) with fine matrix Gdn-HCl extracts further purified by gel filtration chromatography on Sepharose CL-CB, equilibrated in 4M Gdn HCl/50 mM Tris, pH 7.0:[23] Complete restoration of the bone induction cascade with prominent vascular invasion induced by purified proteins from fine matrix.[23] Fine matrix thus contains osteogenic proteins, but the geometry of the inductor blocks the bone induction cascade. Undecalcified sections, original magnification (C,D) x125; (E,F) x200.

These are materials that *per se*, in their own right, retain the prerogative of inducing desired and specific morphogenetic responses when implanted in heterotopic extra skeletal sites, where there is no bone, and without the exogenous application of the soluble osteogenic molecular signals of the TGF-β supergene family.[2,3,34,41,42]

Before discussing and highlighting the mechanistic insights of the "*geometric induction of bone formation*"[43,44] we re-present microphotographs we generated after experimentation in the non-human primate *Papio ursinus* (Fig. 1).[41] The study involved heterotopic intramuscular implantation of coral-derived macroporous bioreactors in the *rectus abdominis* muscle.[41]

Serial undecalcified sections were prepared from specimen blocks harvested on days 30, 60 and 90. Sections showed large plumped hyperchromatic cells interpreted as differentiating osteoblasts directly apposed to the hydroxyapatite surface (Fig. 1). The presented images summarize more than twenty-seven years of systematic research on osteoinductive biomaterials, and lucidly highlight the direct effect of the substratum upon cell differentiation and the induction of the osteogenic phenotype.[41]

The geometric induction of bone formation

In the late eighties we were exposed to the unique finding that coral-derived calcium phosphate-based macroporous bioreactors induced the morphogenesis of bone when implanted in the *rectus abdominis* muscle of the Chacma baboon *Papio ursinus*.[2,3] Of great significance was the observation that the heterotopic induction of bone formation was initiated without the exogenous application of the soluble osteogenic molecular signals of the TGF-β supergene family.[45]

The realization that the insoluble signal, ie. the substratum alone, could initiate the induction of bone formation, modified the tissue engineering paradigm: soluble signals, when recombined with insoluble signals or substrata, initiate the cascade of bone formation by induction.[14-18] The serendipitous discovery of osteoinductive biomaterials *per se*[2,3] paved the way to the design of novel substrata to explore the effect of surface topography on cell shape and differentiation. Topographical geometric surface' modifications regulate the molecular machinery controlling the expression of selected mRNA species of the osteogenic phenotype, resulting in tissue induction and morphogenesis.[46]

The influence of geometry on tissue induction and bone formation has been demonstrated by the classic papers of Reddi's group that highlighted the critical role of geometry of the substratum on the induction of bone formation (Fig. 2).[21-24] A new set of experiments were thus designed using coral-derived calcium phosphate constructs pre-loaded with doses of highly purified naturally derived osteogenic fractions, purified greater than 50,000-fold[23] for implantation in the subcutaneous space of Long-Evans rats.[28]

The constructs were of two geometric configurations, i.e. blocks in disc form (7 mm in diameter, 3 mm in height) and granules (400-620 µm in diameter) of porous hydroxyapatite replicas.[28]

Reconstituted specimens with or without osteogenic proteins (50 µg osteogenic proteins fraction per implant) were bioassayed in the subcutaneous space of Long-Evans rats at bilateral sites over the pectoralis fascia and harvested on days 7, 11 and 21 after implantation[28] (Fig. 3).

Strikingly, the induction of bone formation was observed only in - the hydroxyapatite discs treated with osteogenic proteins (Fig. 3). Induction of bone formation failed to occur in implants of particulate/granular hydroxyapatite even when the particulates had been pre-treated with highly purified osteogenic fractions. Bone did not form in any of the coral-derived configurations without osteogenic proteins.[28]

New experiments were later initiated using the *rectus abdominis* muscle of *Papio ursinus* to exploit the hydroxyapatite-induced osteogenesis model to test the osteoinductive potential of macroporous constructs with different geometric configurations, and without the exogenous applications of the osteogenic molecular signals of the TGF-β supergene family.[47]

Coral-derived bioreactors in block/cylinder configurations were implanted in the *rectus abdominis* muscle and tested against granular particulate coral-derived constructs implanted contra laterally.[47] Specimens harvested on days 60 and 90 provided histological material that unequivocally showed the influence of the geometry of the substrate on the induction of bone formation (Fig. 4).[47-53] Coral-derived constructs in block/cylinder configuration did induce the spontaneous and intrinsic induction of bone formation within the macroporous spaces.[47] Particulate/granular coral-derived constructs in general failed to initiate the induction of bone formation (Fig. 4).

One particulate/granular coral-derived specimen showed the induction of bone formation within a concavity of the implanted hydroxyapatite substratum (Figs. 4B,C). The reported images were instrumental in the identification of the concavity as an initiator of the bone induction cascade in macroporous coral-derived bioreactors when implanted in heterotopic sites of *Papio ursinus* (Fig. 4).[47-53] The observation also suggested the revision of all available histological records of macroporous bioreactors previously implanted in the *rectus abdominis* of *Papio ursinus* (Figs. 5,6).

The induction of bone formation by coral-derived and sintered macroporous hydroxyapatites-based bioreactors: the concavity, the shape of life

Histological re-evaluation and re-analyses was completed on old as well as newly cut and stained sections from coral-derived hydroxyapatite specimen blocks, and including newly prepared undecalcified sections.

The assessments lead to the formulation of the hypothesis that the induction of bone formation by coral-derived calcium phosphate-based macroporous bio-

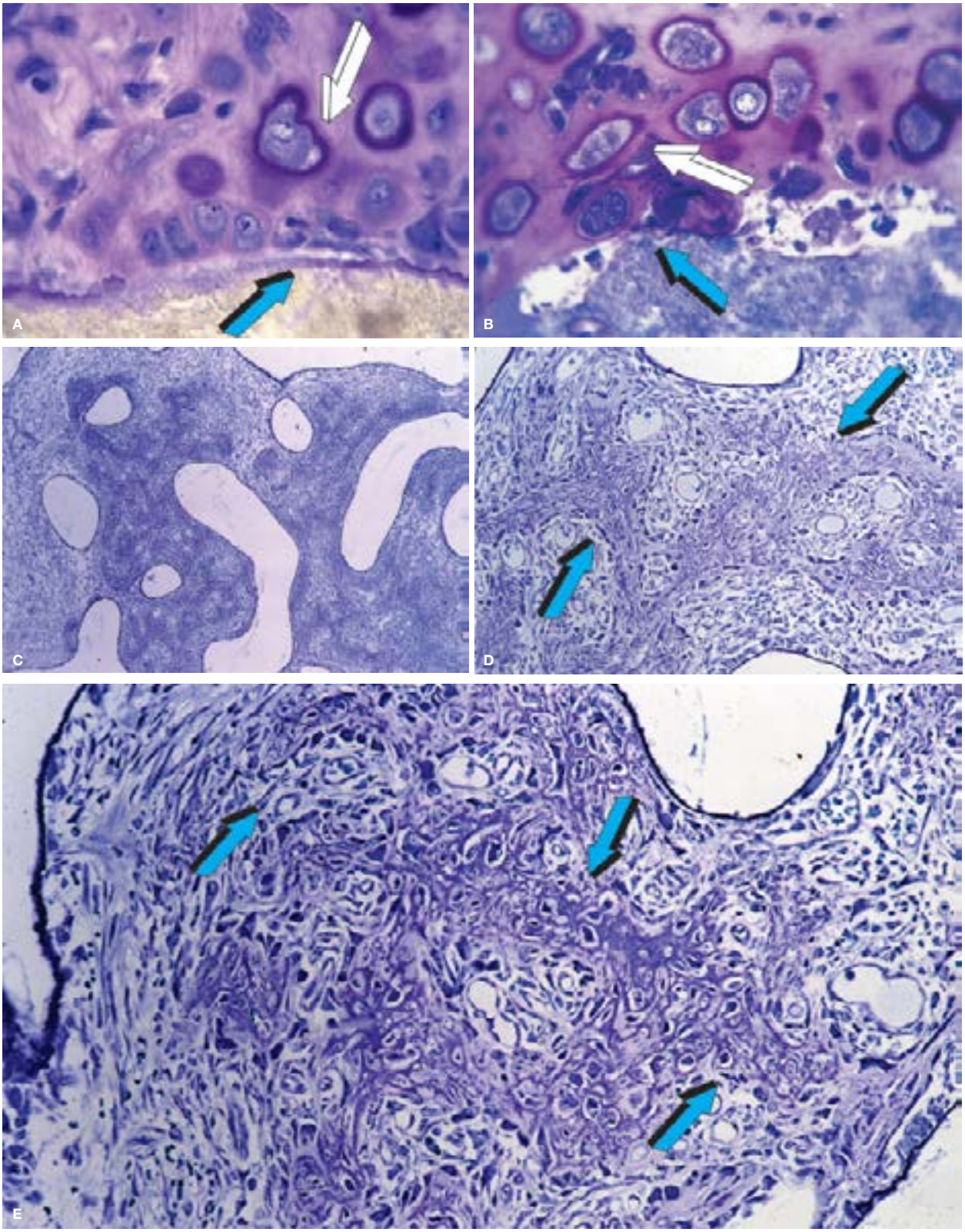

Figure 3. Tissue induction and morphogenesis by geometrically different coral-derived hydroxyapatite constructs pre-loaded with highly purified bovine osteogenic fractions implanted in the subcutaneous apace of Long-Evans rats. Tissue specimens, harvested on day 7, 11 and 21 after heterotopic implantation, were subjected to undecalcified histological analyses and alkaline phosphatase assay.[28]
(A,B) Differentiation of large chondroblastic cells (white arrows) with cartilaginous matrix secreted at the hydroxyapatite interface on day 7 after heterotopic implantation (light blue arrows).[28]
(C,D,E) Other specimens, also on day 7, show the direct induction of intramembranous bone formation regulated by the angiogenetic and morphogenetic vessels of Trueta' and Aristotle' definition.[18,30,54] Trabeculae of newly formed bone populated by contiguous osteoblasts (light blue arrows) form around the morphogenetic vessels[18,27,30] that construct the primordial osteonic structure of long-lived vertebrate's' bones. The remarkable and florid induction of bone formation as shown in **(C,D,E)** markedly contrast with the lack of bone differentiation (not shown). Additional experiments were conducted to exclude that the specific activity of osteogenic proteins bound to the substrata could be affected by the geometric configurations of the coral-derived substrata[28]. The binding and/or release of recombinant human bone morphogenetic protein was not affected by the geometry of the coral-derived substrata since rhBMP-2b[28] binds equally well to coral-derived constructs in granular and disc configurations.[28] Undecalcified sections stained with toluidine blue.[28]
Original magnification: **(A,B)** x200; **(C)** x75; **(D)** x175; **(E)** x200; **(F)** x 175.

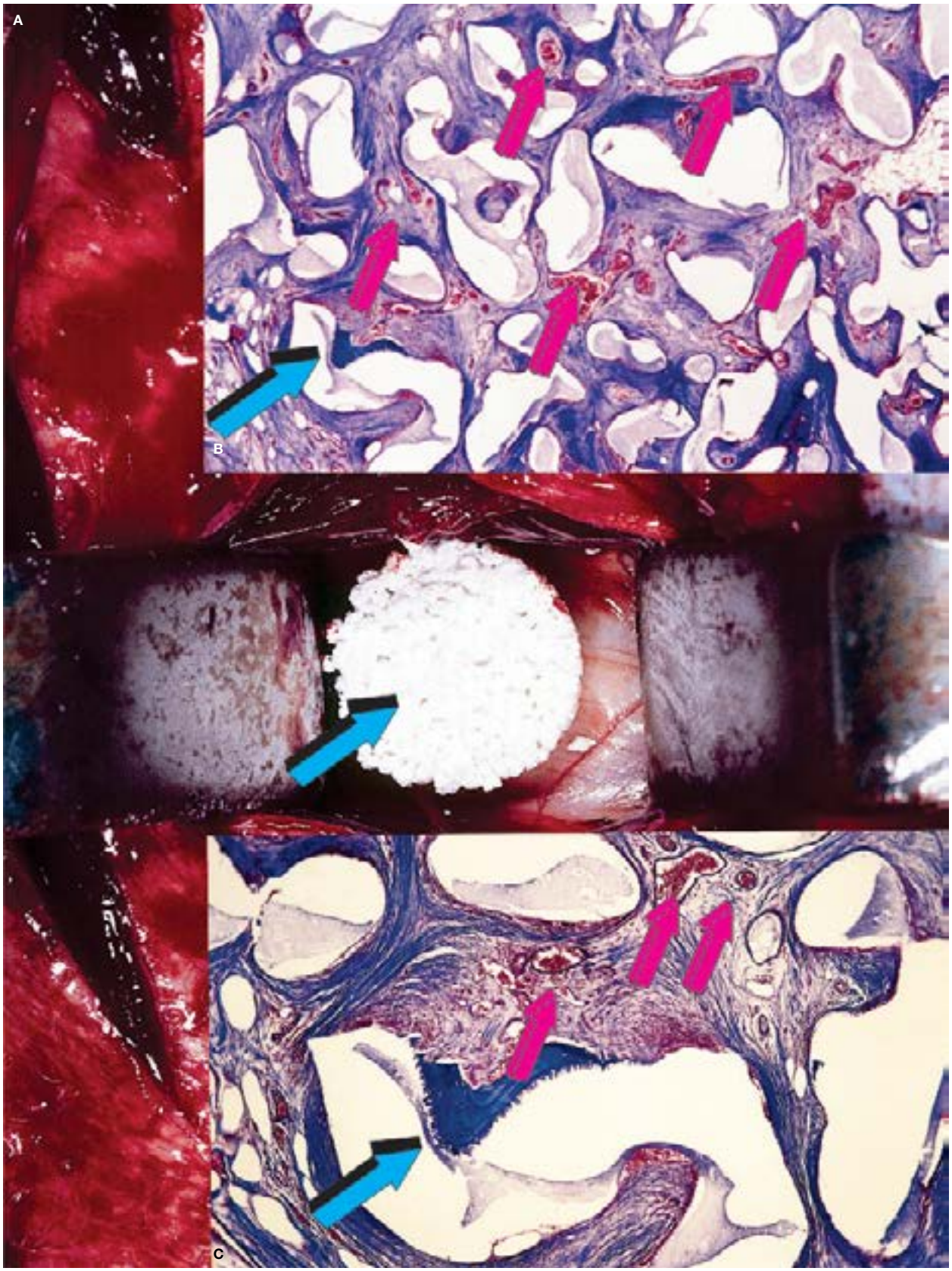

Figure 4. The effect of substratum' geometry on the induction of bone formation by coral-derived macroporous constructs implanted in the *rectus abdominis* muscle of adult *Papio ursinus* and harvested on day 60 and 90 after heterotopic implantation.[47] Coral-derived constructs were rods 20 mm in height and 7 mm in diameter, and particulate/granular construct pelleted for heterotopic implantation **(A)** (light blue arrow) in intramuscular pouches created by sharp and blunt dissection of the *rectus abdominis* muscle[47].
B. Histological analyses of particulate/granular coral-derived constructs show the lack of bone differentiation across the specimen. One section only (light blue arrow) showed the minimal yet positive induction of bone formation in a concavity of the particulate/granular coral-derived construct **(C)** (light blue arrow). This particular section and digital image sparkled the interest on the geometric induction of bone formation and called for a mandatory revision of several previously cut specimens which again highlighted the critical role of the substratum geometry on the induction of bone formation without the exogenous applications of soluble osteogenic proteins of the transforming growth factor-β (TGF-β) supergene family.[45] Decalcified sections cut at 6 µm, original magnification (B) x75; (C) x175.

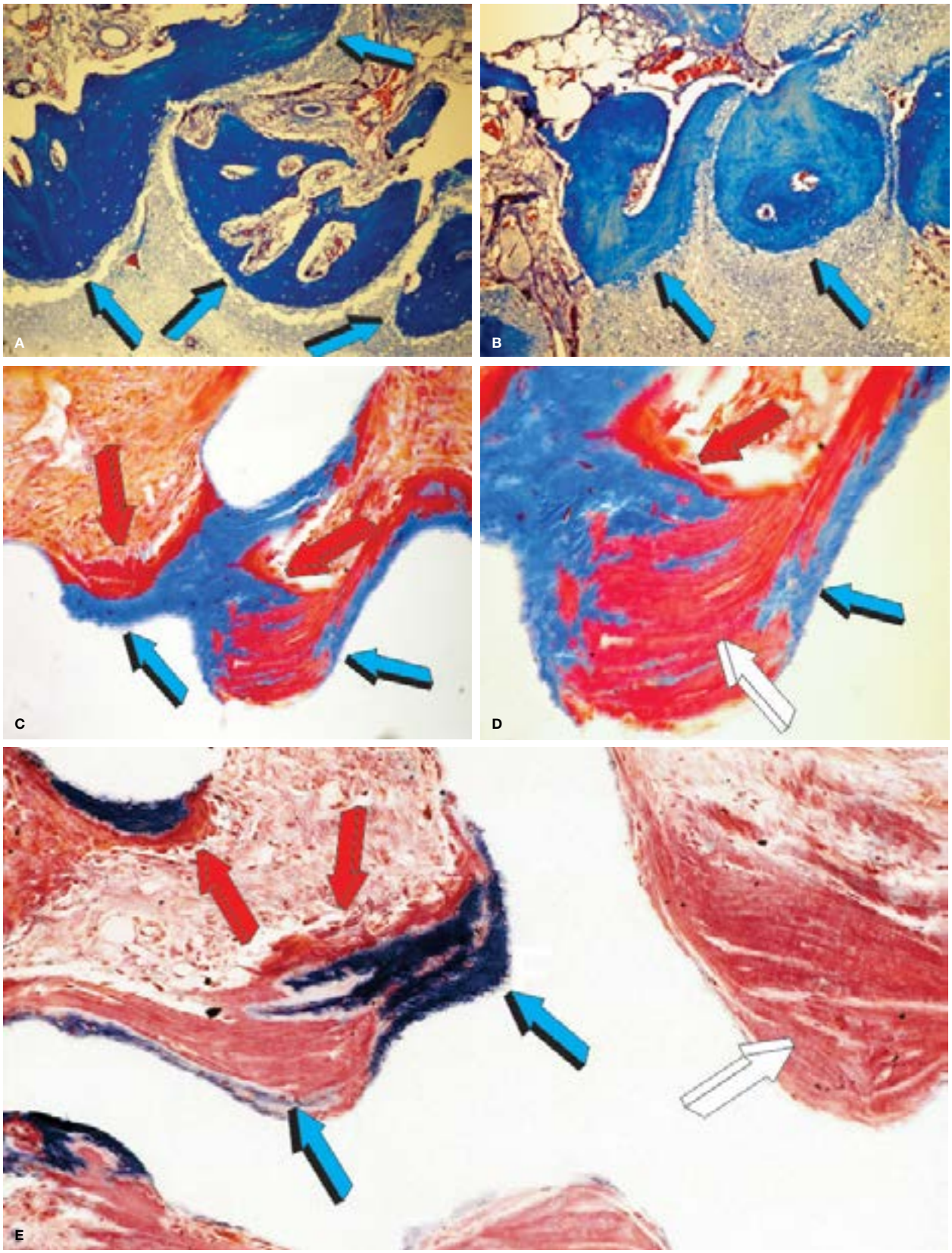

Figure 5. The "intrinsic" induction of bone formation regulated by the geometry of the substratum. Macroporous rods of coral-derived bioreactors were implanted in the *rectus abdominis* muscle of *Papio ursinus* and harvested on day 90 after implantation.[2,3,20,29,47-50] Revision of several specimens harvested from the *rectus abdominis* muscle of *Papio ursinus* in the late eighties and early nineties were re-examined and often re-cut to expand on the biological meaning of the images presented in Figs. 4B,C. These invocated the concavity as a critical geometric signal to initiate the induction of bone formation in macroporous coral-derived constructs. A,B. (light blue arrows) Induction of substantial bone formation within concavities of the macroporous bioreactors. C,D,E. Undecalcified sections illustrating the induction of mesenchymal condensations at the hydroxyapatite interface (white arrows) predating mineralization (light blue arrows) of the collagenous condensations with the differentiation of osteoblastic-like cells secreting newly formed osteoid matrix (red arrows) onto the mineralized condensations. Bone initiates within the concavities of the implanted substratum without the exogenous application of the osteogenic proteins of the TGF-β supergene family.[47-50] Decalcified sections cut at 6 µm, original magnification (A,B) x45; (C,D,E). Undecalcified sections cut at 6 µm stained free-floating with Goldner's trichrome, original magnification (C) x 75; (D) x125; (E) x80.

