JOURNAL OF DENTISTRY 42 (2014) 915–928

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/jden

Review

Tissue engineering in dentistry

Ensanya Ali Abou Neel [a,b,c,*], *Wojciech Chrzanowski* [d,e], *Vehid M. Salih* [c,h], *Hae-Won Kim* [e,f,g], *Jonathan C. Knowles* [c,e]

[a] *Division of Biomaterials, Operative and Aesthetic Department Biomaterials Division, King Abdulaziz University, Jeddah, Saudi Arabia*
[b] *Biomaterials Department, Faculty of Dentistry, Tanta University, Tanta, Egypt*
[c] *UCL Eastman Dental Institute, Biomaterials & Tissue Engineering, 256 Gray's Inn Road, London WC1X 8LD, UK*
[d] *The University of Sydney, The Faculty of Pharmacy, NSW 2006 Sydney, Australia*
[e] *Department of Nanobiomedical Science & BK21 Plus NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan 330-714, Republic of Korea*
[f] *Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan 330-714, Republic of Korea*
[g] *Department of Biomaterials Science, College of Dentistry, Dankook, University, Cheonan 330-714, Republic of Korea*
[h] *Plymouth University Peninsula School of Medicine & Dentistry, Drake's Circus, Plymouth PL4 8AA, Devon, UK*

ARTICLE INFO

Article history:
Received 3 May 2014
Received in revised form
15 May 2014
Accepted 17 May 2014

Keywords:
Tissue engineering strategies
Biomimetic scaffolds
Dentine-pulp complex
Bioengineered teeth

ABSTRACT

Objectives: of this review is to inform practitioners with the most updated information on tissue engineering and its potential applications in dentistry.
Data: The authors used "PUBMED" to find relevant literature written in English and published from the beginning of tissue engineering until today. A combination of keywords was used as the search terms e.g., "tissue engineering", "approaches", "strategies" "dentistry", "dental stem cells", "dentino-pulp complex", "guided tissue regeneration", "whole tooth", "TMJ", "condyle", "salivary glands", and "oral mucosa".
Sources: Abstracts and full text articles were used to identify causes of craniofacial tissue loss, different approaches for craniofacial reconstructions, how the tissue engineering emerges, different strategies of tissue engineering, biomaterials employed for this purpose, the major attempts to engineer different dental structures, finally challenges and future of tissue engineering in dentistry.
Study selection: Only those articles that dealt with the tissue engineering in dentistry were selected.
Conclusions: There have been a recent surge in guided tissue engineering methods to manage periodontal diseases beyond the traditional approaches. However, the predictable reconstruction of the innate organisation and function of whole teeth as well as their periodontal structures remains challenging. Despite some limited progress and minor successes, there remain distinct and important challenges in the development of reproducible and clinically safe approaches for oral tissue repair and regeneration. Clearly, there is a convincing body of evidence which confirms the need for this type of treatment, and public health data worldwide indicates a more than adequate patient resource. The future of these

* *Corresponding author at*: Operative and Aesthetic Department, Division of Biomaterials, Faculty of Dentistry, King Abdulaziz University, P.O. Box: 80209, Jeddah Zip Code: 21589, Saudi Arabia. Tel.: +966 596820208.
E-mail addresses: eabouneel@kau.edu.sa, e.abouneel@ucl.ac.uk (E.A. Abou Neel).
http://dx.doi.org/10.1016/j.jdent.2014.05.008

therapies involving more biological approaches and the use of dental tissue stem cells is promising and advancing. Also there may be a significant interest of their application and wider potential to treat disorders beyond the craniofacial region.

Clinical Significance: Considering the interests of the patients who could possibly be helped by applying stem cell-based therapies should be carefully assessed against current ethical concerns regarding the moral status of the early embryo.

1. Introduction

Tissue loss due to trauma, disease or congenital abnormalities is a major health care problem worldwide. When this occurs in the craniofacial region, it induces serious physiological and psychological consequences on patients. Reconstruction of the craniofacial area to its aesthetic and functional level is therefore a desire of affected patients.[1] This review addresses the concentrated research effort in methods for oro-facial reconstruction from using medical devices and tissue grafts to a more explicit tissue engineering approach. It is an approach that utilises specific biodegradable synthetic or natural scaffolds as well as advanced molecular techniques in order to replace tissue function. The types of scaffold and methodologies used to enable cells to function in an appropriate manner to produce the required extracellular matrix and ultimately a tissue of a desired geometry, size and composition are briefly considered here.