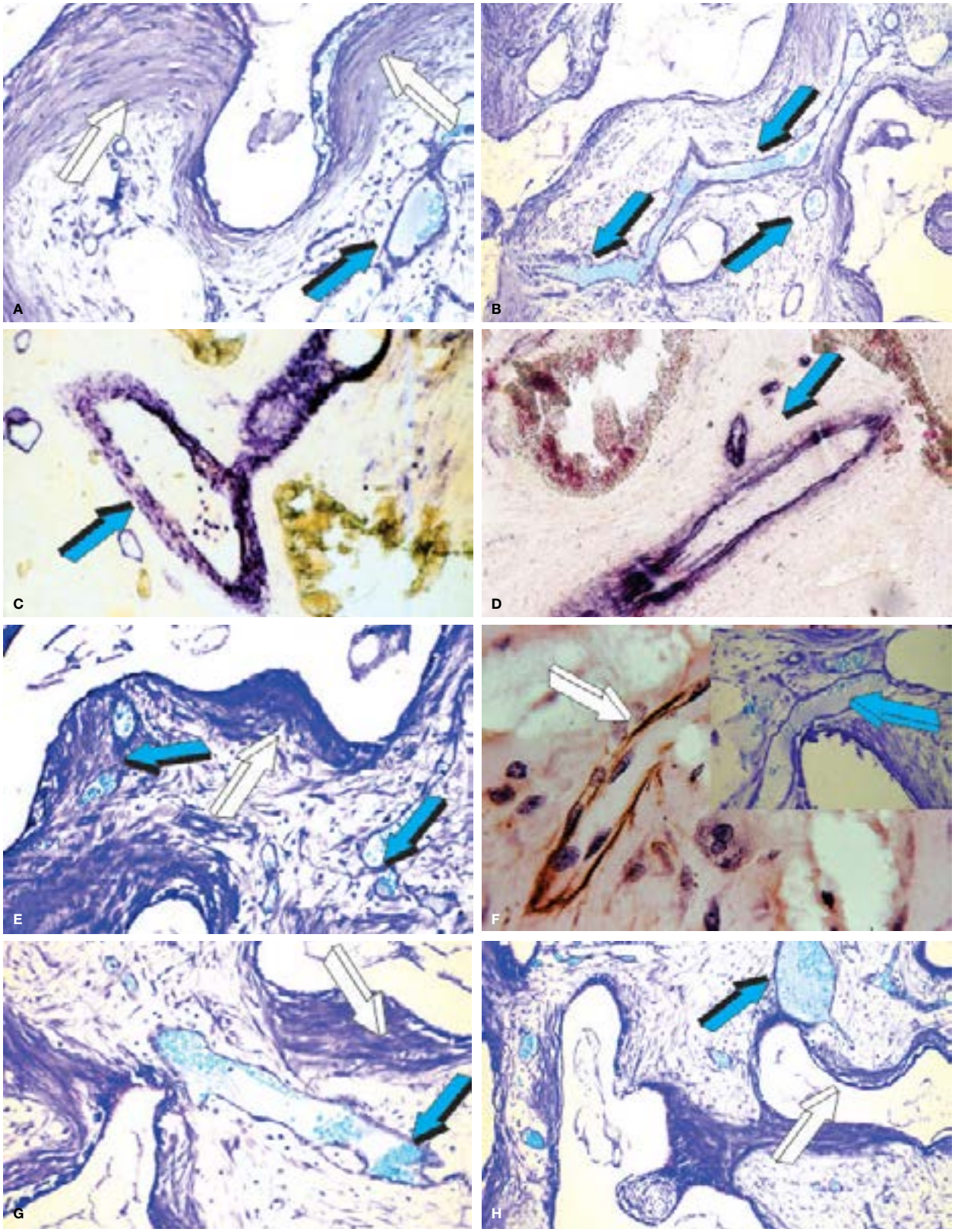

Figure 6. Induction of collagenous condensations and angiogenesis with differentiation of osteogenetic vessels on days 30 and 60 after heterotopic intramuscular implantation of coral-derived bioreactors harvested on days 30 and 60. **A,B.** Concavities of the substratum are invaded by fibro vascular tissue that organize as mesenchymal collagenous condensations within the invaded macroporous spaces (white arrows). Invasion of the macroporous spaces by capillary sprouting in close relationship with the substratum (light blue arrows). Angiogenetic vessels show intense alkaline phosphates activity **(C,D)** elongating to almost touch the implanted bioreactor (light blue arrows) **(E). F.** Invading capillaries immunolocalize laminin within the basement membranes (white arrow). Angiogenic and osteogenic proteins bind to type IV collagen of the capillaries' basement membrane.[16,18] Type IV collagen may thus function as a storage and delivery system by sequestering both angiogenic and osteogenic proteins.[16,18] **G,H.** Continuous remodelling of collagenous condensations (white arrow) within the concavities of the macroporous spaces of coral derived constructs on day 60 after heterotopic implantation. Note the prominent vascular invasion and capillary sprouting (light blue arrow) within the macroporous spaces tightly connected with the differentiating and remodelling mesenchymal condensations. Decalcified and undecalcified sections (C,D,E) cut at 6 µm, original magnification **(A,B)** x 65; **(C,D)** Undecalcified sections cut at 6 µm stained free-floating with Goldner's trichrome, original magnification **(C,D,F)** x 75; **(G,H)** x 60.

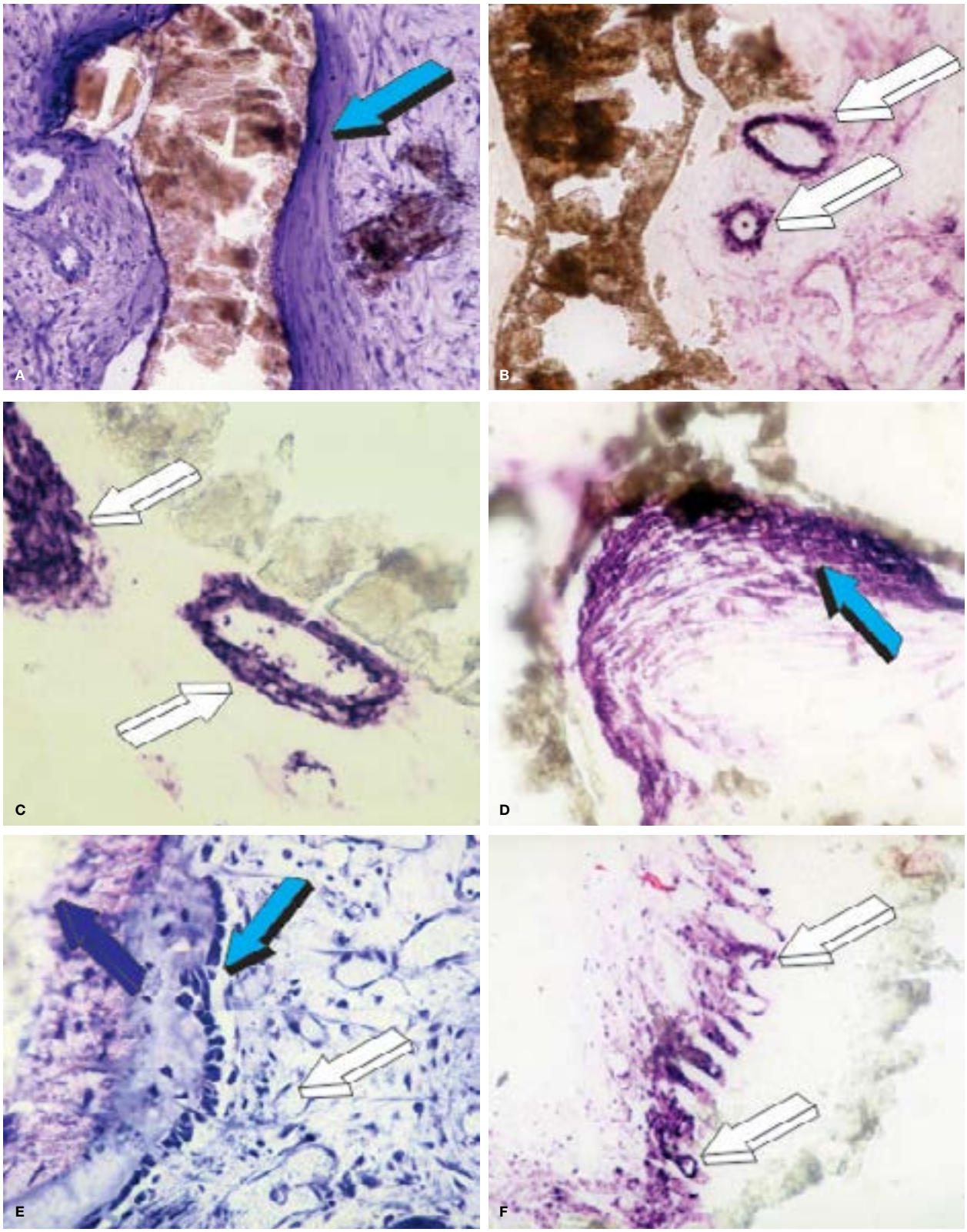

Figure 7. Collagenous condensations, morphogenesis of the "osteogenetic vessels" of Trueta' definition[54] and the induction of bone formation tightly bound to the coral-derived hydroxyapatite surface on day 30 and 60 after heterotopic *rectus abdominis* implantation.[41]
A. Collagenous condensations align directly against the hydroxyapatite substratum on day 30 (light blue arrow).
B. Serial section specular image of **(A)** showing prominent alkaline phosphatase staining of invading capillaries (white arrows **B**) but lack of alkaline phosphatase staining of the collagenous condensations, day 30 (light blue arrow **A**). **C.** Capillaries attached to the hydroxyapatite substratum show multilayers of alkaline phosphatase positive cells in the wall of the invading and embracing vessels on day 30 (white arrows).
D. Induction of collagenous condensations on day 60 depicting prominent alkaline phosphatase staining of the condensations particularly at the transformation microenvironment (light blue arrow) as illustrated in Fig. 1. **E,F.** The induction of bone formation on day 60. Newly deposited osteoid matrix is surfaced by contiguous plumped osteoblastic-like cells secreting osteoid (light blue arrow). **E.** The mineralized newly formed matrix is tightly attached to the hydroxyapatite substratum (dark blue arrow), so the osteoid matrix that is anchored to the mineralized bone by penetrating fibres. Several capillaries (white arrow) are invading the contiguous space of the newly formed bone. **F.** Adjacent section 20 µm apart from image depicted in **(E)**, day 60, showing alkaline phosphatase staining of secreting contiguous osteoblasts. Undecalcified sections cut at 6 µm stained with toluidine blue in 30% ethanol; original magnification (A,B) x100; (C) x210; (D) x125; (E,F) x 175.

reactors is initiated within the concavities of the substratum. We then conceived that the concavity is a powerful morphological, cellular and molecular inductive signal for the induction of bone formation[43-53] (Figs. 5,6).

A time study was henceforth implemented using coral-derived constructs, harvested on days 30, 60 and 90, in the endeavour to identify some of the mechanisms of the intrinsic and/or spontaneous induction of bone formation.[41] Decalcified and undecalcified sections were subjected to histomorphometrical and immuno histochemical analyses, the latter characterizing the developing collagenous condensations (Fig. 7D), the vascular invasion (Figs. 6C,D; 7B,C), the further remodelling of the collagenous condensations (Fig. 6E), and the induction of bone formation (Figs. 7E,F). Sprouting capillaries within the macroporous spaces showed strong alkaline phosphatase staining on several layers of the cells on the walls of the vessels (Figs. 6C,D;7C). These stained capillaries represent the classic "*osteogenetic vessels*" as defined by Trueta.[54]

Osteogenetic vessels invaded the macroporous spaces and were in intimate association with the macroporous constructs, providing osteogenetic precursor cells which would attach to the substratum for further differentiation into the osteoblastic-phenotype[41] (Figs. 6C,D;7B,C).

The basement membrane of invading capillaries showed immunolocalization of laminin, an extracellular matrix basement component (Fig. 6F). Laminin displays amino acid motifs that directly control the phenotypic differentiation and modulation of osteoblastic-like cells *in vitro*.[18,33]

By day 60, collagenous condensation (Fig. 6A) of immunolocalized type I collagen was seen, with alkaline phosphatase staining[41] (Fig. 7D). Staining of invading capillaries which were directly facing the hydroxyapatite substratum was prominent on day 30 (Figs. 6C,D; 7B,C) with later induction of bone formation (Figs. 7E,F).

The newly formed bone faced a highly vascular invading mesenchymal tissue by day 60, with alkaline phosphatase positive cells secreting bone matrix (Figs. 7E,F). Immunolocalization of the extracellular matrix of the invading capillaries included laminin and type IV collagen (Fig. 6F).

Almost thirty years ago, the question that arose was how to correlate the geometric signal of the concavity with the spontaneous induction of bone formation? The concavity as cut in macroporous calcium phosphate-based macroporous constructs is similar to the concavities generated by osteoclastogenesis during the remodelling cycle of the cortico-cancellous osteonic bone[55,56] (Fig. 8). There is thus a profound biomimetism of the "*geometric induction of bone formation*"[43,44,50] with the remodelling cycle of the cortico-cancellous osteonic bone[55,56] (Figs. 8C,D).

The critical role of the concavity initiating and controlling the induction of bone formation in primates was later established using crystalline sintered hydroxy-apatite substrata both as macroporous bioreactors (Figs. 9,10) and as crystalline highly sintered hydroxy-apatite discs prepared with a series of concavities on both planar surfaces (Fig. 11A).

Constructs were implanted heterotopically in the *rectus abdominis* muscle of *Papio ursinus*.[48,49,51] A substantial amount of bone was formed by day 90 within the concavities of both coral-derived (Figs. 9A,B) and highly crystalline hydroxyapatite substrata (Figs. 9C,D).

The induction of bone formation was comparable between substrata with occasionally substantial induction of bone by sintered macroporous constructs (Fig. 9D). Classically, the induction of bone formation was seen on day 30 in the concavities of macroporous sintered bio-reactors (Fig. 10).

Histological sections cut across highly sintered crystalline discs showed the initiation of bone formation only within the concavities as early as day 30 post implantation (Figs. 11B,C).

Immunohistochemical studies showed intracellular cytoplasmic immunostaining of OP-1, also known as BMP-7, within resting differentiating mesenchymal cells at the hydroxyapatite interface (Fig. 11D). Sections taken at 30 days also showed the immuno-localization of OP-1, secreted and embedded onto the hydroxyapatite substratum (Fig. 11E).

Sections cut on day 90 after implantation showed the critical role of the concavity microenvironment in controlling the induction of bone formation with bone remodelling and the induction of hematopoietic marrow (Fig. 11F).

Geometrically modified crystalline hydroxyapatite-coated titanium implants do initiate the spontaneous induction of bone formation in heterotopic intramuscular sites

The induction of bone formation by macroporous sintered constructs produced with a series of repetitive concavities (Figs. 12A,B) was repeated with solid titanium cylinders configured with a series of concavities across the titanium surface which was coated with highly sintered crystalline hydroxyapatite[53,57,58] (Fig. 12C).

The implant body has an outer surface which defines a plurality of concavities, each having a maximum diameter of about 1600 µm, and a maximum depth of about 800 µm. Concavities are spaced apart a distance of about 800 µm[53,57] (Fig. 12C). A Metco 9MB plasma spray gun operating with an Ar/H2 plasma at 35 kW was used to deposit a coating thickness of about 60 to 80 µm of hydroxyapatite powder (Metco-Plasma Technick product AMDRY 6020).

The hydroxyapatite was of high crystallinity and low porosity, resulting in a film highly adherent to the titanium surfaces.[53,57] Prior to spraying the titanium substrata had been roughened by grit blasting with alumina grit to produce the following surface profiles: Ra>3 µm, R1>20 µm, R->15 µm (Figs. 14A,B).[53,57]

The hydroxyapatite coated titanium implants were inserted orthotopically in edentulous mandibular ridges and along the exposed ventral tibiae in a number of adult *Papio ursinus*[53,57] (Fig. 12C) (Animal Research Ethics Committee – AREC – applications: 2010/36/04; 2011/28/05).

Hydroxyapatite-coated titanium geometric constructs were also implanted in the *rectus abdominis* muscle to test the osteoinductive activity of the concavity micro-environment as sculpted in titanium constructs coated with crystalline hydroxyapatite.

Harvested specimens implanted in the *rectus abdominis* muscle on day 5 after intramuscular implantation[53,57] were prepared for SEM analyses and provided critical insights into the early cellular events initiating within the concavities of the hydroxyapatite-coated titanium substrata (Fig. 13).

Plasma hydroxyapatite-coated constructs without geometric configurations showed limited, if any, cellular attachment along the exposed planar sintered hydroxyapatite coating[53,57,58] (Figs. 13C,D).

In marked contrast, titanium constructs coated with crystalline highly sintered hydroxyapatite with concavities across the planar surface showed the prominent induction of cell proliferation and alignment within the exposed concavities to the *rectus abdominis* intramuscular micro-environment (Figs. 13E,F; 14A).

Mesenchymal cellular elements secreted collagenic material around the edges of the concavities (Fig. 14A), bridging the concavities which were exposed to the *rectus abdominis* intramuscular microenvironment.

Cellular bridging with the establishment of cellular tractional forces resulted in the deposition of collagenous material across the concavities (Fig. 14A), indicating that secreting fibroblast-like cells were moving back and forward along the secreted collagen fibres, continuously laying down collagen across the edges of the concavities.

This was later mineralized as bundle bone across the margins of the concavities on the highly sintered crystalline hydroxyapatite discs (Figs. 14B,C).

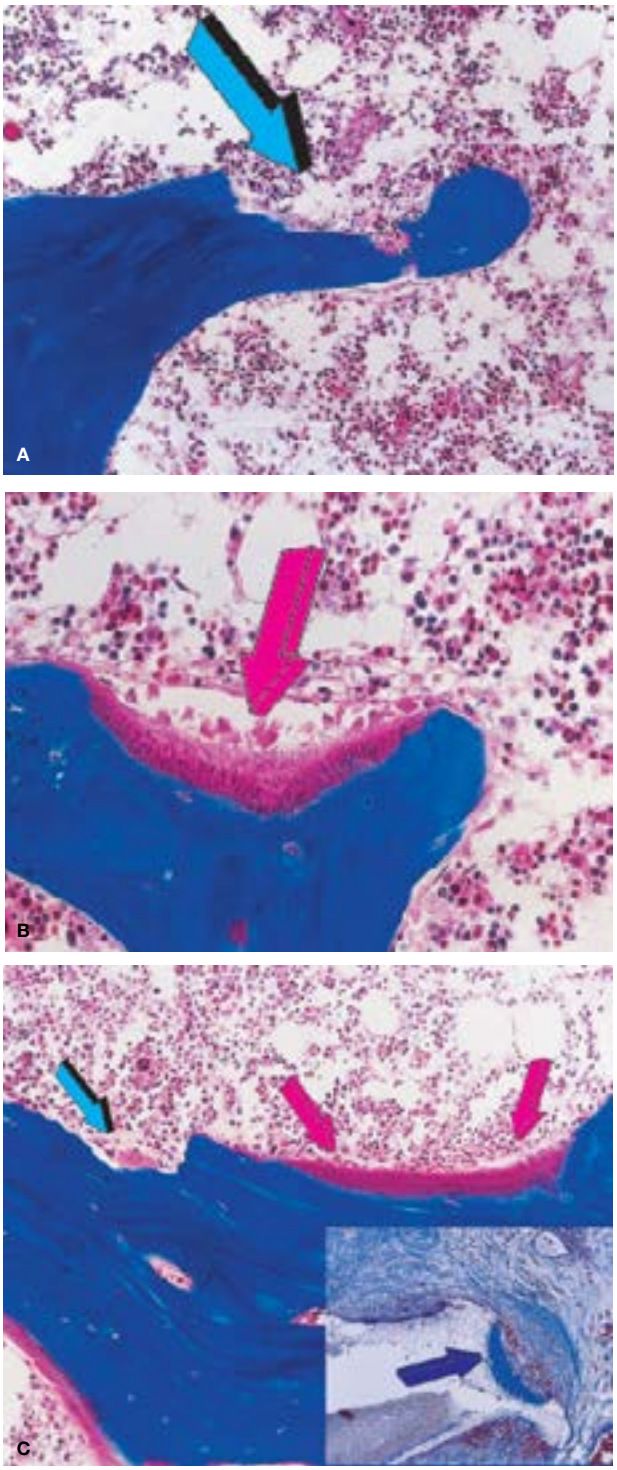

Figure 8. Anatomical, functional and molecular biomimetic correlation of the induction of bone formation within concavities cut by osteoclastogenesis in the primate cortico-cancellous trabecular bone vs. the concavities as assembled in macroporous highly sintered hydroxyapatite constructs. The remodelling cycle of the cortico-cancellous bone is characterized by the resorption phase as initiated by osteoclastogenesis.[55,56]

A,C. At any given time and area of the trabecular bone microarchitecture, osteoclastogenesis sculpts specific geometries in the form of concavities excavated within the trabecular bone (light blue arrows in A,C). The geometry in the form of a concavity is a specific cue imprinted during the remodelling phases of the cortico-cancellous bone of primates.[18,55,56] Osteoclastogenesis sets the release of Ca^{++} and other factors including bone morphogenetic proteins from the resorbed matrix. The deposition phase then follows with angiogenesis and osteoblast recruitment which, when attached to the resorbed lacunae of the bone matrix, synthetize osteoid matrix (B,C magenta arrows). The intrinsic and/or spontaneous induction of bone formation (D dark blue arrow) is initiated by a local peak of Ca^{++} activating stem cell differentiation and the induction of bone formation.[46] Osteoclastogenesis is a critical event controlling the initiation of bone formation by coral-derived macroporous constructs, and it is required to set nano-patterned geometric topographies, which, together with Ca^{++} release, induce angiogenesis, cell differentiation, osteoblasts cell matrix deposition with bone morphogenetic proteins expression and synthesis.[63] Expression of BMPs follows the embedding of the secreted gene products into the implanted matrices with the induction of bone formation as a secondary response.[46] Preloading coral-derived constructs with the osteoclast inhibitor zoledronate zometa or the calcium channel blocker verapamil hydrochloride blocks and reduces significantly the induction of bone formation.[46] Zoledronate-treated specimens showed a delay of the induction of mesenchymal collagenous condensations at the hydroxyapatite interface, with minimal *BMP-2* expression on day 15. Notably, the loss of osteoclast binding resulted in up-regulation of Noggin on day 15, correlating to minimal induction of bone formation by 60 and 90 days after heterotopic *rectus abdominis* implantation.[46] Undecalcified sections cut at 6 µm stained free-floating with Goldner's trichrome, original magnification (A,B,C,D) x 175.

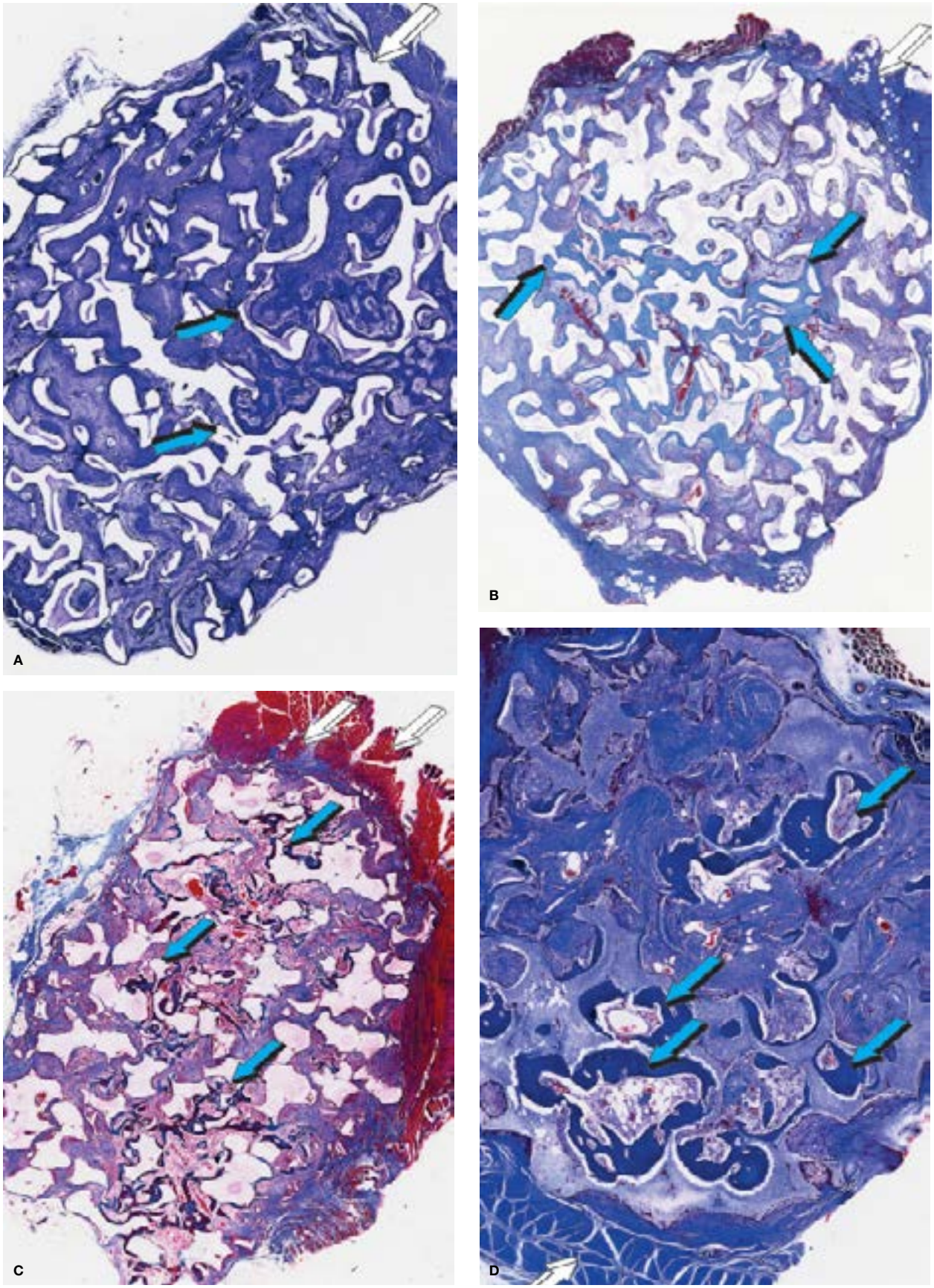

Figure 9. Tissue induction and morphogenesis by calcium-phosphate-based macroporous constructs 90 days after heterotopic implantation in the *rectus abdominis* muscle (white arrows) of the non-human primate *Papio ursinus*.

A,B. Low power views of coral-derived bioreactors. Bone formation by induction (light blue arrows) across the macroporous spaces. Collagenous condensations form across the specimens tightly attached and aligned along the surfaces.

C,D. Induction of bone formation across the macroporous spaces (light blue arrows) by sintered crystalline macroporous constructs. Decalcified sections cut at 6 µm stained with toluidine blue in 30% ethanol (A) and with Goldner's trichrome (B,C,D); original magnification (A,B,C,D) x 7.5.

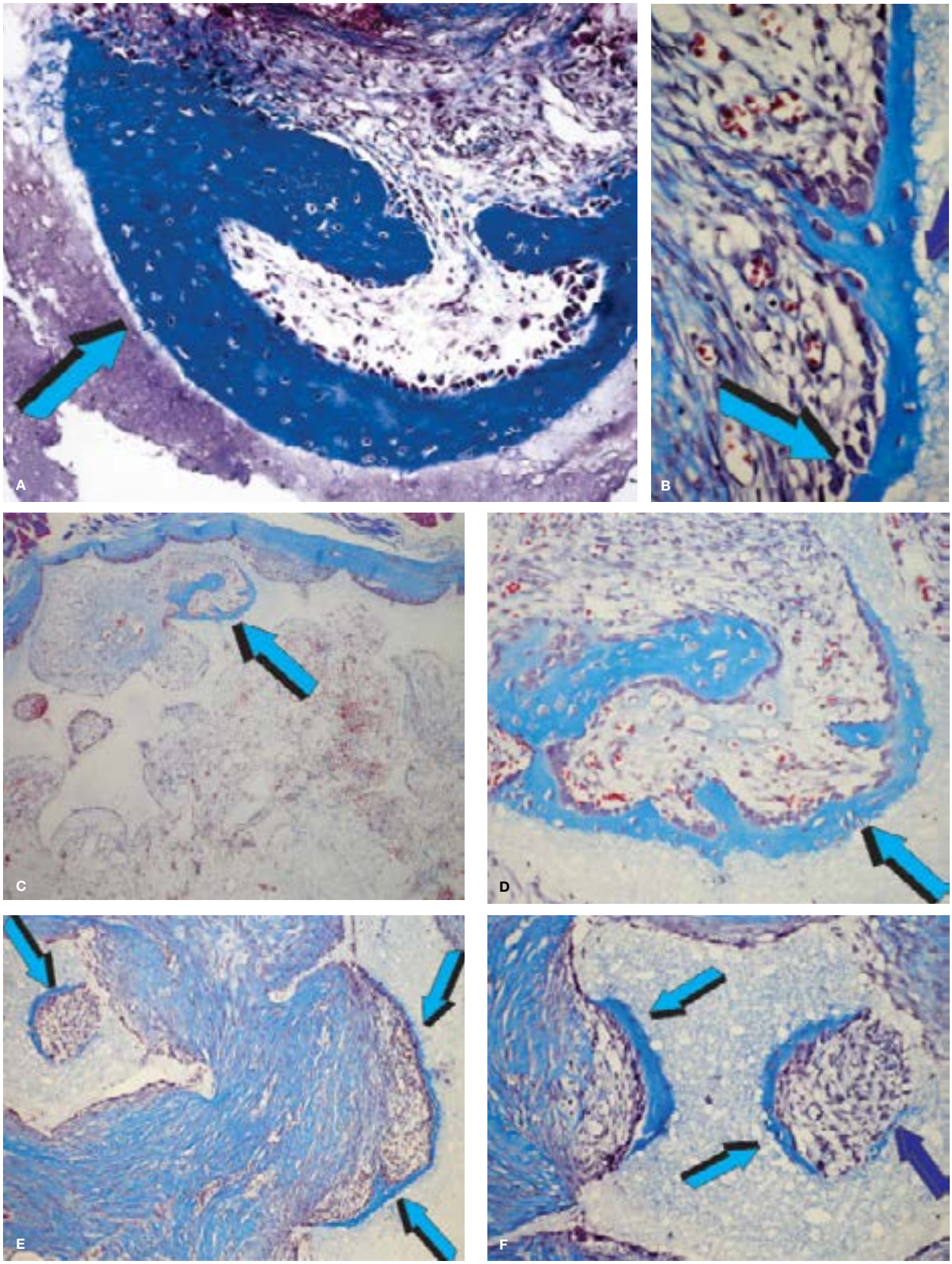

Figure 10. The geometric induction of bone formation by repetitive sequences of concavities (light blue arrows) assembled in macroporous constructs of highly sintered crystalline hydroxyapatite biomatrices after heterotopic implantation in the *rectus abdominis* muscle of the non-human primate *Papio ursinus*.

A. Bone formation within a concavity of the substratum surfaced by contiguous osteoblasts facing a highly vascularized matrix on day 90.
B. The newly formed bone by day 30 is tightly attached to the substratum (light blue arrow) with contiguous plumped osteoblasts (light blue arrow).
C,D. Induction of bone formation within the concavity of the highly crystalline bioreactor showing cellularity of the newly formed bone (**C,D** light blue arrows) tightly attached to the crystalline substratum (**D**).
E,F. Precision induction of bone formation exclusively in concavities of the highly crystalline biomatrix (light blue arrows) on day 30 after heterotopic implantation. Bone forms intrinsically within concavities of the bioreactor (light blue arrows) 30 days after implantation in the *rectus abdominis* muscle. Decalcified sections cut at 6 µm stained with toluidine blue in 30% ethanol. Original magnification **(A)** x35; **(B)** x90; **(C)** x17; **(D)** x90; **(E)** x27; **(F)** x31.

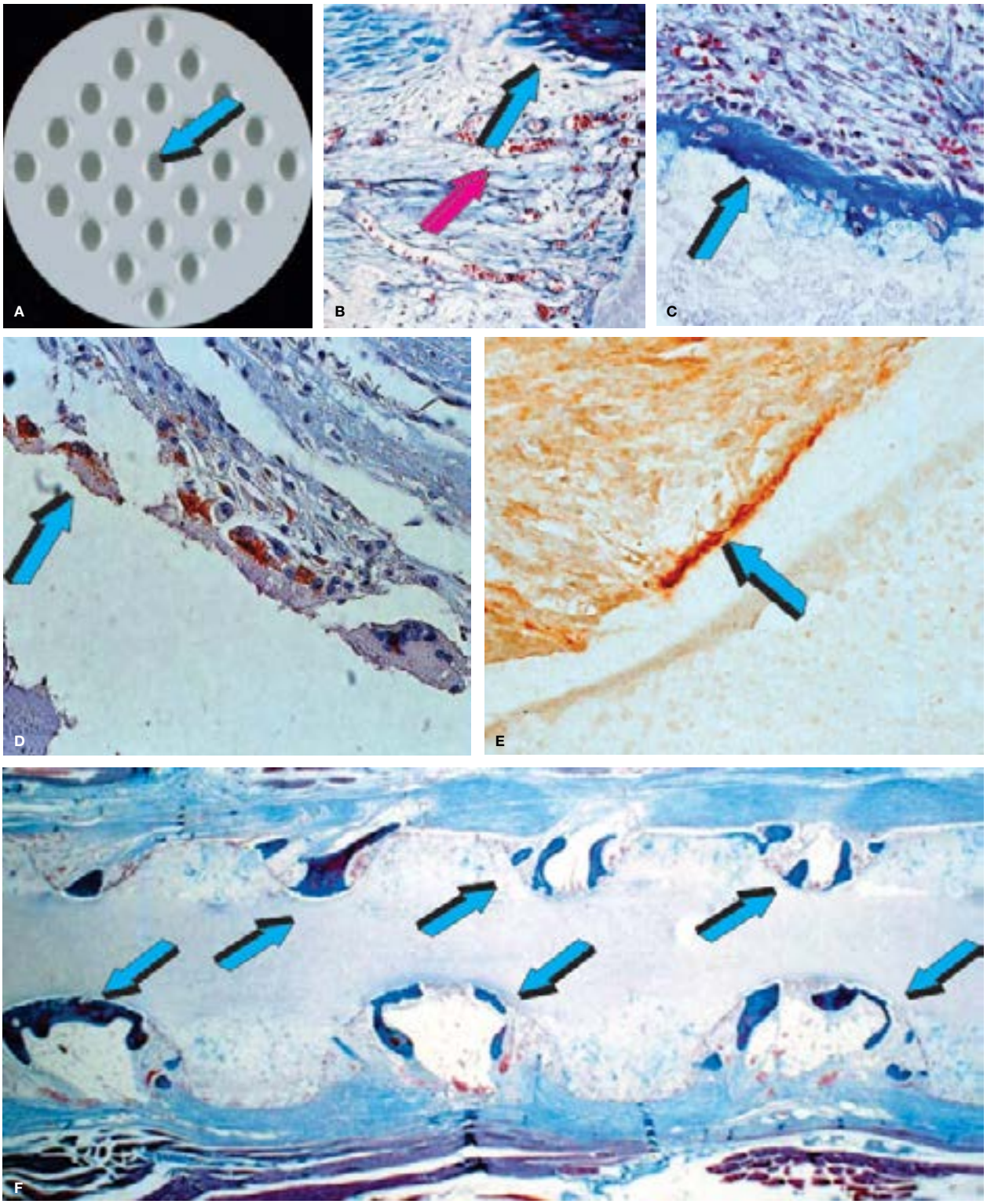

Figure 11. The "*concavity motif*" and the direct regulation of the spontaneous induction of bone formation by the geometry of the substratum: self-inductive geometric cues by sintered crystalline hydroxyapatites in disc configurations with concavities on both planar surfaces (**A** light blue arrow).[43-53] Discs, implanted in heterotopic intramuscular sites of the Chacma baboon *Papio ursinus*, were processed for histological analyses on days 30 and 90.[43-53] (**B**) Capillary sprouting and prominent angiogenesis almost touching the hydroxyapatite surface (**B** magenta arrow) with the induction of bone formation within the concavity of the substratum (**B** light blue arrow).
C. Spontaneous induction of bone formation at the base of a concavity (light blue arrow) of a sintered highly crystalline hydroxyapatite disc harvested on day 30 after heterotopic implantation. Newly formed bone by day 30 with contiguous plumped secreting osteoblasts. The newly formed bone matrix with embedded osteocytes tightly attach to the sintered hydroxyapatite surface.
D. Immunolocalization of osteogenic protein-1 (OP-1) within the cytoplasm of mesenchymal cells differentiating into osteoblastic-like cells attached to the substratum (light blue arrow).
E. Synthesized OP-1 is expressed, secreted and embedded within the highly sintered crystalline substratum (light blue arrow).
F. Reproducible and constant induction of spontaneous bone formation within concavities of the substratum harvested on day 90 after heterotopic implantation. Bone, with the induction of haematopoietic none marrow, forms exclusively with the concavities of the substratum (light blue arrows), and without the exogenous application of the osteogenic soluble molecular signals of the TGF-β supergene family.[53-58] Decalcified sections cut at 6 µm stained with Goldner's trichrome (**B**,**C**); original magnification x125; (**D**,**E**) x95; (**F**) x17.

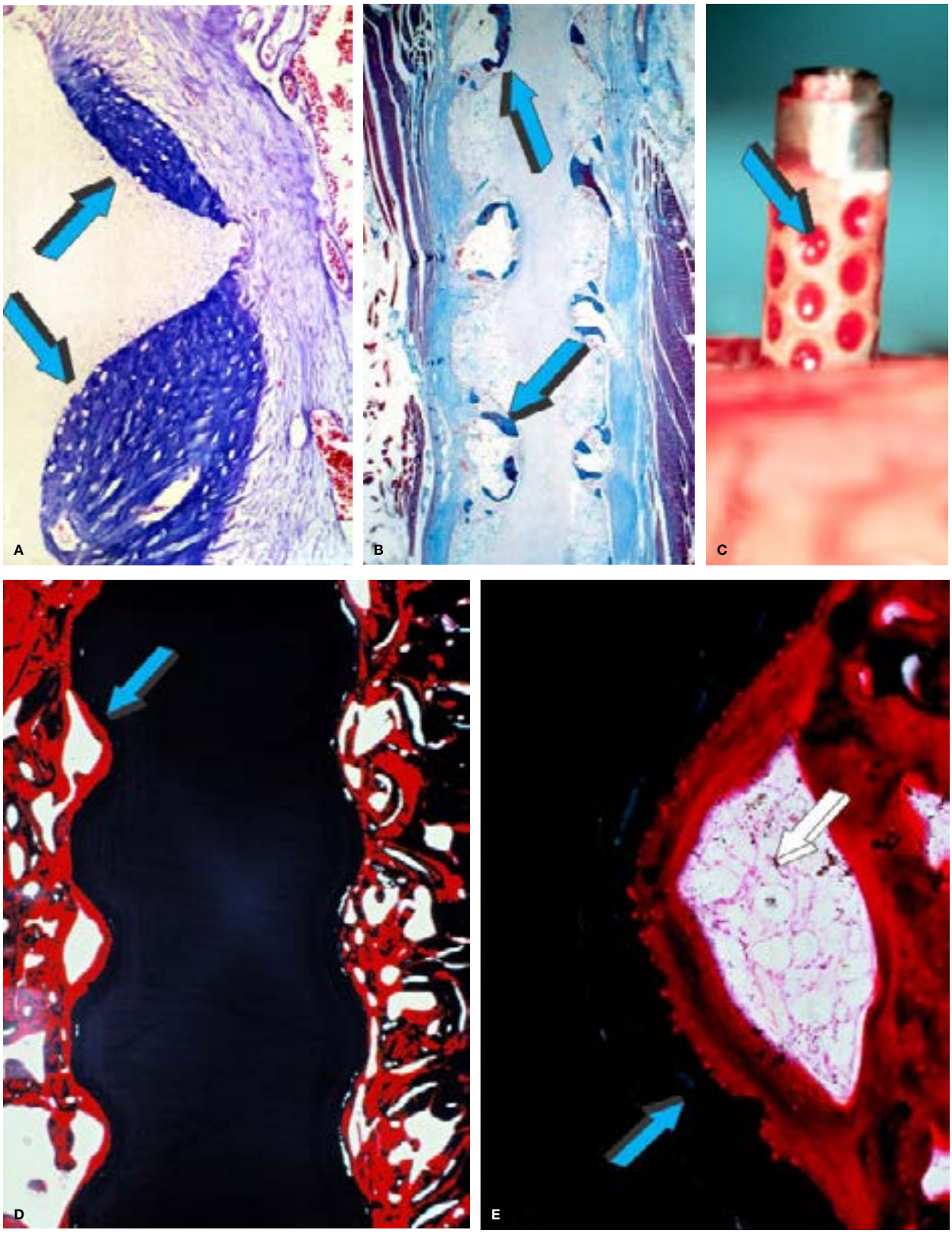

Figure 12. Biological and morphological rationale for translating the geometric induction of bone formation into solid titanium implants coated by highly crystalline sintered hydroxyapatite by plasma spraying methods.[43-53]
A. Spontaneous intrinsic induction of bone formation in concavities (light blue arrows) of a highly sintered hydroxyapatite construct on day 90 after heterotopic intramuscular implantation in *Papio ursinus*.[51-53]
B. Sintered crystalline discs with concavities on both planar surfaces initiate the spontaneous induction of bone formation precisely in the concavities of the substratum only.
C. Translation of the geometric induction of bone formation in preclinical studies in *Papio ursinus* after manufacturing hydroxyapatite plasma sprayed titanium implants inserted in the ventral aspect of the tibia and into mandibular edentulous ridges of *Papio ursinus*.[51-53;57,58] Absorption of plasma and plasma products within the concavities (light blue arrow) of the geometric construct.
E,F. Tissue induction and osteointegration of geometric titanium' constructs on day 90 after orthotopic implantation. Undecalcified tissue blocks were processed in ascending concentration of Technovit 7200 VLC (Heraeus Kulzer GmbH, Wehrheim, Germany) and embedded in the same resin. Undecalcified sections cut, ground and polished to 40-60 µm were stained with a modified Goldner's trichrome.[53,54]
Sections were prepared using the EXAKT precision cutting and grinding system (EXAKT Apparatebau, Nordested, Hamburg, Germany).[57,58]

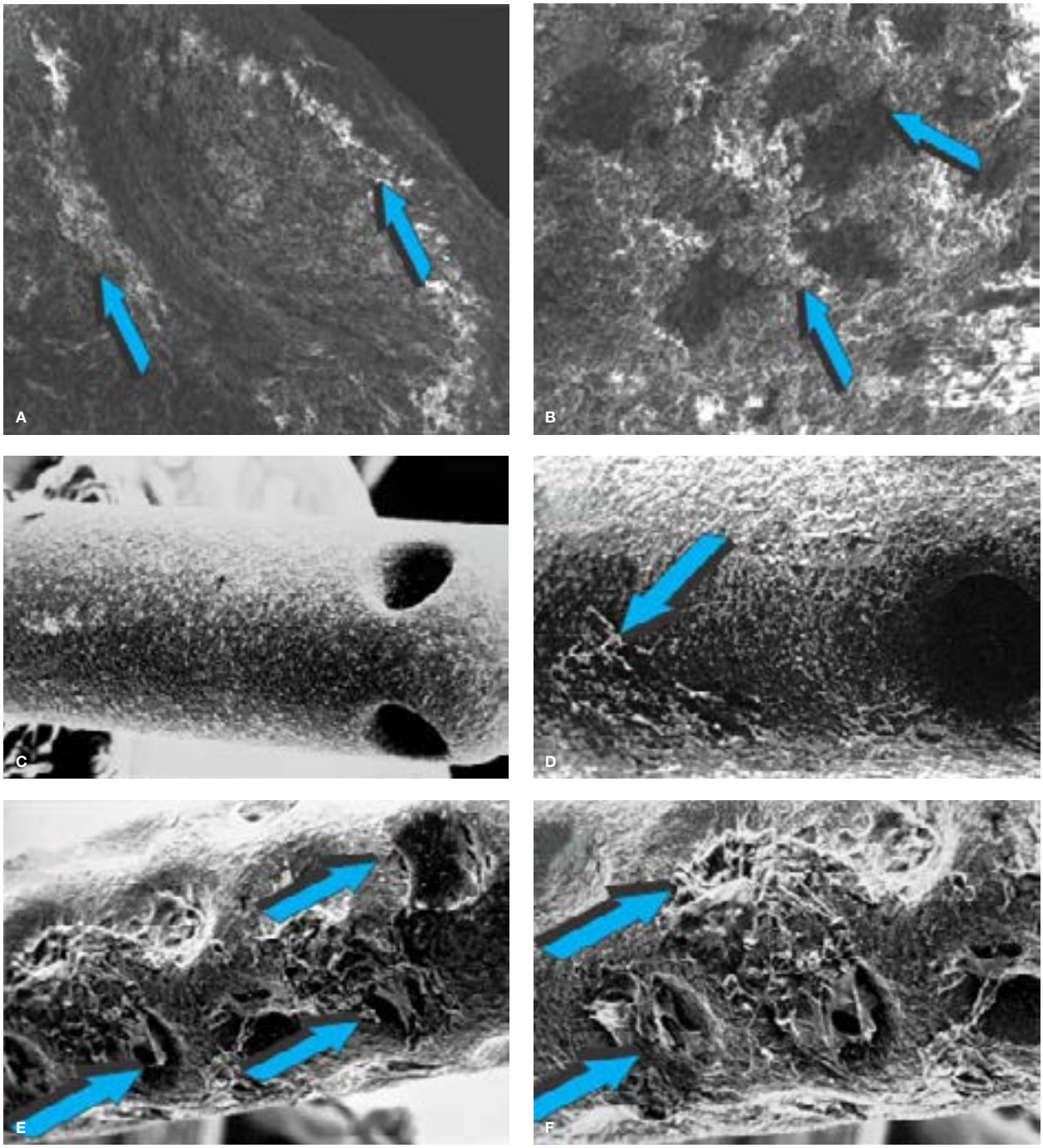

Figure 13. SEM analyses of hydroxyapatite-coated titanium implants. A,B. Image in A show the concavity of the substratum (light blue arrows). B. Coated titanium constructs show nanopatterned geometric modifications in the form of lacunae, pits and concavities of the plasma sprayed titanium constructs.
C,D,E,F. SEM analyses of titanium specimens harvested on day 5 after heterotopic implantation in the *rectus abdominis* muscle of the non-human primate *Papio ursinus*.[68]
C,D. Planar coated implant with minimal if any cellular activities along the surface (light blue arrow).
E,F. A very different image of biological cellular activities (light blue arrows) along the geometric substratum of the hydroxyapatite-coated titanium constructs. Myoblastic/pericytic cellular trafficking along the concavities of the heterotopically implanted constructs with resident cells attaching and stretching along the substratum nanotopography of the highly crystalline hydroxyapatite coating (light blue arrows). Harvested specimens on day 5 after *rectus abdominis* implantation were carbon-coated (5 nm thick) and examined on a FEI Nova Nanolab SEM (FEI Company, Oregon, USA), at 30 kV.