There has been a clear and distinct hypothetical shift in regenerative medicine from using medical devices and whole tissue grafts, to a more explicit approach that utilises specific bioactive, biodegradable synthetic or natural scaffolds combined with cells and/or biological molecules, to create a functional replacement tissue in a diseased or damaged site. Every era in medical research over the past 50 years, involving the use of biomaterials in order to replace tissue function, has been distinct and identified by particular developmental successes and materials. For example, in the 1950s, there was a predominant use of metal implants and associated devices with little thought offered to the effects on local tissues, let alone the cells. Throughout the '70s and '80s, there was a significant increase in the use of polymers and synthetic materials where researchers considered both biological and material properties. More recently, there has been a distinct and concentrated effort in the design and use of both natural and degradable scaffolds and advanced biological consideration of the materials.

There has been an evolution from the use of biomaterials to simply replace non-functioning tissue to that of utilising specific materials, which will nurture, in three dimensions, a fully functioning and structurally acceptable regenerated tissue. Thus, the simple needs to accomplish the replacement of a functioning joint using wholly metal prostheses in the '60s has been markedly enhanced to concentrate on biological aspects of the damaged or diseased tissue to be replaced by repaired, or better still, totally regenerated tissue. There was a very naïve belief that materials were typically 'inert' and it has been rightly suggested that this is a misleading interpretation, as it became clear that materials could indeed change physically and chemically following implantation. Certainly from a biological perspective, no material should be considered (or indeed is) inert.

This review will therefore deal with the significant advancements that have been made in the tissue engineering field as well as its future potential.

2. Strategies of tissue engineering

In this section, cell injection, cell induction and cell seeded scaffold will be briefly described as different but inter-related approaches of tissue engineering. These approaches depend on the use of one or more key elements e.g., cells, growth factors and matrix[2] to guide tissue regeneration.

2.1. *Cell injection therapy*

Since the tissue formation resulted from cellular action, injection of inherently intelligent cells, stem cells in particular, into the defect have been suggested to regenerate tissues. The effectiveness of this therapy however is limited by low engraftment and inadequate localisation of injected cells particularly in areas showing continuous movement e.g., beating heart.[4] Immunological rejection and the ability of the injected cells to maintain their phenotype are other challenges.[3] For adequate localisation and prevention of direct contact with the immune system, using a delivery vehicle to carry and deliver the material has been attempted.[4] It has been observed that cells encapsulated into a delivery vehicle were able to proliferate and differentiate.[5] Thanks to these advantages, this strategy seems to be promising in bone and cartilage repair.[6] It also opened new opportunities to reduce the morbidity and mortality rate caused by heart failure in ischaemic heart patients.[7] But again, the delivery vehicle has to be made from a smart material that can be easily injected but finally solidified at body temperature. Furthermore, the release of cells has to be controlled by the need of the body.

For this strategy, stem cells are the most successful candidate. According to their potency, stem cells are classified into totipotent (generate all differentiated cells in an organism e.g., fertilised egg), pluripotent (form the three germ layers; ectoderm, endoderm and mesoderm e.g., embryonic stem cells), multipotent (differentiate into several cell lines but with more restricted number of phenotypes e.g., mesenchymal stem cells), oligopotent (differentiate into a few cell types e.g., myeloid stem cells) and unipotent cells (i.e., differentiate into

one cell type e.g., skin stem cells).[8] According to their origin, stem cells are classified into embryonic and adult (somatic). Embryonic stem cells have a great potential use in regenerative medicine as they can be maintained indefinitely in an undifferentiated state in culture. Embryonic stem cells showed a major advantage in medical research, understanding the range of transformation of such cells can help in the correction of many mutational errors. While the necessity of using and manipulating embryonic stem cells to produce fully differentiated cells for tissue regeneration is inexpressible, the ethical and legal view points of using the embryo or foetal tissues as a source of these cells must be weighed.