Therefore, the concavity is a prominent signal for cell attachment, orientation, palisading and differentiating events both *in vitro* and *in vivo*.[43-53] Mouse-derived fibroblasts (NIH3T3) and pre-osteoblasts (MC 3T3-E1) were seeded onto coral-derived calcium carbonate/calcium phosphate constructs in disc configuration (7 mm in diameter, 3 mm in height) simulating an *in vitro* bioreactor for cell attachment and differentiation.[57,58]

The concavities of the bioreactor effectively induced cellular orientation and alignment of MC 3T3-E1 cells along the geometric cues of the macroporous bioreactors (Fig. 15A).

EXACT precision cutting and grinding provided undecalcified sections at 170 µm, which showed the tight osteointegration of the hydroxyapatite-coated titanium with the newly formed remodelling bone on day 90 within the concavities of the substrata (Figs. 12D,E; 15C,D).

EXACT cut and ground undecalcified sections of specimen blocks harvested from the *rectus abdominis* muscle 31 months after implantation showed the induction of bone formation with mineralized newly formed bone surfacing the sintered hydroxyapatite coating, and covered by osteoid seams directly facing the *rectus abdominis*' microenvironment (Fig. 16).

This resulted in the first biotechnologically bioinspired biomimetic osteoinductive construct with the unique prerogative of being *per se* osteoinductive without the exogenous application of the osteogenic soluble molecular signals of the TGF-β supergene family, and it was immediately available for translation in clinical contexts.[53,57,58] We later mechanistically resolved the induction of bone formation within concavities of calcium phosphate-based macroporous constructs by invoking the critical role of osteoclastogenesis in releasing Ca^{++} for the induction of angiogenesis and of the osteoblastic phenotype.

This is accompanied by an up-regulation of a specific subset of BMPs with an embedding of the secreted gene products within the concavities of the substratum, initiating the induction of bone formation as a secondary response.[46]

Geometric induction of tissue morphogenesis: substrata' stiffness and elasticity guide cell differentiation

The question that remains and should now drive biomaterial science, molecular medicine and tissue engineering alike, is how to construct biomimetic matrices that in their own right set into motion specific inductive phenomena without the exogenous application of recombinant human morphogens.

The goal is to construct intrinsically osteoinductive biomaterial surfaces, possibly defined as self-inductive surfaces, that *per se*, intrinsically, initiate tissue induction and morphogenesis. Discoveries in the last century showed that the geometry of the inductor, i.e. demineralized tooth dentine or different geometries of demineralized bone matrix particles, did have a profound influence on the induction cascade [21-24] (Fig. 2).

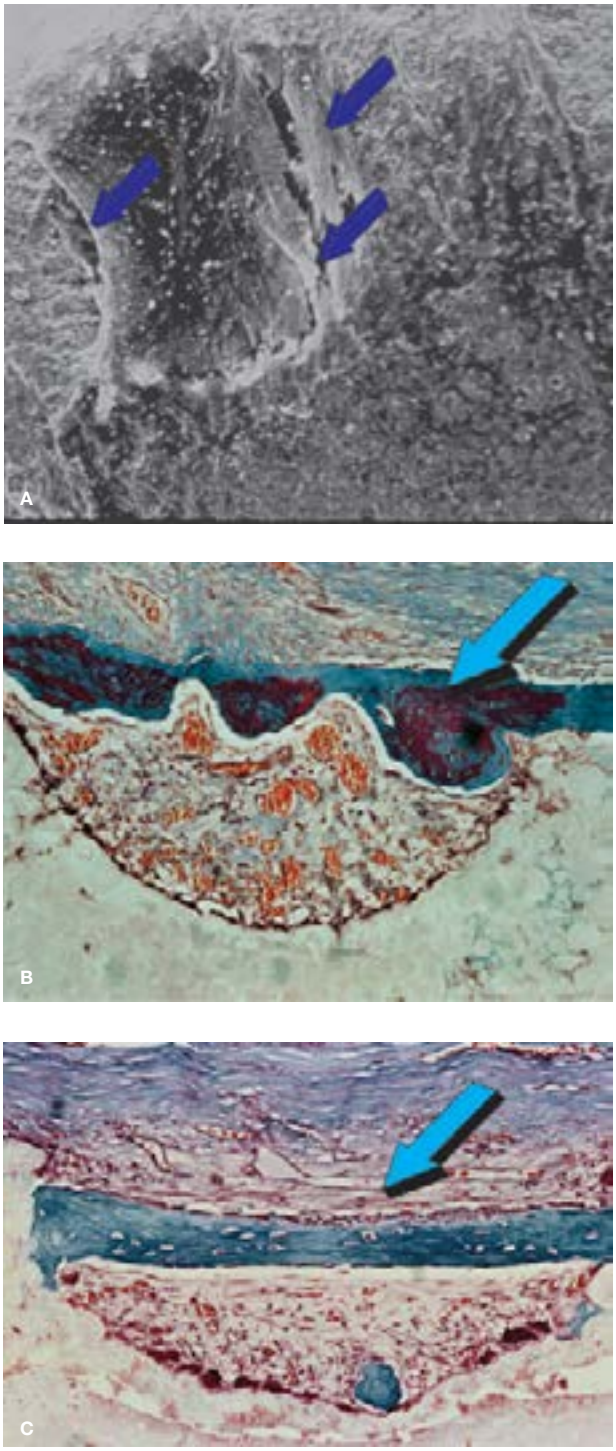

Figure 14. A. Collagenous condensations (dark blue arrows) stretching across a concavity of a hydroxyapatite-coated titanium implant 5 days after implantation in the *rectus abdominis* muscle of the non-human primate *Papio ursinus*. The scanning electron macroscopy (SEM) image depicts tractional patterning forces of fibroblastic/myofibroblastic-like cells secreting collagen fibres whilst moving from the edges of the concavities across the concavities of the hydroxyapatite-coated substratum. The SEM image (A) predates thus the spontaneous induction of bone formation across the concavity bioreactors on day 90 bridging the margins of the exposed concavities exposed to the *rectus abdominis* striated muscle (B,C).
B. The bridging bone (light blue arrow) separate the vascular microenvironment subjacent to the newly formed bone from the external *rectus abdominis* microenvironment (light blue arrow) with several macrophage/poly-nucleated osteoclastic cells surfacing the hydroxyapatite interface.
B,C. Transformation of collagenous condensation across concavities into bone matrix 90 days after heterotopic implantation in the *rectus abdominis* muscle of the non-human primate *Papio ursinus*.
(B,C) Decalcified sections cut at 6 µm stained with toluidine blue in 30% ethanol. Original magnification (B,C) x37.

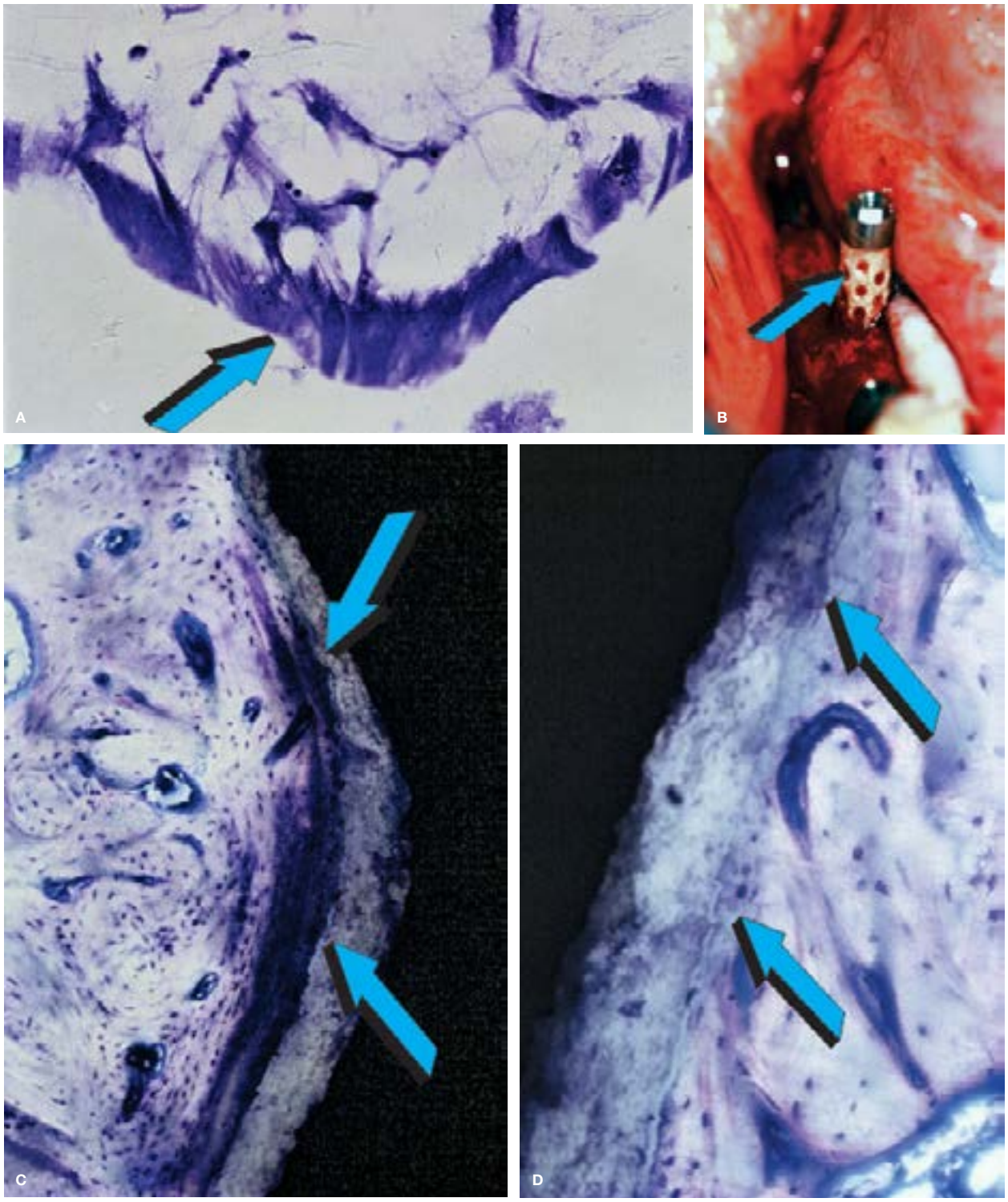

Figure 15. Further conceptualization of the pre-clinical translation of the geometric induction of bone formation into self-inductive hydroxyapatite-coated titanium implants.

A. Patterned attachment, alignment and orientation of MC 3T3-E1 cells grown *in vitro* onto geometric cues of coral-derived hydroxyapatite constructs: the effect of the geometric cue on *in vitro* cell alignment and patterning.[57,58]

B. Pre-clinical translation in edentulized mandibular ridges in *Papio ursinus* with selected uptake of plasma and plasma products by the concavity (light blue arrow).

C,D. High magnification of undecalcified osteointegrated sections along concavities of the hydroxyapatite-coated titanium constructs (light blue arrows). The digital images show the tight bond of the newly generated bone within concavities tightly osteointegrated with the plasma sprayed crystalline hydroxyapatite, perfectly matching the newly generated bone (light blue arrows). (C,D) undecalcified Exakt' sections, original magnification (C,D) x175.

Half a century later a start has been made in the mechanistic assignment of the geometric influence on gene expression and cell differentition. A series of lucid and clear contributions have recently emerged highlighting that "*stem cells feel the difference*",[60] that "*tissue cells feel and respond to the stiffness of their substrate*",[61] and that "matrix elasticity directs stem cell lineage specification".[62]

We believe that the findings that "*soft matrices that mimic brain are neurogenic*" and that "*comparatively-rigid matrices that mimic collagenous bone prove to be osteogenic*"[60] is the most significant contribution so far to the understanding of physical and geometric forces controlling the stem cell microenvironment and the induction of tissue morphogenesis.[60-62]

Selected genes are found to be up regulated including *BMP-2* and *BMP-7*, *RUNX2* and *BGLAP*.[61-83]

There are also focal adhesion (FA) structures controlling actin contractility and the mechano-transduction pathway by focal adhesion kinase (FAK) ultimately up regulating the transcriptor factor *RUNX2*, controlling osteoblastic differentiation and matrix synthesis.

In his Cell paper[62], Disher asks the final question: "*how do stem cells feel or sense matrix elasticity and transduce that information into morphological changes and lineage specification?*".

Actin structures are linked to FA structures and provide the pathway of force transmission from the cell to the elastic matrix.[62] Disher further provides evidence that one of the cell's cytoskeletal motors, the non-muscle myosin (NMM) II isoform, is involved in sensing the matrix elasticity that guides lineage cell specification.[62]

Important research by Curran et al.[70] has shown that controlled modifications of surface topographies guide mesenchymal cell differentiation *in vitro* by expressing and up-regulating *collagen type I*, *osteocalcin*, *osteonectin*, *osteopontin* and *Cbfa1*. *Cbfa1* is a key regulator of osteoblasts differentiation *in vivo* but also of osteoblastic function.[85]

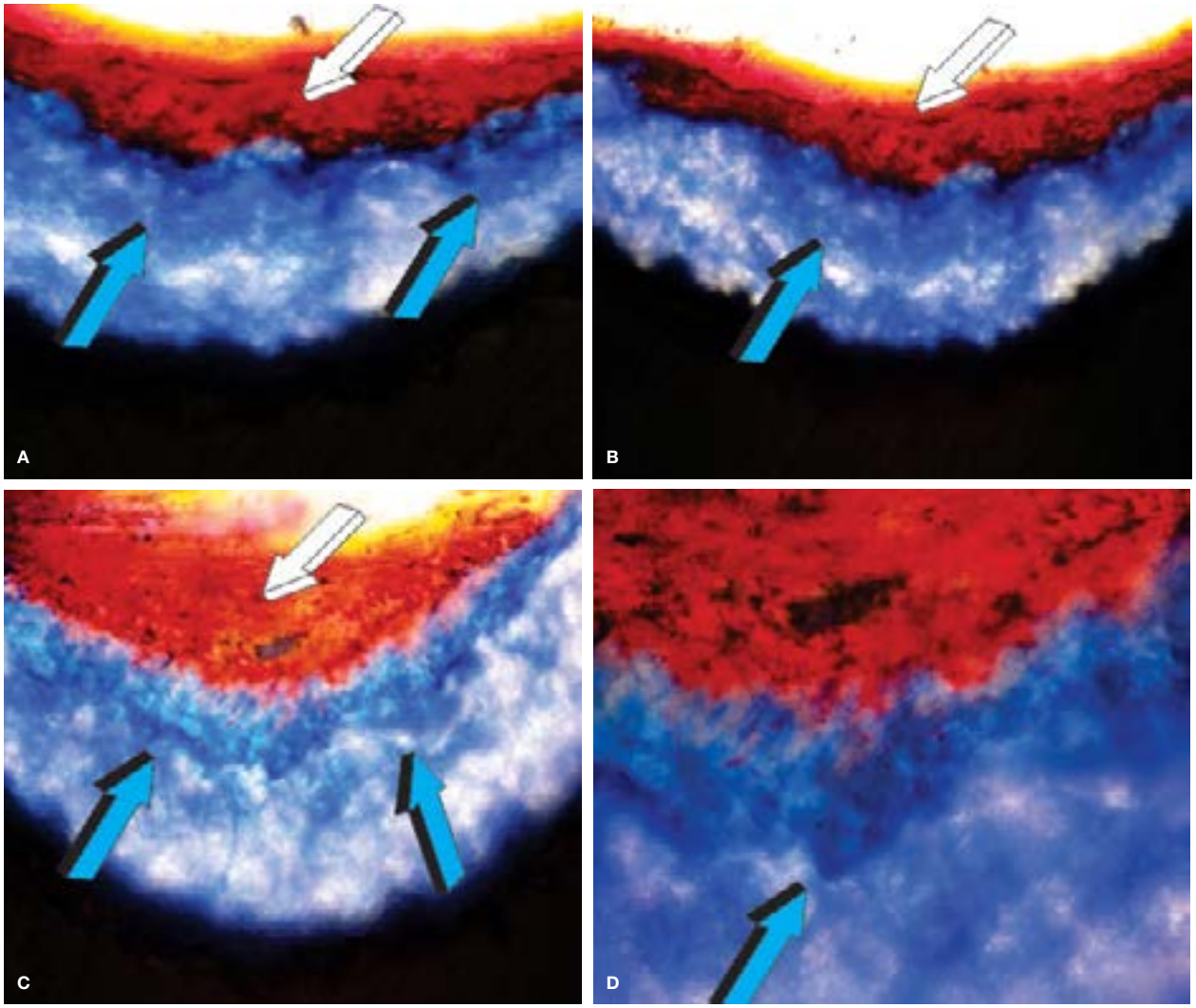

Figure 16. First generation titanium implants with geometric self-inductive configurations: Constructs were implanted in the *rectus abdominis* muscle of *Papio ursinus*[57,58] and harvested 31 months after heterotopic implantation. **A,B,C,D.** Undecalcified sections representing the concavities prepared in the titanium constructs show the induction of bone formation (light blue arrows) tightly attached to the hydroxyapatite plasma sprayed over the titanium geometric construct. Osteoid seams (white arrows) surface the newly formed mineralized bone. Undecalcified sections prepared by the Exakt diamond saw at 40-60 µm. Original magnification (**A,B,C**) x175; (**D**) x225.