2.2. *Cell induction therapy*

Due to the limitations with cell injection therapy, there has been a clear and distinct shift to recruiting the circulating body cells to regenerate the tissues. With respect to osteoinduction, an important consideration when dealing with craniofacial bone regeneration, it is very important to understand the underlying biological mechanisms that facilitate osteinduction. This is highlighted very elegantly in the review of Miron and Zhang.[9] Furthermore, the ideal design of any osteoconductive material would mean that no exogenous biological components would be needed in order to induce osteogenesis. However, exogenous factors are still utilised in the form of injecting the signalling molecules e.g., growth/differentiation factors, to modulate the cell behaviour. Example of these factors include; fibroblasts growth factors-2 and 9 (FGFs-2 and -9),[10] transforming growth factors β1 (TGF-β1),[11] vascular endothelial growth factors (VEGFs),[12] recombinant human growth/differentiation factor-5 (rhGDF-5)[13] and bone morphogenetic protein.[14] Although, this therapy was effective in regenerating some tissues,[15] the expense of purification and the development of an appropriate carrier to deliver these factors to their target sites limit its scope.[16] Taking a step back, injection of the genetic information instead has been thought to produce a population of progenitor cells to over-express the growth/differentiation factors necessary for modulating cell behaviour.[17] Choosing gene(s) for the required protein(s), timing of gene expression, type of gene vector (viral or non-viral), and method of gene delivery (systematic or local) have to be considered when employing gene therapy.[18]

2.3. *Cells seeded scaffolds*

Combining all the previous attempts together led to the emergence of another strategy to engineer tissues. This strategy depends on the isolation of appropriate cell population from a biopsy taken from the patient or a donor. And the most likely candidate for such therapies remains the Mesenchymal Stem Cell (MSC). The potent immunomodulatory and anti-inflammatory properties of human oral mucosa-/gingiva-derived MSCs places them as a very strong potential cell source for MSC-based therapies for wound repair and a wide range of inflammation-related diseases. Zhang et al.[19] quite correctly asked, whether these MSCs differ from bone marrow stem cells in terms of host defence immune response, because of their specific anatomic location in the oral cavity? Answering such queries will substantially enhance our understanding of the biological properties of oral mucosa-/gingiva-derived MSCs and their important roles in tissue regeneration and cell-based therapy of immune- and/or inflammation-related diseases. In addition, MSCs although initially considered as having the potential to differentiate into only tissue-specific cells for regenerative medicine, are now being recognised as an essential cell type that possesses important immunomodulatory properties capable of treating a variety of immune-related diseases. MSCs can thus regulate the intensity of immune response by inducing T-cell apoptosis, which could have great therapeutic

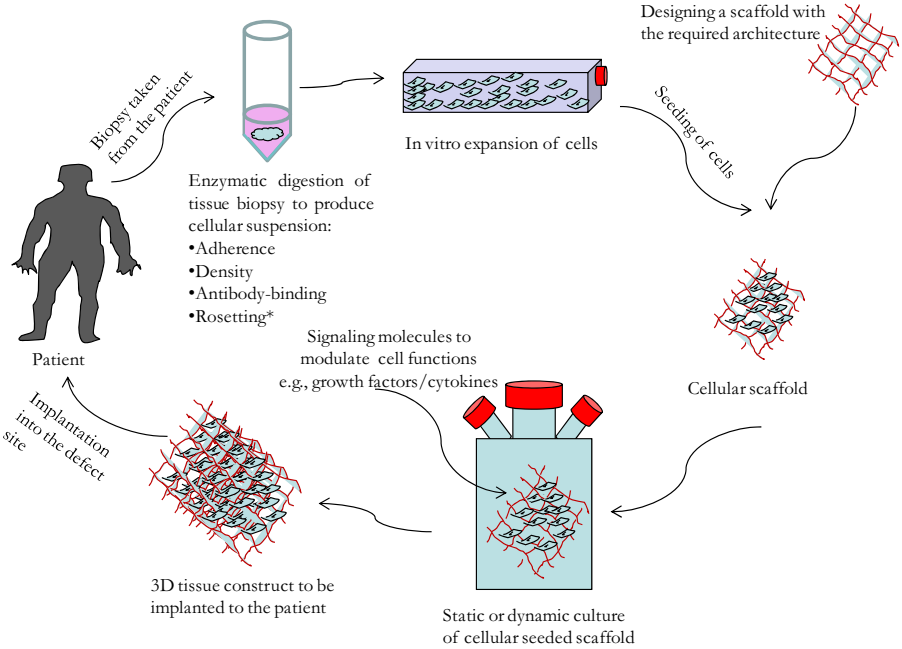

Fig. 1 – Diagrammatic representation of cell-matrix tissue engineering strategy. * Different methods used to produce cellular suspensions from a tissue biopsy are described in details by Tomlinson et al.[23]

potential when utilising biomaterials for tissue engineering applications.[20] The isolated cells will then be expanded in culture and finally seeded within or onto a natural or synthetic scaffold that define the shape of the tissue and supports cells during their growth[21] (Fig. 1). Ideally, cells adhere to the scaffold, proliferate, differentiate and form the required tissue. Then the newly formed ''organoid'' can be then transplanted into the patient. Another option for this strategy relies on implantation of acellular scaffolds into the defect while the body cells can populate the scaffold to form the new tissue in situ. Gupte and Ma[22] clearly recognised that three-dimensional scaffolds artificially create a multi-scale environment capable of directing cell adhesion, proliferation, and importantly, differentiation. These authors also clearly recognised significant technical challenges which need to consider the synergistic integration of key structural cues with relevant biological molecules for cell-based therapies in order to achieve properly functioning dental and craniofacial tissue regeneration.

3. Engineered orofacial tissues

Orofacial structures are very unique in their development and function. Orofacial bones, for example, are derived from both neural crest and paraxial mesoderm; the skeletal bones however derived from mesoderm. Furthermore, orofacial bones undergo significant stress and strain produced from different muscles of mastication and respond differently to growth factors and mechanical stimuli.[24] Furthermore, orofacial tissues have limited and variable capacity for regeneration. Unlike alveolar bones, cementum has a very slow regenerative capacity.[25] Unlike enamel, dentine can regenerate. As it is encased in dentine and has limited apical blood supply, the pulp has a limited capacity for regeneration.[26]

Statistics on tooth loss indicated that in US >20 million people are missing all of their teeth, and >100 million have lost 11–15 teeth.[27] Dental implants have been advocated as tooth replacement; lack of adequate bone support and the proximity to anatomic structures e.g., maxillary sinus and inferior alveolar canal are the most frequently encountered problems. Using bone grafts to provide bone support has been attempted; the success however was limited.[28] Tissue engineering, therefore, found an interest as the clinically relevant approach to regenerate dental tissues as well as the whole tooth. The first attempt involved the application of calcium hydroxide for regeneration of dentine and pulp in traumatically exposed teeth.[29] The field of tissue engineering has then grown tremendously to the development of fully functional bioengineered tooth.[30] This section covers the progress made to reach a destiny where a fully functional bioengineered tooth becomes a reality. It also covers the tissue engineering attempt to replace soft tissues (skin, mucosa, muscles and salivary glands), bone and temporomandibular joints (TMJ). Each section was ended by the authors' opinion as discussed later.

3.1. *Dentine-pulp complex*

The regeneration of the dentine-pulp complex, obtained with pulp capping materials (e.g., calcium hydroxide, mineral trioxide aggregates, Biodentine®), has been correlated with the stimulation of differentiation of the pulp progenitor cells into odontoblast-like cells[29] or secretion of TGF-β1[31] which plays a key role in angiogenesis, recruitment of progenitor cells, cell differentiation and finally mineralisation of the injured area. Stem cell therapy has been attempted for regeneration of the dentine-pulp complex. Dental tissues are a very rich source of stem cells. Examples of these tissues include e.g., pulp,[32] apical papilla,[33] human retained[34] or exofoliated deciduous teeth,[35] oral mucosa and gingiva.[19] Subcutaneous injection of stem cell-sheet derived pellet at the back of a mice induced the formation of the dentine-pulp complex.[33] Encapsulated stem cells were also used for dentine-pulp regeneration; examples of materials employed for cell encapsulation include enzyme-cleavable, customised self-assembled peptide hydrogels,[36] PEGylated fibrin hydrogels[37] or biodegradable lactide and glycolide.[11] The encapsulated cells were also effective in dentine-pulp regeneration. For example, Gelfoam-encapsulated dental stem cells stimulated the formation of the dentine-pulp complex in pulpless root canals in young permanent incisors in beagles.[38] Cell-free scaffolds e.g., Emdogain gel[39] or combination of Emdogain and platelet rich plasma[40] stimulated the regeneration of the dentine-pulp complex. Growth factors [e.g., fibroblast growth factor basic (FGF), transforming growth factor β1 (TGF-β1) and endothelial growth factor (EGF)] have been also included within the scaffolds to modulate the function of stem cells.[11]

Due to the size and confinement of the pulp within the root canal(s), cell therapy and/or injectable hydrogels represented the common strategic approach for engineering the dentine-pulp complex. With this approach, however, the highly organised and specialised nature of such complex e.g., presence of different cell layers in a specific order and dentine on the periphery of pulp, has not been considered. Thorough investigations are required to develop a technology that allows designing such hierarchical structure while injecting the hydrogels scaffolds to shape the dentine-pulp complex and to allow preferential arrangement of different type of cells and hence the tissues in the innate order.