Submicron topographically patterned ridges and grooves modulate osteogenic differentiation in human mesenchymal stem cells by up-regulating *RUNX2* and osteocalcin.[63-65]

Functionalization of the topographies of surfaces has been proposed by McNamara's group[76,77] who report that *in vitro* cell responses to 15 nm high nanopillars resulted in larger focal adhesion, up-regulation of the osteogenic transcription factor *RUNX2* and *osteocalcin*, contributing to the greater osteogenic capacity of the nanopatterned substrata.[75,76]

Similarly, nanotopographic cues control the fate and differentiation of adult stem cells when using scalable nanopatterns with defined densities of nanoposts - ultimately "*proposing engineering synthetic tissues with complex geometric organizations*".[81]

Geometrically modulated elastomeric substrata do mechanistically regulate cell function with an impact on cell morphology and stem cell differentiation.[81-83] Recent reports have shown the effect of subcellular geometry in regulating stem cell differentiation.[86] Micropillar geometrical arrays control cell differentiation and the osteogenic phenotype, altering the geometry of the cell nuclei,[86] a new cue that regulates cell differentiation at the subcellular level.[86]

PERSPECTIVES AND LIMITATIONS: BEYOND MORPHOGENS AND STEM CELLS

The theme of geometry in the regulation of cell differentiation, cellular transformation and the induction of the osteogenic phenotype was set last century by the seminal discoveries of Reddi's group.[21-24]

This critical contribution to the geometric influence on differentiation and transformation of fibroblasts is however still poorly understood, and even more poorly cited in the so many articles flourishing at IOSA, reporting the effect of geometry on the pathways of cell differentiation. These include, but are not limited to, the geometric control of capillary architecture 87 as well as geometric cues for directing stem cell differentiation.[80] Indeed tissue geometry also determines the sites of branching morphogenesis in the mammary gland.[88]

The geometric control of capillary morphogenesis[87] introduces yet again the Trueta[54] "osteogenetic vessels" providing evidence that "angiogenesis is a prerequisite for osteogenesis."[54] Following those seminal studies of Trueta on the identification of osteogenetic vessels, recent reports highlight that signalling by endothelial Notch activity promotes endothelial cell proliferation, vessel growth in post- natal long bones and initiates both angiogenesis and osteogenesis in bone.[89]

In further studies, Kusumbe et al.[90] uncovered structurally different capillary subsets with different morphological, molecular and functional properties. These molecularly distinct capillary subsets mediate growth of the bone vasculature, generate distinct molecular microenvironments, and maintain perivascular osteoprogenitors. This coupling of angiogenesis to osteogenesis,[90] provides further molecular evidence for the Trueta "osteogenetic vessels".[54] Watari et al.[79] showed that the combination of soluble molecular osteoinductive media together with topographical cues set by 400 nm pitch surface topography significantly enhanced the expression of osteogenic genes including *RUNX2* and *BGLAP*.[78] Vlacic-Zischke et al.[80] and Ahn et al.[81] further reported the spatial control of adult stem cell differentiation by nanotopographic cues.[80,81]

The latter study showed that the induction of osteogenesis markers by mesenchymal stem cells and/or pericytes is dependent on the geometric density of the nanoposts on the substratum, providing evidence that the nanotopography of the substratum controls stem cell differentiation and outcome.[81]

Modifying the geometries and nanotopographies of surfaces has been and still is a fascinating scenario of tissue and molecular biology. *In vitro* work, however, together with results in animal models, including non-human primate species, may well not adequately reproduce the results of the application of morphogens as conducted in *Homo sapiens*, where the capacity to regenerate has been severely dwarfed through evolution by either the discarding or acquiring of gene pathways inconsistent with tissue induction and regeneration.

What it is that makes the primate *Homo sapiens* heal with difficulties and uninspiringly when compared with animal models, including non-human primates?
What did *Homo sapiens* genetically lose or gain along the continuously unravelling of the marvellous molecular and biological adventure of human evolution?
Only a concerted genetic and molecular approach comparing animal phyla genomes will break the boundaries of super healing. Until that day is reached, tissue engineering and regenerative medicine in man will remain a dream.

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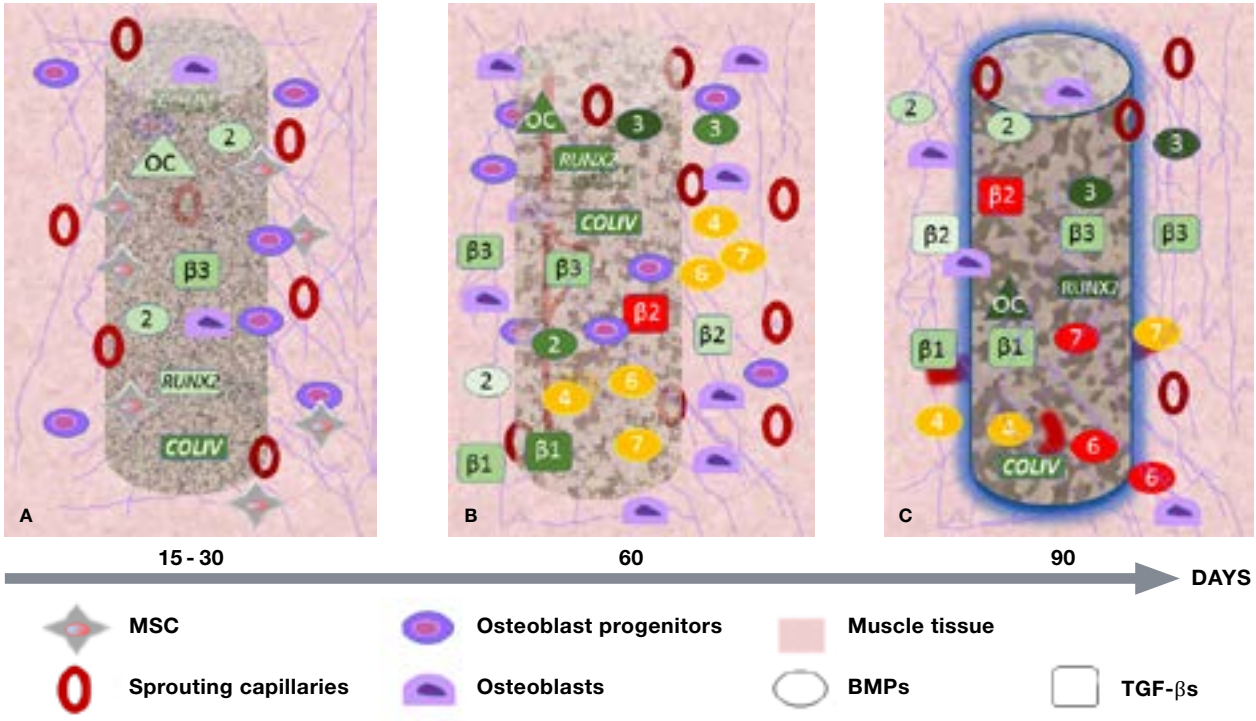

Figure 17. Schematic representation of the spontaneous induction of bone formation by calcium carbonate coral – derived macroporous bioreactors. Macroporous bioreactors, implanted in the *rectus abdominis* muscles of the baboon *Papio ursinus*, were harvested on day 15 (**A**), 30 (**B**) and 90 (**C**).

The interaction of the macroporous device with its unique geometry and the responding microenvironment of the *rectus abdominis* muscle initiate the bone induction signaling cascade. The relative expression profiles of key genes involved in the bone induction cascade are represented. Upregulated genes are indicated in green with the increase in intensity indicating increased expression levels. Genes colored in yellow exhibited no change in expression relative to the control tissue. Down regulated genes are indicated in red.

On day 15 (**A**) peripheral spaces of the 7% HA/CC bioreactors are invaded by vascular connective tissue and parallels a pronounced upregulation of *Col IV* gene expression. Resident mesenchymal stem cells (MSCs) populations are stimulated to differentiate along the osteoblastic lineage as indicated by the upregulation of OC and transcription regulator of the bone forming expression programme.

On day 60 (**B**), newly formed bone has formed in the cavities of the bioreactors. The spaces are now surfaced with osteoblasts that face the highly vascularized matrix. The bone induction cascade is represented at the molecular level by strong upregulation of *BMP2*, *BMP3*, and *TGF-B*$_1$ expression and moderate *TGF-*β$_3$ expression. The expression levels of *BMP4, 6* and *7* remain unchanged relative to the muscle control tissue. By day 90 (**C**) bone levels in the device has increased significantly and the remodeled blocks of lamellar bone can be seen throughout the macroporous spaces (newly formed bone shown in blue with dark grey areas representing newly formed bone in the device). Prominent capillary sprouting is also present. The importance of the responding microenvironment is highlighted by the expression of members of the *BMP* and *TGF-*β family of genes in the muscle tissue adjacent to the implanted device. The differential expression pattern of the *BMP* and *TGF-*β genes over the time course show that the spontaneous induction of bone in the heterotopic sites of the Chacma baboon is the result of the temporal and spatial expression of several genes whose activity have been carefully orchestrated by the intrinsic properties of the implanted calcium carbonate coral-derived macroporous bioreactors.

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Prosthodontic rehabilitation in an elderly patient with a hemi-maxillectomy defect

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MM Mothopi-Peri[1], DG Howes[2], CP Owen[3]

CASE REPORT

Abstract

Prosthodontic rehabilitation in patients with maxillectomy defects can be challenging. When rehabilitating with conventional means, problems of retention and stability are a common occurrence. Implant rehabilitation offers better outcomes in this regard but can be costly for the majority of patients especially in developing countries such as South Africa.

This treatment can also be contraindicated for patients with uncontrolled medical conditions such as diabetes mellitus. This case report illustrates a continuum of treatment in an elderly male patient with a hemi-maxillectomy defect. His successful management was made possible by positive changes that occurred in his life over a certain period of time.

Keywords

Maxillo-facial prosthodontics; maxillary defects; lifestyle-appropriate treatment.

INTRODUCTION

Maxillectomy defects are caused by congenital diseases, cancers, trauma, burns and infections. Infections can be due to diseases such as diabetes mellitus and HIV/AIDS. Rehabilitation of patients with maxillofacial defects poses challenges to both the clinician and the patient. Prosthodontic rehabilitation of elderly patients has been reported in the literature, and some authors have described patients with diseases such as diabetes mellitus.[1]

Treatment protocols ranged from conventional to implant supported prostheses.[2] Most of these reports, however, failed to highlight the importance of other patient factors such as age, medical history, socio-economic status, biologic and psychological status.

The purpose of this case report is to present a continuum of treatment for an elderly diabetic patient with a hemi-maxillectomy defect. It will illustrate the need for a dynamic treatment-planning approach in maxillofacial rehabilitation, mainly because of the possibility of changes in the patient's conditions and situations.

CASE HISTORY

A 71 year old male patient presented with a hemi-maxillectomy defect as a result of a resection carried out as part of managing mucomycosis. During the surgical procedure he lost teeth from the last molar in the first quadrant to the canine in the second quadrant, also leaving him with a defect in the right maxillary buccal vestibule (Fig. 1) which caused an oroantral communication.

The patient's medical history included Type II diabetes mellitus, asthma, hypertension and urinary problems. At initial presentation he was on medication for all these conditions and had a frail appearance.

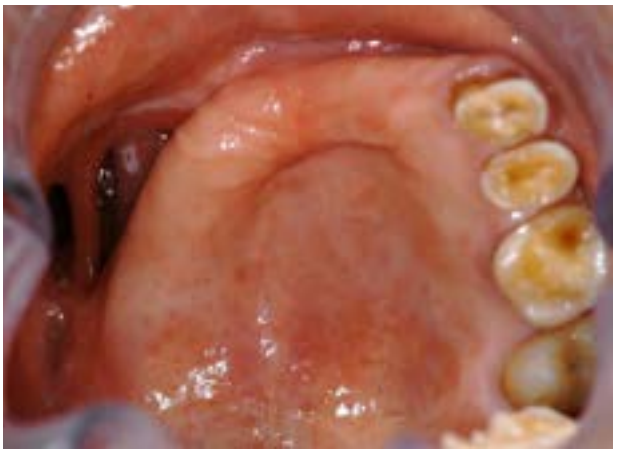

Figure 1. Maxillary arch after surgical resection.

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2. **Dale G Howes:** Supervised the treatment and revised the paper - 30%
3. **C Peter Owen:** Consulted, co-wrote and revised the paper - 30%

The defect and partial edentulousness caused difficulty in mastication, drinking, and speech; which in turn caused his withdrawal from social activities. Due to his medical condition and socio-economic status at the time, non-invasive 'appropriatech'[3] treatment options were carried out for him.

A removable maxillary denture with acrylic obturation was constructed. Retention was not optimal, and over the next five years, three dentures/obturators had to be constructed (Fig. 2). This impacted negatively on the patient's daily activities and quality of life.

During this time the patient's medical condition improved and this warranted consideration for alternative treatment options which would improve his deteriorating oral condition. With motivation, funds were obtained which enabled implant planning and treatment.

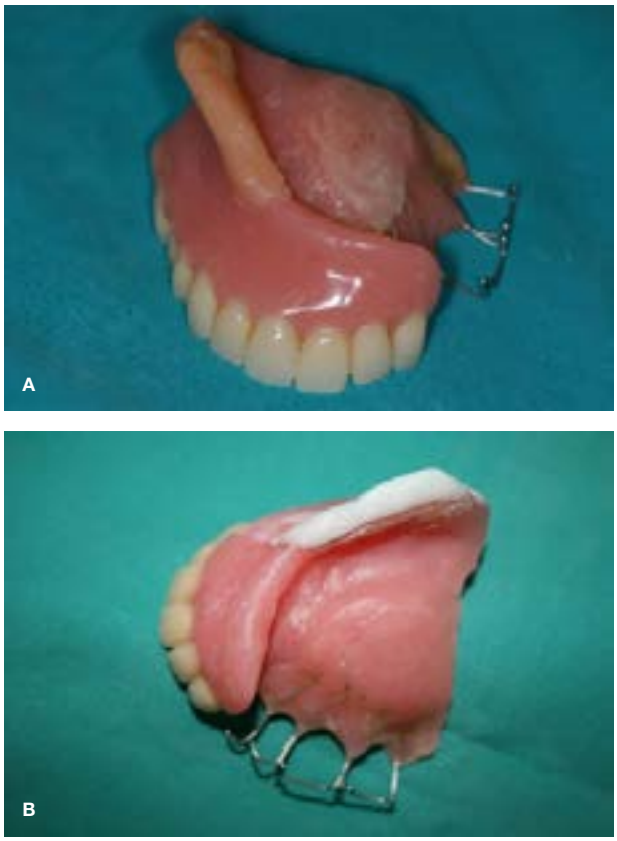

Figure 2. Examples of removable obturators.

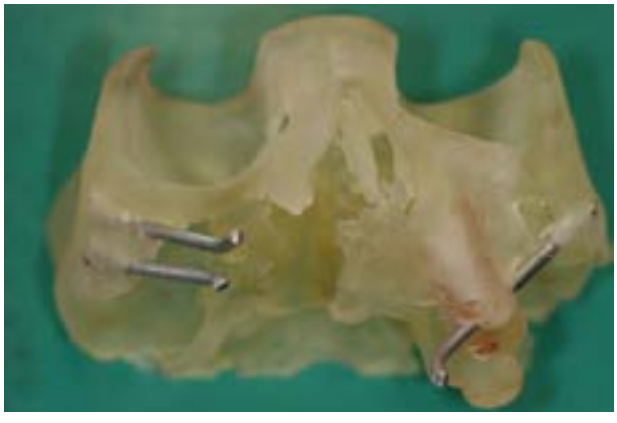

Figure 3. Rapid prototype model, showing positions of oncology implants on the right and the zygomatic implant on the left.

Planning was carried out using cone beam computed tomography, and the production of stereolithic models through rapid prototyping (Fig 3). It was decided to extract teeth in the second quadrant except for the third molar which was to be used as a guide to maintain the occlusal vertical dimension. Implants were placed as follows:

- Two oncology implants (Southern Implants, Irene, South Africa) in the right zygoma.
- One co-axis implant (Southern BAT 12d13) on the left side in the first premolar site.
- One zygomatic (Southern) implant on the left side.

The patient was then rehabilitated with a bar retained overdenture (Fig. 4).

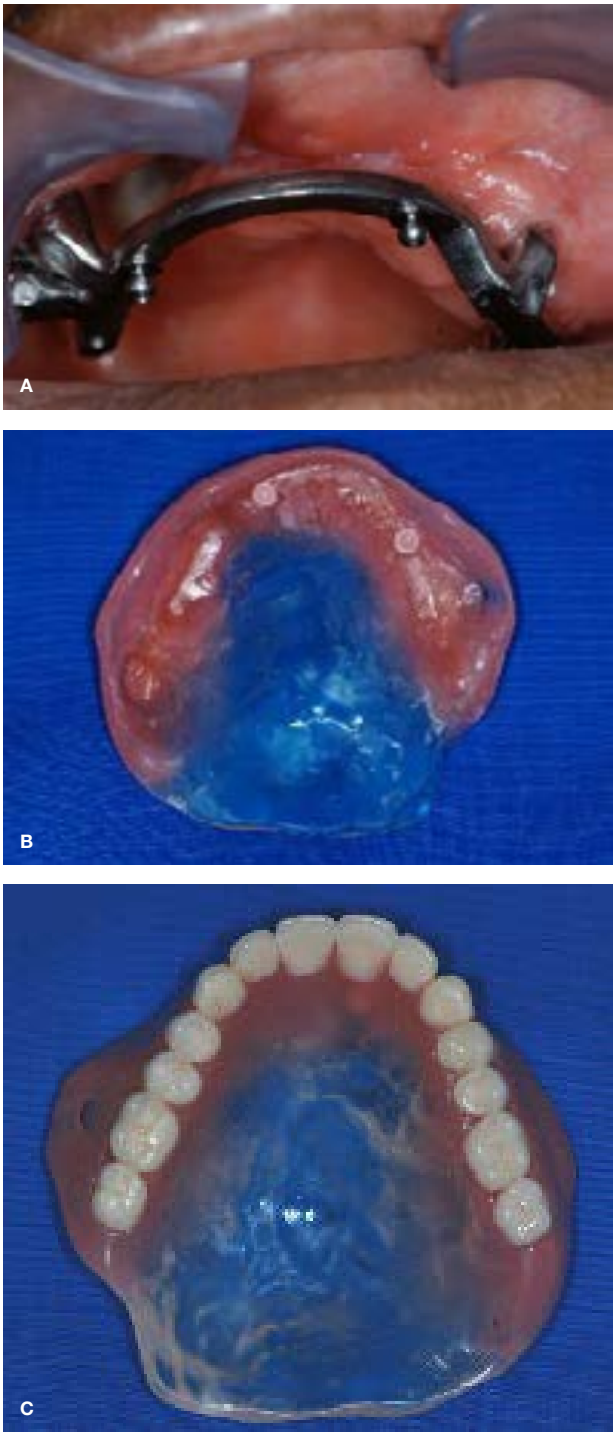

Figure 4. Bar-retained overdenture obturator.

After a year the patient reported difficulty in manipulating the prosthesis and a lack of retention. The bar-retained prosthesis was then converted to a fixed implant supported prosthesis with a separate removable obturator (Fig. 5). This design allowed the obturator to be removed and cleaned without difficulty.[4]

DISCUSSION

Conventional means of rehabilitation are cost-effective and therefore affordable to the majority of patients, especially in a developing country.[5] But they tend to have problems of stability and retention as exemplified in this patient, negatively affecting mastication, deglutition and speech.

Although implant rehabilitation is not a contraindication for elderly and diabetic patients, it is a prerequisite that patients are healthy and well controlled during treatment.[1]

The patient under discussion here benefited from the initial appropriatech cost-effective approach i.e. removable partial dentures (RPDs) with acrylic obturation. When he experienced problems with the RPDs and his health condition improved, implant rehabilitation was considered but could only happen when funds were made available. It was then possible to plan and place implants and rehabilitate with a bar-retained overdenture. When the patient experienced challenges with this, his prosthesis was converted to a fixed implant supported prosthesis with a separate removable acrylic palate/obturator.

CONCLUSION

This case is presented as an example of the dynamic nature of treatment planning, which allowed for a continuum of treatment to be carried out. It demonstrates the importance of versatility when treating patients in maxillofacial prosthodontics, not only between patients but within an individual as well[6] so that treatment is patient mediated and appropriate to prevailing lifestyle and condition.

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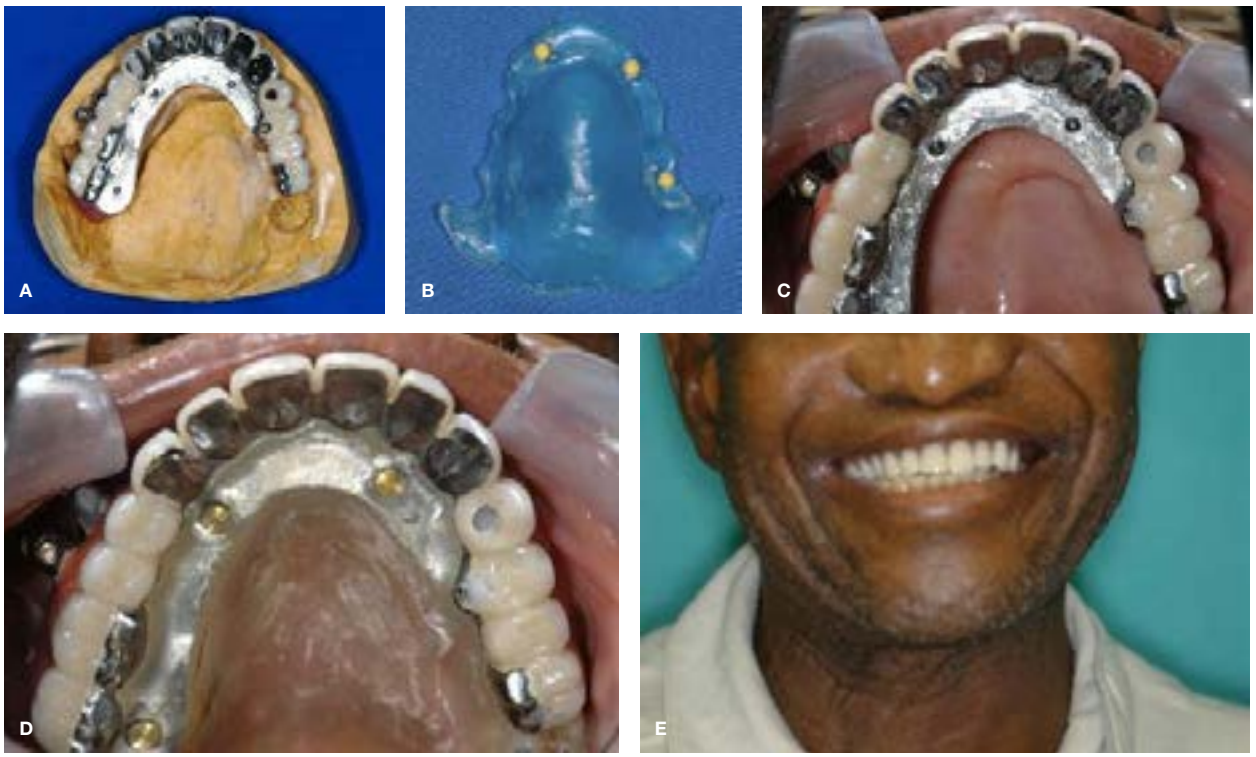

Figure 5. Fixed framework on model (**A**); separate obturator (**B**); Framework in mouth (**C**) and with obturator in place (**D**); final smile (**E**).