3.2. *Periodontium*

Periodontitis is a widespread condition of inflammation that causes destruction of tooth supporting connective tissues (gingiva, alveolar bone, periodontal ligament and root cementum) and eventually teeth loss (Fig. 2a). Regeneration of tooth supporting structures i.e., cementum-periodontal ligament-bone interfaces and structures are very challenging and require the synergy of all cellular and molecular events involved in regeneration of these complex tissues. This section covers the progress from guided bone/tissue regeneration to the most recent advances in tissue engineering employed to replace the lost tooth supporting structures in an attempt to maintain natural dentition.

Guided tissue/bone regeneration membrane (GTR/GBR) utilises occlusive membranes to maintain the defective space, selectively encourage the appropriate cells to regenerate the lost tissues and support the newly formed tissues.[41] GTR/GBR was employed to treat periodontal[42] and alveolar[43] defects as well as to maintain integrity of alveolar bone following teeth extraction.[44] Several synthetic polymers were used as

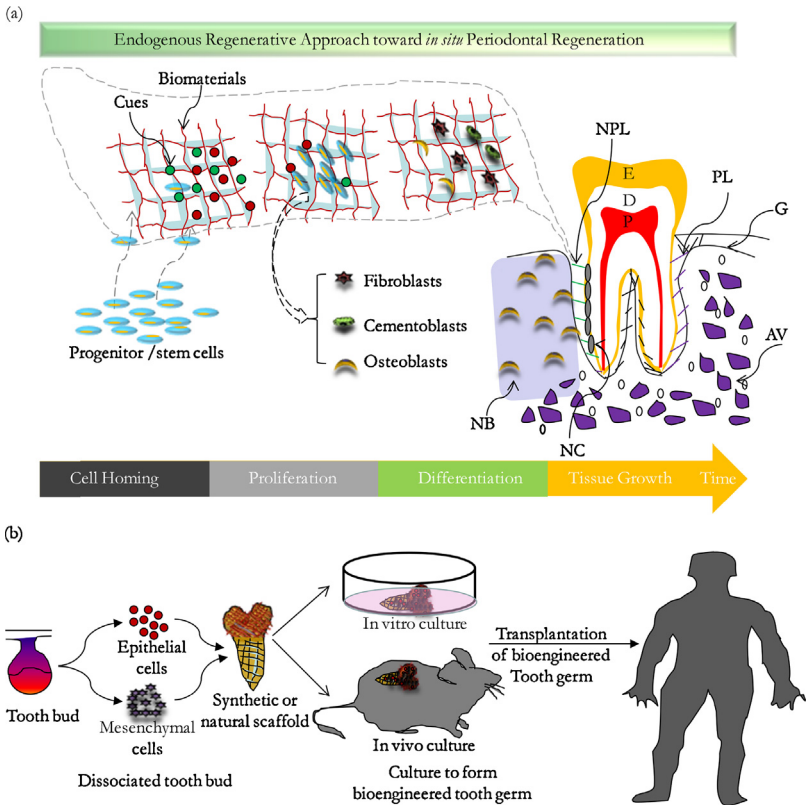

Fig. 2 – A schematic representation of (a) the endogenous approach used for regeneration of periodontal tissues adopted from.[61] E: enamel, D: dentine, P: pulp, G: gingival, PL: periodontal ligament and AB: alveolar bone NPL: new periodontal ligament, NB: new bone, NC: new cementum and (b) a strategy to engineer a whole tooth.