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Oral Medicine Case Book: An intra-oral solitary Schwannoma of the lower lip, a rare diagnosis

SADJ September 2019, Vol. 74 No. 8 p448 - p450

G Abrahams[1], MT Peck[2], LXG Stephen[3], J Opperman[4]

CASE REPORT

A 25-year-old clinically healthy female presented to the Oral Medicine Clinic at the University of the Western Cape, with a swelling on her lower lip. The swelling started following a knife injury to the affected area and was present for almost three years. No treatment was sought for the initial injury and the lesion has slowly increased over time. The lump was asymptomatic but the patient requested that it be removed for cosmetic reasons.

She had no significant medical history and her extra-oral examination revealed bilateral submandibular lymphadenopathy. Intra-orally, a 15x15mm exophytic soft tissue lesion was seen on the lower labial mucosa, across the midline. The lesion firm, broad based, non-tender on palpation and was the same colour as the surrounding mucosa (Figure 1).

In addition, a small scar was noted across the midline of the lesion possibly from her previously reported assault. She had poor oral hygiene with visible soft plaque and calculus deposits detected on all her molars as well as multiple carious lesions and missing teeth.

The palpable bilateral lymph nodes could be attributed to the multiple lower carious teeth. The differential diagnosis included a mucocele, salivary gland neoplasm, benign soft tissue neoplasm (based on rate of growth and clinical appearance), traumatic fibroma, or a swelling secondary to the initial trauma.

ACRONYMS

MRI:	Magnetic Resonance Imaging
CT:	Computed Tomography

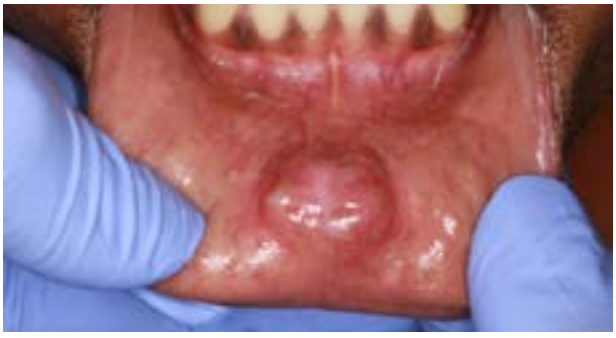

Figure 1. Initial clinical presentation.

Management and diagnosis

An excisional biopsy was performed under local anesthesia. A curvilinear incision was made superficially across the midline of the lesion. Thereafter, blunt dissection was performed to separate the lesion from the surrounding tissue. Following complete enucleation, primary closure was obtained using vicryl sutures.

Postoperative instructions were given and a follow up appointment scheduled one week later. The healing was uneventful at the follow up appointment and the patient was referred to the relevant departments for prophylaxis and management of the carious lesions.

The macroscopic examination revealed an oval, firm well-circumscribed mass measuring 13 x 8.5mm (Figure 2). Microscopic examination showed a well circumscribed, encapsulated neoplasm comprising bland spindle-shaped cells with hyperchromatic nuclei, exhibiting a palisaded arrangement surrounding acellular eosinphilic areas (Verocay bodies) (Figure 3). A predominant "Antoni A" pattern was demonstrated throughout; with, focal "Antoni B" areas also present (Figure 4).

Immunohistochemical staining with S100 protein showed nuclear and cytoplasmic positivity in the spindle-shaped cells, confirming the Schwannian origin of the neoplastic cells (Figure 5).

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2. **Mogammad T Peck:** Review - 10%
3. **Lawrence XG Stephen:** Review - 10%
4. **Johan Opperman:** Histology diagnosis - 10%

DISCUSSION

Schwannomas, also referred to as neurilemmoma or neurinoma, are rare encapsulated benign neural neoplasms of Schwann cell origin.[1-7] Schwann cells form a thin barrier around each extracranial nerve fiber, and wrap larger fibers with an insulating membrane, forming the myelin sheath in order to enhance nerve conductance.[8] First described by Verocay in 1910, these tumours were initially referred to as 'Neurinomas'. Thereafter, in 1935, the term 'Neurilemmoma' was proposed due to the nerve sheath elements associated with these tumours.[4,7,9,10]

Schwannomas occur along the course of somatic, cranial and autonomic nerves, and are more frequently associated with sensorial nerves.[5,6,9] Schwannomas do not arise from cranial nerves I and II, because these nerves lack Schwann cells.[11-13] Schwannomas arise in association with a nerve trunk and as it grows, displaces the nerve. Nerve impingement can become symptomatic.[2,3,5,13] Approximately 25%-45% of all extracranial Schwannomas occur in the head and neck region[1-7,9-12,16-20] with only 1% seen intra-orally.[6,7,12,16-19] Intra-oral Schwannomas are rare, with the tongue being the most common site affected, followed by the palate, floor of the mouth, buccal mucosa and lip.[3-5,7,9,15,16] Furthermore, Schwannomas of the lip is the rarest reported clinical entity and is therefore generally not included in the differential diagnosis of a lower lip swelling.[1,3,6,10,12,14,17,20]

Schwannoma of the lip was first described in 1969 and since then only a few number of Schwannoma cases of the lip have been reported.[12,18] Moreover, as the Schwannoma of the lip enlarges, it also causes obvious aesthetic disfigurement for the patient and can lead to emotional distress.[20] On occasion, it can arise centrally within bone, most commonly in the posterior mandible with concomitant bone expansion with associated pain and paresthesia.[5,7] The tumour can affect individuals of all age groups but is most commonly found in the 2nd and 3rd decades of life, with no gender predilection.[7,18]

The aetiology remains unknown. Some causative factors such as rare genetic predisposition, spontaneous growth, external injury, and chronic inflammation have been postulated.[20] Schwannomas are not usually associated with a history of trauma, even though post traumatic cases have been reported.[7,13,17] Moreover, with the history of trauma in our case as well as the location, a Schwannoma was not considered as a differential diagnosis.

Clinically, Schwannomas are typically an asymptomatic solitary, freely mobile, submucosal mass characterized by slow growth and a smooth surface.[1,3,11] Clinical symptoms are variable depending upon the location, other symptoms may arise, such as dysphonia, nasal obstruction, dyspnea, dysphagia and oral pain.[6,12,13,20]

Schwannomas are usually solitary lesions but infrequently may be multifocal. Multifocal lesions also occur in 1) multiple localised neurilemmomas; 2) in association with neurofibromas, in von Recklinghausen's

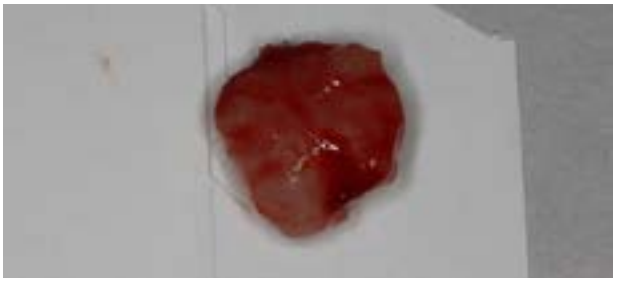

Figure 2. Macroscopic presentation of lesion following surgical excision.

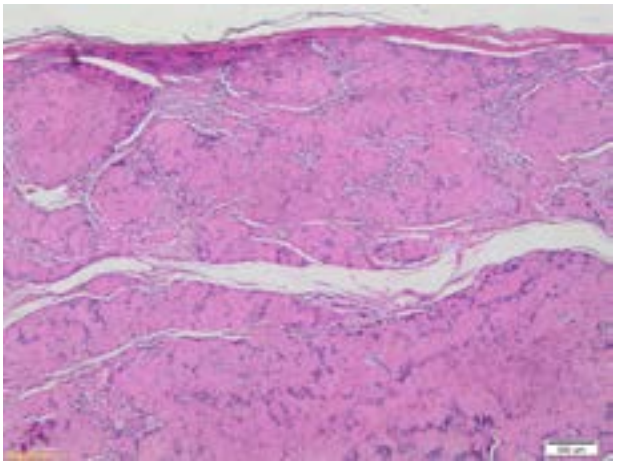

Figure 3. 4x magnification of neoplasm showing streaming fascicles of spindle-shaped cells.

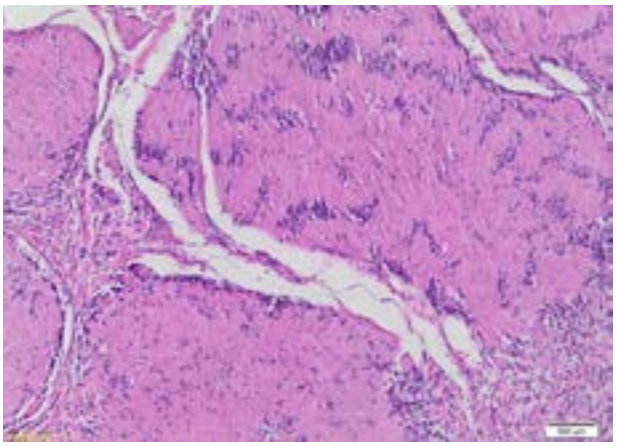

Figure 4. Streaming fascicles of spindle-shaped cells forming palisaded arrangement ("Antoni A area) around central acellular, eosinophilic areas representing Verocay bodies (yellow arrow) and "Antoni B" area (red triangle).

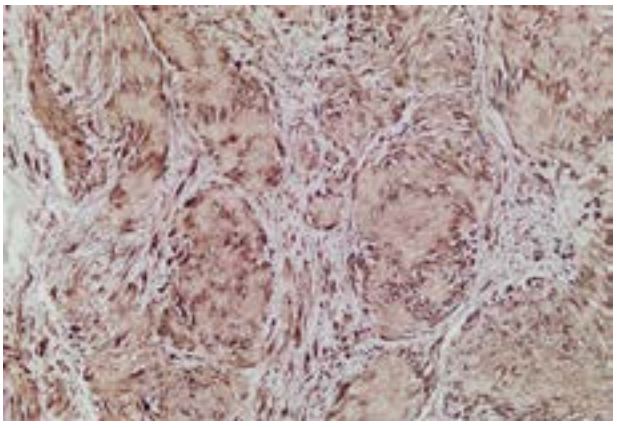

Figure 5. 10 x magnification of S100 positive stained nuclear and cytoplasmic components of spindle-shaped cells.

disease and in 3) Schwannomatosis, a non-hereditary disease characterized by multiple subcutaneous and intradermal Schwannomas along with variety of intracranial tumours.[16,21]

Histologically Schwannomas are usually encapsulated and shows proliferation of spindle shaped-shaped cells, which assume two different patterns. The hypercellular areas, so-called Antoni type A, consists of spindle shaped cells organised in a palisaded pattern usually around eosinophilic areas, forming the so-called Verocay bodies. The hypocellular areas, so-called Antoni type B, are less organised and the spindle cells are randomly arranged within a loose myxomatous stroma.[2] The prognosis of Schwannoma is favourable and no malignant transformation has been reported, local recurrences are rare and this has been associated with incomplete enucleation of the tumour.[1,10,16]

The preoperative diagnosis of intra-oral Schwannomas are challenging. The most commonly applied imaging method is magnetic resonance imaging (MRI).[6] MRI or computed tomography (CT) may be significant in outlining tumour margins, composition and identifying any associated tumour infiltration. These imaging techniques are useful guide for surgical mapping prior to biopsy, which is required to arrive at a definitive diagnosis.[12,20]

Surgical resection of Schwannomas located on the lips is difficult. The intricate neuronal innervation of the lower lip requires precision during surgical removal, to prevent damage. Minimal damage during excision may cause significant morbidity such as impaired speech, aspiration, dysarthria, dysphagia, and paresthesia.[20] A diagnosis of lip Schwannomas of the lip is very rare and is often misdiagnosed in early lesions. A differential diagnosis of lip Schwannomas is often only regarded until a more progressive stage of growth and subsequent cosmetic disfigurement occurs. To our knowledge there have been 3 previous reports of Schwannomas arising in a previous site of trauma.[7,13,17]

Even though Schwannomas are not bluish in colour, a differential diagnoses of a mucocele should be included, as the colouration of a mucocele can vary depending on the lesion size, proximity to the surface and the elasticity of the overlying mucosa.[23] We recommend other differential diagnoses to include a traumatic neuroma, labial minor salivary gland tumours, traumatic fibroma, or a swelling secondary to the initial trauma and a lipoma.[16-18] Albeit rarely encountered, it is our recommendation that intra-oral Schwannomas be included in the differential diagnosis for asymptomatic, well-circumscribed nodules or masses of the lower lip.[7,13,17,22]

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Bite mark analysis in a case of child abuse

SADJ September 2019, Vol. 74 No. 8 p451 - p453

VM Phillips[1], D Avelino[2]

CASE REPORT

As the Forensic Odontology consultant of the Western Cape, I (VMP) was requested by a Lt. Col. of the South African Police Services to examine a child, NL, who was alleged to be a child abuse victim and who had bite marks on her body.

The examination took place at Karl Bremer Hospital, Bellville, in the Paediatric Ward. In attendance were the authors, a nursing sister, Lt. Col. and a Dt. Sgt of the SAPS. The assault on the child had taken place three weeks prior to the forensic odontology examination. According to the Police Officers there were three adults, two females, one male, and two children in the house at the time of the reported assault on the child.

The complainant ZP said the two children present were four years and six months of age respectively. These children did not undergo dental examination. The carer of the child UP was arrested as the possible perpetrator of the assault lesions on the child's body.

The Investigating Officer was requested to present the adults for dental examination at the Dental Faculty of the University of the Western Cape at Tygerberg. There were three females; ZP, UP and the biological mother GL, as well as one male, GE.

Photographs were taken of the teeth of each adult and dental impressions were taken for comparison of their teeth with the bite marks. No dental impressions were necessary of ZP as she had no upper teeth. Each suspect signed an informed consent document with regard to the dental examination.

ACRONYMS

ABFO: American Board of Forensic Odontology

EXAMINATION OF THE CHILD VICTIM

NL was a 2 year old child. She was wearing a 'nappy' and a hospital gown. The gown was removed for examination purposes and the child placed on a hospital examination bed. The authors examined the child's body for bite mark lesions.

Numerous lesions were seen on the back, abdomen and legs of the child. These lesions were judged to be in the late stages of healing and had left poorly distinctive bruises on the skin.

One of the lesions situated on the left abdomen was more visible and had defined healing puncture wounds corresponding to a bite mark (Fig. 1). The other lesions on the right side of the back and on the right leg were not well defined (Fig. 2), but were consistent with bite marks.

Photographs were taken of the lesions using a NIKON digital camera and an ABFO millimetre scale for size reference.

The various lesions on the skin of the child were relatively indistinct; some however showed definitive patterns of bite marks. The bite mark on the left abdomen (Fig. 2) was more noticeable and was used to investigate and to test correlation with the biting patterns of the adult suspects.

The sizes of the bite marks were consistent with adult dental arches and therefore the children could be dismissed as possible suspects.

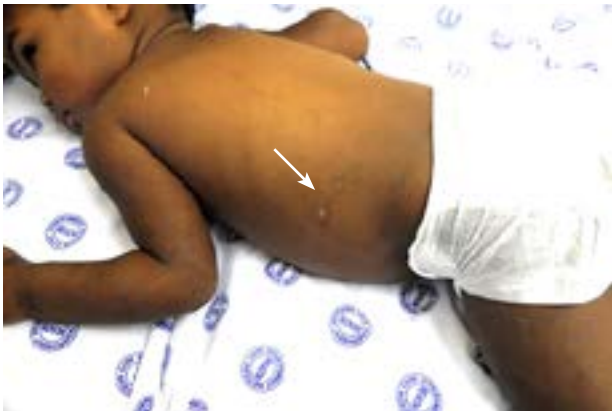

Figure 1. A bite mark on the left abdomen of the victim.

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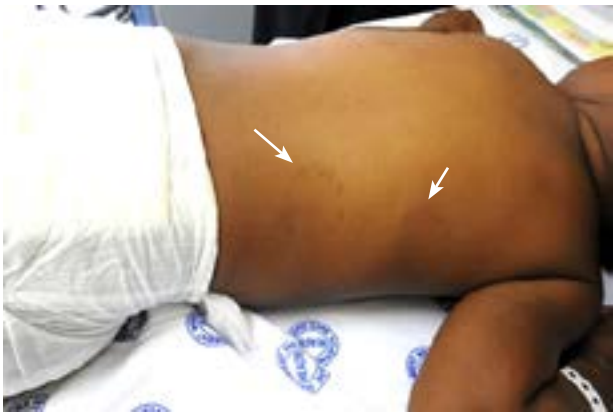

Figure 2. Two other bite marks on the right back of the victim. These were less distinct.

Analysis of the bite mark

Most of the bite marks on the body of the child were indistinct and in various stages of healing. The history of the assault showed that these lesions were approximately three weeks old during which the lesions had undergone healing.

One of the bite marks situated on the left abdomen of the child showed puncture wounds in stages of healing (Fig. 3). Between these puncture marks there was a faint curved pattern of bruises.

These puncture lesions were significant enough to analyse the biting patterns of the three adults and to facilitate correlation with the tracings of their upper and lower dental arches.

Only the upper teeth of the three suspects were investigated as the cause of the bite mark as the bruises due to the lower teeth were too indistinct.

Figure 3 shows the bite mark on the left abdomen with an ABFO scale *in situ*.

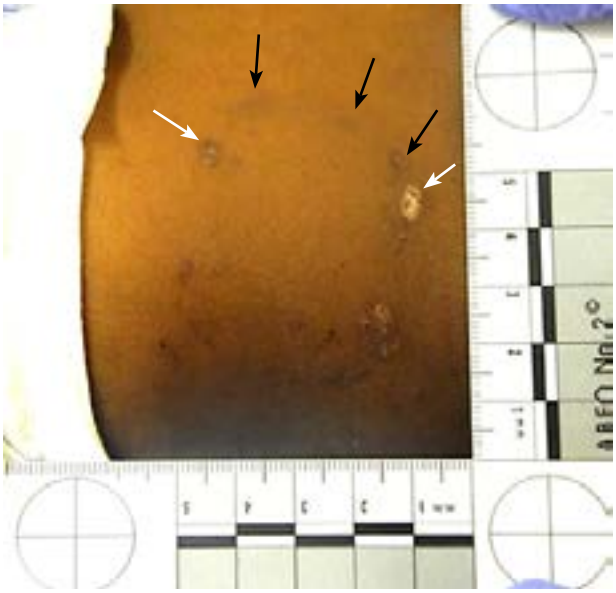

Figure 3. The bite mark on the left abdomen of the victim showing healing of the puncture wounds which were due to maxillary teeth (White arrows) and the indistinct curved bruise pattern due to The Incisors (Black arrows).

Examination adult suspects

Three adult suspects who may have inflicted the bite marks on the child were brought to the Dental Faculty of the University of the Western Cape for dental examination.

Written consent for the dental examination was obtained for each suspect. Examination of ZP (the complainant) showed that she had no upper teeth and this eliminated her as a possible bite perpetrator.

The suspect that was arrested (UP) was the first biting pattern to be analysed. A tracing of her biting pattern of her upper teeth was superimposed over the photograph of the bite mark of the left abdomen of the child (Fig. 4).

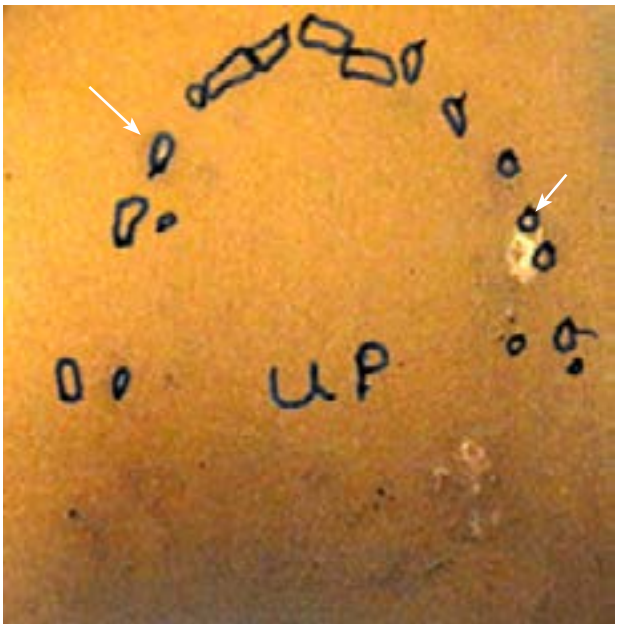

Figure 4. The tracing of the biting pattern of UP superimposed on the lesions of the bite mark.

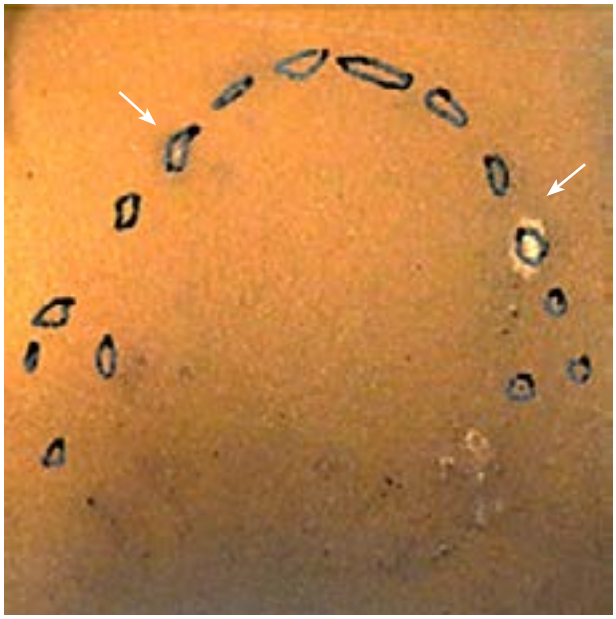

Figure 5. The tracing of GL's biting pattern of her upper teeth showed an accurate match with the bite mark.

When the left canine is placed on the puncture wound the right 1st premolar almost overlays the right puncture wound. This showed a possible correlation and that the bite mark could have been inflicted by UP's upper teeth.

The biological mother GL was examined and the tracing of her biting pattern was superimposed over the photograph of the bite mark (Fig. 5). There was an accurate alignment of her upper teeth with the bite mark on the child.

The third suspect GE only had two maxillary canines and his dental arch was much narrower than the bruise pattern of the bite mark.

DISCUSSION

Bite marks are associated with an oval pattern of bruises or puncture wounds on the skin of the victim. These lesions are due to the force of the biting surfaces of the upper and lower teeth into the skin of the victim.

The degree of definition of a bite mark is dependent on the force with which the bite is inflicted. The analysis is dependent on the time span between the biting of the victim and when the bite mark is examined. The longer the time span the less distinct the bruises because healing of the bite mark lesions takes place.

The skin is a malleable medium and is subject to distortion when a force is applied. This is the reason that bite mark analysis is not an exact science, but an adjunct in the forensic analysis of trauma.

The method of analysis of bite marks depends on the matching of the biting pattern of the suspect with a 1:1 photograph of the bite mark being investigated. Ideally the bite mark would show the bruises caused by the upper and lower anterior teeth in an oval or curved pattern.

In this case healing of the superficial lesions had taken place leaving only the distinct puncture wounds due to the canines or first premolar teeth. The examination of the bite marks on the body of the child took place approximately three weeks after the assault.

The time span resulted in poor definition of the bite marks for comparison purposes because healing of the lesions had occurred.

One of the bite marks, however, showed distinct puncture wounds. This bite mark, situated on the left abdomen of the child, was used to investigate the biting patterns of the three suspects by the superimposition of their biting patterns of their upper teeth over a 1:1 photograph of the bite mark.

The results of the analysis of the biting pattern of UP's upper teeth with the bite mark showed that she may have bitten the child. Her dental arch of her upper teeth, however, show a slightly wider arrangement of the teeth (Fig. 4) compared with the puncture wounds and the position of the upper right teeth did not match the bite mark accurately enough. There is a moderate degree of probability that UP inflicted this bite mark on the child.

The analysis of the biting pattern of GL's upper teeth with regard to the bite mark showed that the dental arch of her upper teeth matched the puncture wounds. The upper left canine tooth and the upper right canine and 1st premolar teeth matched the puncture wounds (Fig. 5).

CONCLUSION

The results of the analysis of the biting patterns of the three suspects showed that the most likely person to have inflicted the bite on the left abdomen of the child was her biological mother GL. Her biting pattern matched the bite mark accurately. There was a high degree of probability that the perpetrator of the bite mark on the abdomen of the child was inflicted by the child's mother (GL).

Declaration

No conflict of interest declared.

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What's new for the clinician: excerpts from and summaries of recently published papers

SADJ September 2019, Vol. 74 No. 8 p456 - p461

Compiled and edited by V Yengopal

1. Efficacy of Endo-Ice followed by intrapulpal ice application as an adjunct to inferior alveolar nerve block in patients with symptomatic irreversible pulpitis - a randomized controlled trial

Koteeswaran V, Ballal S, Natanasabapathy V, Kowsky D.
Clin Oral Invest. 2019; 23: 3501.

Endodontic treatment among patients with symptomatic irreversible pulpitis (SIP) in lower molars is often associated with pain as the failure rate of the inferior alveolar nerve block (IANB) ranges between 44% and 81%.[1]

A wide range of additional methods have been employed to improve the anesthetic efficacy of IANB in SIP. The percentage of success of these techniques is highly variable.

The techniques include the use of pre-anesthetic medication, increasing the volume of anaesthesia, intramucosal administration of drugs like ketorolac, tramadol, and magnesium sulphate, adding mannitol to lignocaine, intraosseous, intraligamentary, and intrapulpal anesthetic injections.[1]

Cryoanalgesia has been successfully used in the field of medicine for managing both acute and chronic pain. The application of cold decreases the tissue temperature, resulting in vasoconstriction, reduction in the tissue metabolites, and downregulation of inflammatory mediators and neuropeptides, thereby reducing pain.

Cryoanalgesia is a simple, relatively inexpensive and non-invasive adjuvant for pain management. Cryoanalgesia has numerous applications in dentistry. Topical cold application (in the form of Endo-Ice) at the injection site prior to palatal anaesthesia has been employed to reduce the pain on injection. However, to date, there are no clinical trials evaluating the effect of cold when used for supplementary anaesthesia in reducing intraoperative pain during pulp extirpation. Koteeswaran and colleagues (2019)[1] reported on a trial that sought to evaluate the efficacy of Endo-Ice followed by intrapulpal ice application for relieving pain during pulp extirpation in mandibular molars with symptomatic irreversible pulpitis (SIP). The secondary objective was to evaluate the effect of Endo-Ice followed by intrapulpal ice application in reducing dental anxiety.

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ACRONYMS

DAS-R:	Corah's Dental Anxiety Scale-Revised
IAN:	Inferior Alveolar Nerve Block
SIP:	Symptomatic Irreversible Pulpitis

MATERIALS AND METHODS

After initial screening of 94 patients, a total of 60 patients who met the inclusion criteria participated in this randomized controlled single blinded study. Baseline parameters such as age, gender, vitality status, pre-operative pain, and dental anxiety scores were recorded prior to treatment.

The level of pre- and post-intervention pain was recorded as a continuous data using simple Visual Analogue Scale (VAS). The VAS consisted of a line of 10cm length anchored by two extremes with 0 signifying no pain and 10 representing the worst pain imaginable. The dental anxiety of the patient was measured using the Corah's Dental Anxiety scale-revised (DAS-R) before and after intervention.

Patients were included into this randomized single-blind controlled trial if they were diagnosed with SIP planned for endodontic treatment in mandibular molars, were within the age group of 20-50 years with a

preoperative VAS score > 4 (Moderate to severe pain). Patients not willing to participate in the study, age > 50 years, patients who reported with a history of pain on cold application, immature young permanent teeth, teeth that did not respond to traditional vitality tests, acute apical abscess, calcified pulp chamber and root canals, previously filled root canals, chronic apical abscess with or without sinus, periodontally compromised teeth, cases of necrotic pulp, medically compromised patients, patients with a history of intake of analgesics, steroids, and/or antibiotics in the recent past 48 h were excluded.

Patients were randomly assigned to any of the three following groups:

- Control group (IANB): Standard inferior alveolar nerve block was administered using a self-aspirating 27-gauge needle with 2 ml of 2% lignocaine hydrochloride with adrenaline 1: 80000 (Lignospan Special, Septodont).
- Articaine group (IANB-A): Inferior alveolar nerve block was administered as described in the control group. Supplementary buccal infiltration with 2 ml of 4% articaine with adrenaline 1:100000 (Septanest, Septodont) was administered in the mucobuccal fold using a self-aspirating 27-gauge needle against the tooth of interest, deposited over a period of 2 min (1 ml/min).
- Cold group (IANB-C): Standard IANB was administered as described previously. Two forms of cold (Endo-Ice and Ice sticks) were used. Prior to access opening, Endo-Ice was applied on the buccal, lingual (three seconds per surface), and occlusal surfaces (four seconds) for a total of 10 seconds and access opening was performed immediately. The pulp was exposed and deroofing was done. Following which, Ice sticks (8 m diameter and 2 cm length) were carried with the help of a tweezer and directly placed on the pulp chamber for a period of four minutes (two Ice sticks for two minutes each).

In all the groups, topical anesthetic gel (20% benzocaine) was applied for 60 seconds at the site of injection prior to administration of IANB.

Throughout the trial, the patients were asked to quantify the level of pain to the outcome assessor at 3 intervals, i.e., preoperative, during access opening and immediately after pulp extirpation. The root canals were medicated with Calcium hydroxide and temporarily sealed with IRM.

The patients were given the DAS-R questionnaire and their level of anxiety was noted. The endodontic treatment was completed one week later. Patients who required additional anaesthesia during treatment were administered intraligmentary injection and intrapulpal injection (if required) and were excluded for final analysis.

RESULTS

A total of 60 patients were included for the final analysis (32 females and 28 males). The baseline parameters including age, gender, and preoperative VAS score and dental anxiety scores were not statistically significant among the three groups ($p > 0.05$).

The mean VAS score during access opening was least for articaine followed by cold and control group (p value = 0.02). However, there was no significant mean difference in VAS values between cold and control group (p value = 0.95). The mean VAS scores during pulp extirpation were higher in the control group compared with the cold group and articaine group (p value = 0.001).

The difference in the VAS scores between the two test groups during pulp extirpation was statistically insignificant (p value = 0.99). Further, cold significantly reduced the level of anxiety when compared with the articaine or control group (p value = 0.001).

CONCLUSIONS

The authors concluded that the Endo-Ice application on the tooth surface did not produce adequate anaesthesia during access opening when compared with buccal infiltration with articaine. However, supplementary buccal infiltration with articaine or intrapulpal ice application improved anaesthesia during pulp extirpation in symptomatic irreversible pulpitis (SIP). Additionally, cold significantly reduced the dental anxiety after treatment.

Implications for practice:

Cold may be considered as a simple, cost-effective, chairside supplementary anesthetic technique for effective management of endodontic pain and anxiety.

Reference

1. Koteeswaran V, Ballal S, Natanasabapathy V, Kowsky D. Efficacy of Endo-Ice followed by intrapulpal ice application as an adjunct to inferior alveolar nerve block in patients with symptomatic irreversible pulpitis - a randomized controlled trial. Clin Oral Invest. 2019; 23: 3501.

2. Esthetic, clinical, and radiographic outcomes of two surgical approaches for single implant in the esthetic area - one-year results of a randomized controlled trial with parallel design

Huynh-Ba G, Hoders AB, Meister DJ, Prihoda TJ, Mills MP, Mealey BL, Cochran DL.
Clin Oral Implants Res. 2019; 30: 745-59.

The timing of implant placement in relation to tooth extraction remains a topic of much debate. A consensus conference of the International Team for Implantology (ITI) defined four types of implant placement (Type 1 to Type 4) based on the amount of healing time and the corresponding soft and hard tissue healing obtained following tooth extraction (Figure 1).

ACRONYMS

ITI:	International Team for Implantology
mPI:	Modified Plaque Index
mSBI:	Modified Sulcus Bleeding Index
PES/WES:	Pink and White Aesthetic Scores
PPD:	Probing Pocket Depth
VAS:	Visual Analogue Scale

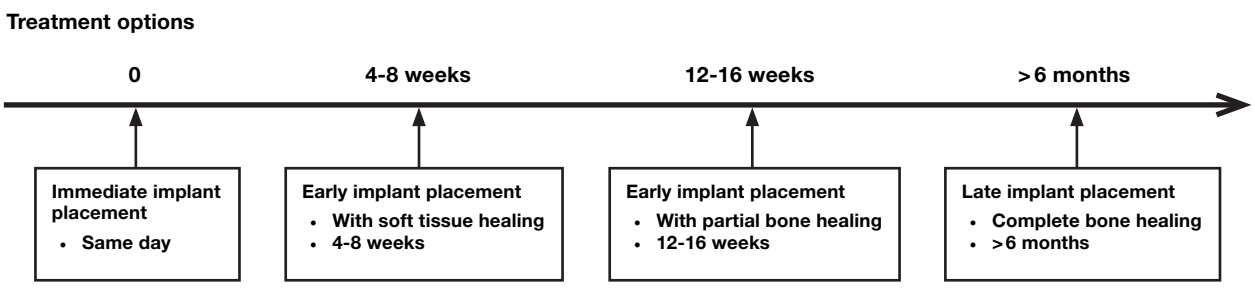

Figure 1. The four treatment options for post-extraction implant placement (ITI consensus statement)

Studies suggested that similar implant survival rates can be obtained irrespective of timing of implant placement. However, the emphasis on aesthetic outcomes in determining clinical success has gradually increased over the years, leading to the development of tools allowing the assessment of implant-supported restoration aesthetics.

Among existing objective aesthetic parameters, those fre-quently used include the pink and white aesthetic scores (PES/WES) and the mid-facial marginal soft tissue level.

Huynh-Ba and colleagues (2019)[1] reported on their follow-up data at one year following final crown delivery, comparing aesthetic, clinical, and radiographic outcomes of immediately (Type 1) and early placed implants (Type 2). The primary outcome was the mid-facial soft tissue level margin and the initial hypothesis upon which the study was designed was that following Type 1 implant placement significantly different mid-facial recession would be observed as compared with Type 2 implant placement.

METHODS

Fifty patients requiring tooth extraction and replacement with a dental implant were enrolled in this study.

Patients were considered for study inclusion if they were 18 years of age or older, had one tooth in either the anterior maxilla or mandible, including incisors, canines, and premolars, requiring extraction leading to a single-tooth gap to be replaced by means of an implant.

Neighbouring teeth had to be periodontally healthy with no interproximal bone loss. The walls of the extraction socket had to be intact. Patients were excluded from the study if they were currently smoking more than 10 cigarettes per day, had significant untreated periodontal disease or a history of treated periodontitis, had a medical condition or were taking medications which affected soft tissue and bony healing (e.g., history of oral/IV bisphosphonates, poorly controlled diabetes, chemotherapeutic and immunosuppressive agents).

Prior to extraction, a customized radiographic film holder was fabricated for each prospective implant site allowing the examiners to record standardized periapical radiographs throughout the study using a long-cone parallel technique. Alginate impressions were made and initial study casts were obtained.

Photographs were taken perpendicularly to the facial surface. The same methodology and settings on a single camera were used throughout the study.

Following tooth extraction and confirmation of the integrity of the alveolar socket, an envelope containing the concealed treatment group allocation was opened by the surgeon to allocate the patient to immediate (Type 1) or early (Type 2) implant placement protocol following the generated random sequence.

All the surgical procedures were performed under local anaesthesia with or without parenteral sedation based on patient preference. Prior to surgery, patients were instructed to rinse for one minute with a 0.12% Chlorhexidine rinse. Implants used were bone level titanium implants with a sand-blasted, acid etched, and chemically modified hydrophilic surface (Straumann®).

In patients randomized to the Type 1 group, the integrity of the socket walls was evaluated and if intact, the surgeon would proceed with implant placement. A limited full-thickness envelope flap (up to 5mm beyond the crest of bone) was reflected to allow for bone measurements and the implant bed was prepared according to manufacturer recommendations.

A conical healing abutment of height and diameter based on the operator's preference was then placed on the implant and the gap between the implant and the internal walls of the socket was grafted using mineralized freeze dried bone allograft (Straumann®), and covered by a resorbable collagen membrane (BioGide®). The flap was coronally advanced and sutured. Primary closure of the wound was not a requirement.

For patients allocated to the Type 2 placement protocol, a collagen plug (Ora-plug®) was placed into the extraction socket and sutures were placed over the extraction site using resorbable sutures.

Following 4–8 weeks of healing, a muco-periosteal flap was elevated which consisted of a full-thickness flap which was extended mesio-distally 1–2 teeth adjacent on each side of the implant site and a single vertical releasing incision on the distal aspect of the flap.

The implant was placed and a simultaneous guided bone regeneration procedure was performed using freeze dried bone allograft (Straumann®) to over-contour the bone on the facial aspect of the implant.

The grafted area was covered with a resorbable collagen membrane (BioGide®) and a periosteal releasing incision was performed to coronally advance the flap. A cover screw or a short conical healing abutment (≤2 mm) was placed based on operator's preference with the requirement that tension-free primary flap closure would be achieved.

Immediately after implant installation and prior to any grafting procedure in both treatment groups, bone measurements were recorded using the following landmarks:

- S = Implant shoulder
- BC = Top of the bone crest
- IB = Internal border of the top of crest
- EB = External border of the top of crest
- D = Base of the defect

All patients received systemic antibiotics:
amoxicillin 500 mg, three times per day for seven days. If allergic to penicillin, patients were prescribed doxycycline 100 mg twice per day for seven days, azithromycin 500 mg on the first day followed by 250 mg once per day for days two through five, or clindamycin 150 mg four times per day for seven days based on surgeon's preference. Choice of pain medication prescribed was based on the operator's preference.

Patients were instructed to rinse with 0.12% Chlorhexidine twice per day for two weeks. Patients were also prescribed 4 mg Medrol Dosepak (methylprednisolone) to limit post-operative swelling based on surgeon's preference.

All patients returned for suture removal and post-operative assessment at 7–14 days after surgery. Further follow-up visits were scheduled at one and two months post-implant placement.

Four months following implant placement, patients returned for the second stage uncovering procedure. Following an uneventful 7–14 days of healing, the patient was referred back to the primary care provider for the restorative phase. The patients were recalled after final crown delivery, three, six, and 12 months.

The aesthetic outcomes were evaluated in two ways:

(a). Soft tissue dimensions were recorded using digital photograph of the casts and the Gingival Status Software to measure the level of the mid-facial mucosal margin and the height of the papillae prior to extraction, three and 12 months after final crown delivery.

(b). The PES/WES as described by Belser et al. (2009) were determined by three independent examiners (ABH, GHB, BSD), two of whom were blinded as to which of the Type 1 or 2 placement protocol was used (GHB, BSD).

A calibration session consisting of the evaluation of 15 anterior implant cases unrelated to the study was performed prior to the assessment.

Clinical parameters including probing pocket depth (PPD) recorded to the nearest millimeter, modified plaque index (mPI), and modified sulcus bleeding index (mSBI) were recorded using an UNC-15 probe at four sites around the implant.

Radiographic bone level changes were evaluated using standardized radiographs made from customized plastic film holders Radiographs were taken immediately following crown delivery as well as at 12 months post-loading.

The distance of the first bone to implant contact in relation to the implant shoulder defined the bone level on the mesial and distal of the implant surface.

Patient satisfaction related to the aesthetic outcome, timing of implant placement, pain, and swelling associated with the procedure was evaluated by filling out a questionnaire at the three months after final crown delivery visit.

Answers were recorded on a visual analogue scale (VAS) ranging from 0 to 10 labelled with “completely

satisfied," "no pain," "no swelling" at the zero point and "not satisfied," "extreme pain," "extreme swelling" at the ten end point. Patients were also asked whether or not the implant restoration affected their ability to speak or to eat.

RESULTS

Fifty patients were enrolled to participate in the study. Four patients were exited prior to randomization:

Two patients were withdrawn due to buccal bone dehiscence and/or fenestration observed at the time of extraction, one patient was withdrawn due to a localized infection and one patient was withdrawn due to adjacent tooth fracture after enrolment and prior to extraction.

After randomization, two patients in the immediate implant placement group (Type 1) were exited. One patient was withdrawn due to lack of primary stability and another one due to unacceptable proximity between the implant and the mental foramen.

Forty-four patients (22 in each group) received a dental implant and nine additional patients were lost to follow-up or did not receive restorations: Two patients (one Type 1, one Type 2) never pursued restorative treatment, two patients (two Type 2) did not report to the three-month follow-up, two patients (two Type 2) did not report to the six-month follow-up, two patients (two Type 2) did not report to the 12-month follow-up and one patient (Type 1) was lost due to fracture of the adjacent tooth prior to 12-month follow-up. Thirty-five patients completed the 12-month post-implant loading evaluation, including 20 Type 1 patients and 15 Type 2 patients.

The reasons for extraction included external root resorption, deep recurrent decay on previously restored teeth and fractures at or below the gingival margin of the tooth. Except for two teeth, all the teeth treated within the study were maxillary anterior teeth.

All the lateral incisors sites (n = 12) received a 3.3 mm diameter implant and the remaining sites a 4.1 mm diameter implant. The implants were of 10, 12 or 14 mm in length.

At the one-year follow-up, all implants were well integrated resulting in a 100% implant survival for both Type 1 and Type 2 implant placement. The surgical procedure was well tolerated by the patients with the exception of two patients in the Type 2 group who experienced significant swelling and bruising following surgery, which resolved by the time of the 2-week post-operative appointment.

At the time of implant placement, significantly greater horizontal defect dimension and vertical defect depths were observed on the buccal and palatal aspect of the implants in the Type 1 group compared with the Type 2 group. Conversely, the level of the buccal bone in relation to the implant shoulder was significantly more apical in the Type 2 group where buccal dehiscences were consistently observed at the time of implant placement. Despite these initial differences at the time of implant placement, resolution of these defects was observed at the time of Stage Two surgery and no significant differences were observed for any of the osseous measurements performed between the two treatment groups.

The overall soft tissue changes were evaluated relative to each of the following parameters: mid-facial gingival margin location and the mesial and distal papillae height.

For the mid-facial gingival margin, some recession was observed from baseline to the 12-month follow-up visit and amounted to 1.03 mm ± 0.24 mm in the Type 1 group and 1.37 mm ± 0.28 mm in the Type 2 group which did not reach statistical significance ($p = 0.17$).

Conversely, the level of the mesial papillae receded significantly more for the Type 2 group (1.08 ± 0.33 mm) at 3 months compared to the Type 1 group (0.55 ± 0.21 mm, $p = 0.02$). At the 12-month follow-up, no significant difference was found between the two treatment groups and both papillae had receded about 1 mm.

The aesthetic outcome of the final restoration was evaluated at the 12-month follow-up visit using the PES/WES criteria previously defined by Belser et al. (2009).

The inter-examiner agreement rate was characterized by an intraclass correlation of 0.87 and 0.85 for Type 1 and 2, respectively. None of the five individual parameters of the PES or WES, nor the total PES or WES were significantly different when comparing the outcome following Type 1 and Type 2 implant placement.

When the threshold for clinical acceptability was set at PES ≥ 6, the percentage of clinically acceptable cases was 55% for the Type 1 group and 40% for the Type 2 group. Taking the same threshold for the WES, the percentages of clinically acceptable cases were 45% and 27% for the Type 1 and 2 group, respectively. None of these differences were statistically significant ($p > 0.05$).

At the 12-month follow-up examination, the recorded peri-implant clinical parameters showed mostly no significant difference between groups in all cases. These parameters reflected non-inflamed tissue with probing depths (PPDs) less than 3 mm, minimal bleeding on probing and limited amounts of plaque.

Radiographic peri-implant bone levels at baseline were on average at 0.1-0.2 mm from the shoulder of the implant with no significant difference between the two groups. These levels remained stable in both groups over the 12-month observation period.

Patients were overall very satisfied in both treatment groups. Both groups reported low levels of pain and swelling associated with the surgical procedure and a similar fraction in both groups reported some impairment in their ability to speak or eat following treatment (Table 7). None of the differences between groups were statistically significant.

CONCLUSIONS

Previously reported results demonstrating that few differences were observed in clinical, aesthetic, and patient-centered outcomes three months after final crown delivery on implants placed according Type 1 or Type 2 implant placement protocols, were further substantiated at the one-year timeline. Additionally, stable radiographic crestal bone levels with no difference between treatment groups were documented.

Implications for practice

This study suggests that immediate and early implant placement protocols are two treatment alternatives with no significant differences in outcomes at one year following crown delivery.

Reference

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Do the CPD questionnaire on page 465

The Continuous 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.

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http://dx.doi.org/10.17159/2519-0105/2019/v74no8a6

The bifidity of **dignity**

SADJ September 2019, Vol. 74 No. 8 p462 - 463

WG Evans

CASE

The concept of Dignity may be considered as integral to the human condition.. and most observers would agree. Where there is less agreement, however, is how each observer would define Dignity.

It is intriguing to realise that despite the universal recognition of the importance of Dignity, there remains considerable debate as to what exactly is implied by the concept.

In the healthcare professions, dignity and ethics are intimately interwoven, as exemplified by Thomas Aquinas who claimed that there was a virtue in acknowledging the dignity of a person, respecting and honouring that status.[1] Aquinas called this virtue *observantia*, claiming that justice depended on the observation of dignity.[1]

That explanation of dignity at once runs against the definitions commonly provided in dictionaries when the emphasis appears to be one of dignity being related to the individual… a type of self respect, a personal attitude.

Roget's International Thesaurus lists several characteristics together with "dignity: stateliness, portliness, courtliness, grandeur, loftiness, nobility, lordliness… etc. and even includes at the end of the list of 19 similar words... the state of sobriety! It is clear that in the healthcare professions there should be a broader understanding and application... and that it is the dignity of the patient which may be the more important.

Once again, Aquinas spells it out.. the action of observantia, which already recognises the relevance of the other person… should be supported by the action of *misericordia*… which is the empathy shown when confronted by those suffering an affliction.[1]

Consider the patient whose personal dignity may be challenged ...and even denied. There is an initial loss of dignity when it is a toothache which has brought the patient to the surgery. Then the requirement that there should be obedience to instructions ...is that why those attending for treatment are called "patients" why not "clients" or "customers"?

The first move when he/she is seated in the dental chair is the placement of a bib …redolent of infant days when drooling was the norm! Now local anaesthesia reduces the ability to control the lips.. and even the most dignified of patients ends up drooling! And has difficulty in articulating clearly! The environment imposes upon the patient and restricts his/her behaviour, resulting in a sense of loss of control, a direct challenge to personal dignity.

It is in the management of this challenging situation that the principles of professional Ethics come into play. Essentially these principles are readily expressed in Thomas Aquila's concept of *misericordia*… which is the empathy felt by the ethical practitioner… but to be fully effective, *misericordia* should be accompanied by *observantia*... the recognition in the first place that the patient is worthy of respect. That combination is important. Should the practitioner follow only *observantia*, there is the risk of according too much autonomy to the patient... whilst *misericordia* alone could result in the opposite... too great a dictatorship by the practi-

tioner and a suffocating of the dignity of the patient.[1] The challenge to the discipline is finding the correct balance so there is action in unity.

Practitioners should be aware of just how easily that balance may be disturbed or disrupted. Nora Jacobson, in searching for a taxonomy of dignity, accumulated a long list of actions which were inimical to the ethical management of dignity.

In every encounter between people.. or between patient and practitioner… there is a assessment of physical and social characteristics... eye contact, dress, age, gender, responses …and inappropriate interpretation may lead to a sense of violation of dignity.[2]

Amongst the disrupting behaviours listed by Jacobson are: Rudeness, Indifference, Condescension, Diminishment, Contempt, Dependence, Restriction, Trickery, Labelling, Vilification, Discrimination.

There are more… the ethical practitioner is alert to these behaviours and will control these reactions. Further, however, Jacobson echoes Aquinas when she observes that dignity is likely to be promoted when there is a combination of effects… the patient in a position of confidence and self assurance, expecting sound treatment, the practitioner in a position of compassion. That favourable situation (solidarity) promotes dignity supported by transparency, friendliness, calm.[2]

It is often in the small courtesies that the respect due to patients may be shown.. the greeting, formal but friendly, sitting down next the patient when discussing treatment and not towering over him/her, keeping the patient informed on the process, ...and of course adhering to the appointment schedule …time is of the essence!

The last word may be that from The Shorter Oxford English Dictionary "Dignity is the quality of being worthy or honourable, worth, excellence." That is fine, we do strive for that quality. Remember however Dignity in the profession is bifid, extending also to include respecting those same qualities in the patient.

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http://dx.doi.org/10.17159/2519-0105/2019/v74no8a7

Maxillofacial Radiology 174

SADJ September 2019, Vol. 74 No. 8 p464

CJ Nortjé

A twelve year old female presented with a bony hard swelling (Fig.1) of the right maxilla of six months duration. The lesion extended from the philtrum to the zygomatic arch, obliterating the nasolabial fold. Intraorally the extension was from the 11 to the 17. There was buccal and palatal expansion and the 15 and 16 were mobile. What is your diagnosis?

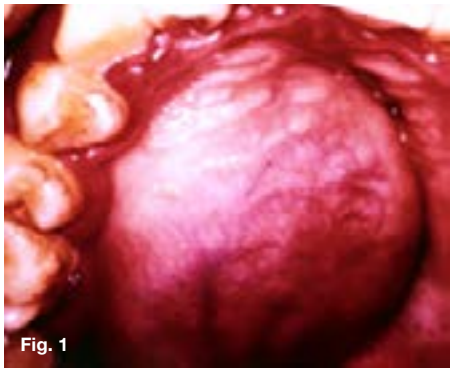
Fig. 1

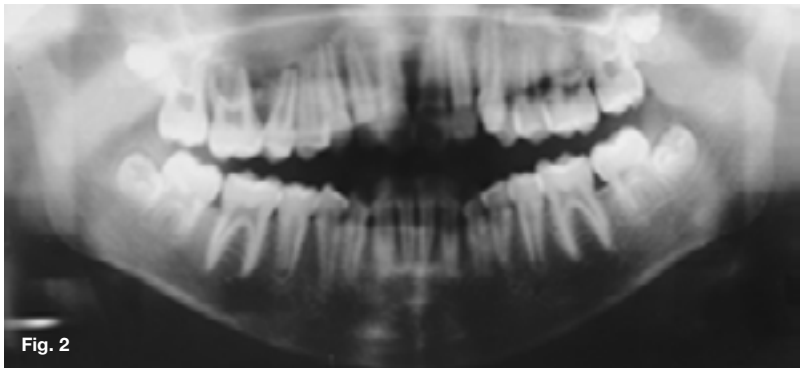
Fig. 2

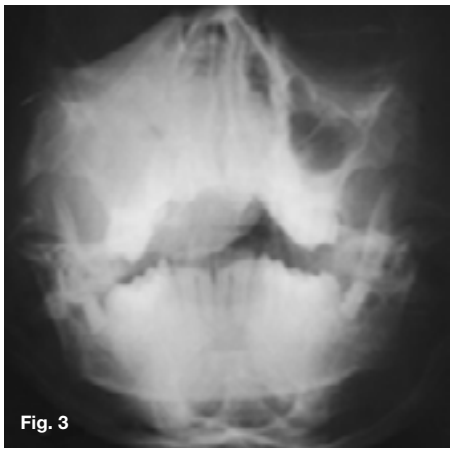
Fig. 3

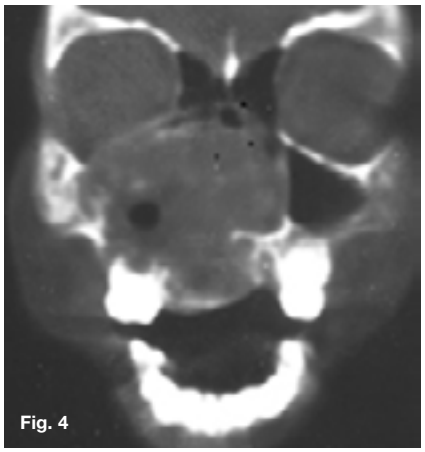
Fig. 4

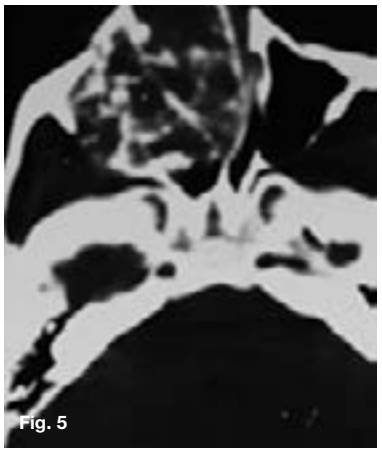
Fig. 5

INTERPRETATION

Figure 2 and 3 revealed an ill-defined radiopaque lesion of the right maxilla extending into the maxillary sinus, the nasal cavity, displacing the nasal septum, the infratemporal fossa and inferiorly into the hard palate and oral cavity. The coronal soft tissue window (Fig. 4) shows the lesion extending superiorly into the ethmoid sinus and orbital floor. Non-contrast axial bone window (Fig. 5) shows a large multilocullar expansile lesion within the maxilla. A histological diagnosis of a plasma cell myeloma (Plasmacytoma) was made. This is an unusual lesion which some authorities believe to be unrelated to multiple myeloma even though the two are microscopically indistinguishable. The lesion affects only a single bone. Infrequently, it is seen in soft tissue, in which case, the term extramedullary plamacytoma is used. The criteria for establishing a diagnosis of solitary plasma cell myeloma are not well defined. Multiple myeloma usually runs an invariably fatal course within two years, or at least becomes widely disseminated in that period. Christopherson and Miller, in reviewing 51 cases of solitary plasma cell myeloma, found a predilection for the sixth decade, although the age range of the patients was from 19 months to 72 years. Males were affected more frequently than females, as in multiple myeloma. The authors also stated that all cases surviving for three years without evidence of dissemination or "metastasis" may be considered true examples of solitary myeloma. Radiographically, the lesion may be seen as a well-defined, unilocular radiolucency with no evidence of sclerotic borders or as a ragged radiolucency similar to multiple myeloma. There is nothing pathognomic or even characteristic of the roentgenographic features of solitary plamacytoma. Plasmacytoma are usually treated with radiation therapy. Lesions have been surgically excised with good results, although this is not the preferred treatment in most instances. Unfortunately, when patients with plasmacytoma of bone are observed on a long-term basis most will develop multiple myeloma.

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Reference

1. Christopherson, WM; & Miller, AJ: A re-evaluation of solitary plasma-cell myeloma of bone. Cancer, 3: 240, 1950.

CPD questionnaire

This edition is accredited for a total of 3 CEUs: 1 ethical plus 2 general CEUs

GENERAL

Inductive surface geometries: Beyond morphogens and stem cells

Introduction and definitions: osteoinduction and osteoinductive biomaterials

1. Identify the INCORRECT statement.
   Morphogens may:
   A. exert an influence on other morphogens to trigger long range patterns of activity
   B. initiate the cascade of pattern formation
   C. initiate tissue morphogenesis
   D. respond to auditory signals

2. Identify the CORRECT statement.
   The induction of bone formation requires:
   A. soluble osteogenic molecular signals only
   B. insoluble signals arising from the substratum
   C. a combination of a and b
   D. neurogenic signals together with soluble molecular signals only

Osteoinductive biomaterials

3. Identify the INCORRECT statement.
   The work of Hulbert et al. demonstrated:
   A. that porosity in biomaterials plays a critical role in the initiation of osteogenesis
   B. that the implanted hydroxyapatites had pore sizes less than 120 µm, an essential for bone formation
   C. that macroporous hyroxyapatites hosted remodelling bone
   D. that the trabecular bone invading an implanted calcium phosphate based matrix was analogous to new compact Haversian bone

4. The defining test for the osteoinductive properties of a biomaterial is to place it between the interfaces of treated defects and to evaluate the osteogenesis.
   A. True
   B. False

5. Identify the INCORRECT statement.
   The "extractable substance" concept introduced by Levander:
   A. was shown to initiate bone formation in a fully developed organism.
   B. has lead to an understanding of morphogenetic substances or morphogens
   C. has not been further investigated
   D. culminates in the tissue engineering paradigm, *"regeneration of tissue is, in other words, a repetition of embryonal development"*

6. Identify the INCORRECT statement.
   Research has shown that:
   A. small quantities of proteins are bound in both the organic and inorganic components of the extracellular matrix of bone
   B. the solubization of these proteins could be achieved through chaotrophic extraction from intact demineralized extracellular matrix of bone
   C. extraction of these proteins resulted in the isolation of soluble molecular signals and an insoluble signal, or substratum
   D. the substratum was a solid plate with no interstices

The geometric induction of bone formation

7. The surface topography of osteoinductive biomaterials has been shown to exert a regulatory effect on the molecular machinery controlling osteogenesis.
   A. True
   B. False

8. Identify the INCORRECT statement.
   Research reported by Ripamonti et al. has shown that the induction of bone in implant sites in muscle:
   A. occurred in implanted macroporous hydroxyapatite discs treated with osteogenic proteins
   B. occurred spontaneously after surgical intervention with no implants
   C. did not occur around implanted particulate hydroxyapatite treated with osteogenic proteins
   D. occurred in implanted macroporous hydroxyapatite discs which had not been treated with osteogenic proteins

9. Identify the INCORRECT statement.
   The concavities on the surface of the implanted coral-derived calcium phosphate-based macro-porous bioreactors have been shown:
   A. to be powerful morphological, cellular and molecular inductive signals for the induction of bone formation
   B. to demonstrate sprouting capillaries showing strong alkaline phosphatase staining on several layers of the cells on the walls of the vessels ("Trueta vessels")
   C. to demonstrate cartilaginous cells
   D. to demonstrate osteogenic precursor cells attached to the surface of the hydroxyapatite substrate

Geometrically modified crystalline hydroxyapatite-coated titanium implants do initiate the spontaneous induction of bone formation in heterotopic intramuscular sites

10. Bundle bone was laid down across the margins of the concavities on the highly sintered crystalline hydroxyapatite discs implanted in the *rectus abdominis* muscle of adult *Papio ursinus*.
    A. True
    B. False

Geometric induction of tissue morphogenesis: substrata stiffness and elasticity guide cell differentiation

11. Identify the INCORRECT statement
There are findings that show:
A. that the micro-morphology of the substrate has no influence on cellular responses
B. that soft matrices that mimic brain are neurogenic
C. that comparatively rigid matrices that mimic collagenous bone prove to be osteogenic
D. geometric forces may be controlling the stem cell microenvironment and the induction of tissue morphogenesis

12. The research on the geometric induction of tissue morphogenesis points towards the possibility of engineering synthetic tissues with complex nano-patterned geometric organizations
A. True
B. False

Prosthodontic rehabilitation in an elderly patient with a hemi-maxillectomy defect

13. Identify the INCORRECT statement.
Effective Prosthodontic rehabilitation in an elderly patient involves:
A. an awareness of the age, medical history, biologic and psychological factors
B. no consideration of socio-economic status
C. versatility in treatment approach
D. treatment appropriate to prevailing lifestyle and condition

An intra-oral solitary schwannoma of the lower lip, a rare diagnosis

14. Identify the CORRECT statement
A. The tumour is most commonly found in the 4th and 5th decades of life
B. Schwannomas are usually associated with a history of trauma
C. Schwannoma' are typically an asymptomatic solitary, freely mobile, submucosal mass characterized by slow growth and a smooth surface
D. Clinical symptoms always include dysphonia, nasal obstruction, dyspnea, dysphagia and oral pain

15. Schwannomas may occur along the course of all somatic, cranial and autonomic nerves.
A. True
B. False

Case report: Bite mark analysis in a case of child abuse

16. Identify the INCORRECT statement.
Bite mark analysis is not an exact science, but an adjunct in the forensic analysis of trauma because:
A. the analysis is dependent on the time span
B. the patient covers up the bite
C. the bruises heal
D. the skin is malleable and distorts under pressure

Maxillofacial Radiology Case 173

17. Plasmacytomoa affects one bone.
A. True
B. False

18. Patients with plasmacytomoa may eventually develop multiple myeloma.
A. True
B. False

Clinical Windows - What's new for the clinician

19. In the Koteeswaran et al. trial, single-blind meant that:
A. only the patient knew what intervention he/she was receiving
B. only the clinician knew what intervention the patients were receiving
C. both patient and clinician knew what treatment was being administered
D. neither the patient nor clinician knew what interventions were being administered.

20. In the Huynh-Ba et al. trial, overall soft tissue changes for the mesial and distal papillae height at 12 months was significantly better in the type 1 group when compared with the type 2 group.
A. True
B. False

ETHICS

21. Identify the CORRECT answer.
Thomas Aquinas proposed that:
A. Justice had no relationship with Dignity
B. Justice depended on the observation of Dignity
C. Dignity depended on the exercise of Justice
D. there was no virtue in either Justice of Dignity

22. Identify the INCORRECT statement:
A. *Observantia* implies acknowledging the dignity of another person
B. *Observantia* implies acknowledging the dignity of self
C. *Observantia* implies recognition of the relevance of another person
D. *Observantia* implies there is a dignity in honouring the status of another person

23. Identify the CORRECT answer:
The bifid nature of the ethics of Dignity may best be expressed by:
A. a combination in which *observantia* is the most dominant characteristic
B. a combination in which *misericordia* is the most dominant characteristic
C. a balanced combination of *observantia* and *misericordia*
D. a haphazard combination of *observantia* and *misericordia*

24. Identify the CORRECT answer:
    Jacobson claims that dignity will be promoted.
    A. by firm control tempered by compassion
    B. by ensuring the patient is confident and expecting good management, whilst the practitioner is compassionate
    C. by ensuring only that the patient is confident
    D. by ensuring only that the practitioner is compassionate

25. The Shorter Oxford dictionary does not appear to recognise the bifid nature of Dignity as practised by the profession.
    A. True
    B. False

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Have you read the Instructions to Authors?	☐
Are you submitting electronically?	☐
Have you provided all author information including first names, affiliations, qualifications, positions held, Department and Institution, ORCID number, contact details?	☐
Is the first author under the age of 35 on submission of the article?	☐
Have you provided all details of the Communicating Author?	☐
Have you submitted questions for the CPD section? (four or five multiple choice, one correct answer)?	☐
Have you submitted details of the contribution of each author... can be percentage or descriptive... or both?	☐
Have you confirmed the status of your paper in terms of any Conflict of Interest?	☐
Have you submitted the Clearance Certificate number when Ethical permission has been required to undertake research or to publish data?	☐
Does the paper adhere to the format requested in Instructions to Author?	☐
Are the references quoted according to Journal policy, both in the text and in the list of references?	☐
Have all authors signed the Letter of Submission?	☐

Smalls Advertising Placement Procedure and Rules

- All smalls advertisements are restricted to a **maximum 100 words** per advertisement.
- All advertisement requests are required in writing, **submit to abayman@sada.co.za**, with full contact details of the advertiser which should include:
  - the wording of the advertisement as you require it to be published;
  - the members professional number; (will not be published);
  - the members contact details (will not be published).
- Advertisement **lifespan is two weeks** from the date of upload.
- Advertisements to be **repeated follow the same process** as the original placement request.
- All advertisements which **exceed a word count of 100** words will be forwarded to our **publishers E-Doc** for further processing as a potential advertisement to be placed in the SADJ electronically or as website advertising. E-Doc will contact you thereafter regarding your requirements.
- **SADA Members** may place advertisements at no cost providing their annual membership fees are either paid in full at the time of their request of a debit order request has been lodged.
- **Non-SADA Member** advertisers will be charged R25 per word for placement of their advertisements.
- Advertisement must be paid in full prior to uploading on the web platform.
- Invoice may be settled telephonically with the use of a credit card to prevent delay of placement.
- **Telephonically processed** payments will result in uploading of advertisement within **24 hours** of settlement.
- Advertiser remains liable for placement costs should payment be dishonoured and invoice remains unpaid.

Contact details:
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Email: abayman@sada.co.za

SADA
THE SOUTH AFRICAN DENTAL ASSOCIATION

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