GTR/GBR membranes; they include polytetrafluoroethylene (PTFE, Gore- Tex®),[45] polylactide (e.g., Vivosorb® & Epi-Gide®), glycolide (Gore Resolut Adapt®) and polylactide/glycolide.[46] Biomimetic materials, collagen in particular, has been advocated as alternative to synthetic polymers; examples of collagen membranes include; Ossixt™, Bio-Gide®, Neomem®, Biomend™, Biomend Extendt™.[47] To enhance tissue regeneration, negatively charged collagen membranes were developed.[48] To control the degradability and hence enhance the osteogenic potential of collagen membranes, immobilisation of hydroxyapatite nanoparticles,[49] alkaline phosphatase[42] or bioactive glass[50] on collagen membranes has been also attempted. The recent advances in the field of tissue engineering utilises growth factors and cytokines for periodontal regeneration.[51] Examples of growth factors used include transforming growth factor β1 (TGFβ1), fibroblast growth factor-2 (FGF-2), bone morphogenic protein-2 (BMP-2), recombinant human bone morphogenic protein-2 (rhBMP-2). Soaking collagen membranes in BMP-2 or TGFβ1 enhanced the cellular activity of human osteoblasts in vitro.[52] Incorporation of FGF-2 enhanced the bone regeneration capacity of collagen membranes in a rat calvarial defect.[53] Contradicting results, however, were obtained clinically. For example, no complete periodontal regeneration was attained with combined therapy of collagen membrane and BMP.[54] On the other hand, the five-year survival rate was 100% with excellent clinical and radiographic outcomes was seen for rhBMP-2 combined with collagen membranes.[55] Although there is some degree of success in treating craniofacial, cleft palate, bone and cartilage defects[56] bacterial infection is a common problem with GTR/GBR membranes. Incorporation of tetracycline,[57] chlorhexidine[58] and zinc[59] could overcome this problem. The antibacterial agent could be very effective provided that its release is well controlled. More recently, developments in bone repair/regeneration using carbon nanotubes or carbon nanotube-based composites (i.e. CNT associated with different biological molecules or polymers) have been identified as a innovative biomaterial for oral tissue regeneration. Indeed, Martins-Junior et al.[60] provided an excellent overview of bone tissue engineering focusing on the potential actions of CNT in bone formation and repair/regeneration.

Regardless of the clinical effectiveness of collagen membranes in combination with bone graft or substitutes or growth factors, the in vivo degradation of collagen could be too fast to enable tissue regeneration in large defects in particular. Space maintenance and tissue occlusion properties could be also challenging in this situation; therefore the utilisation of a membrane with ideal mechanical, degradation properties but still maintaining excellent biocompatibility is still required. For such a case, the application of multilayered membranes combining a layer of flexible synthetic polymer (e.g., polylactide-co-glycolide dimethacrylate) encased between two layers of natural polymers (e.g., collagen) could be an option. The flexibility of the synthetic polymer provides better handling,

adaptation and tissue occlusion. The synthetic polymer's degradation can be controlled by adjusting the molecular weights and the ratio of polylactide to polyglycolide segments. Collagen however provides an excellent biocompatibility and enhanced cellular response. Another direction would be the use of biologically active nanofibrous scaffolds. The resemblance to ECM and the presence of large pores could be an attractive for cells invasion and proliferation. To fabricate nanofibrous scaffolds with a wide range of properties, a combination of both synthetic and natural polymers can be employed.

Endogenous regenerative technology "ERT" depends on key endogenous resources (e.g., cells or growth factors and proteins) for regeneration of functional tissues (Fig. 2b). Cell homing or cell transplantation are meritorious promising approaches, that rely on cells, to completely and reliably restore the periodontium.[62] For cell homing, a material niche (e.g., autogenic growth factors in combination with fibrin and Emdogain and Bio-Oss) is required to recruit the host stem cells to regenerate the periodontium. The choice and deign of each niche component as well as the invasiveness of the clinical procedures would affect the clinical outcome.[61] Cell transplantation could be another option for periodontium regeneration. For example, injection of autogenic gingival stem cells encapsulated within collagen or deproteinized bovine cancellous bone scaffold showed a significant improvement in periodontal tissue regeneration of miniature pigs.[63] Injection of autogenic fibroblasts was found to be safe and effective in restoring the interdental papillae in a randomised controlled study carried out on 20 patients.[64] The use of platelet rich plasma (PRP) as a source of key endogenous growth factors and proteins involved in tissue regeneration has been also employed to reliably regenerate periodontium. PRP increased the proliferation, differentiation and hence odontogenic and osteogenic gene expression of human periodontal ligament and dental pulp stem cells. Combination of PRP with either human cultured periosteum/hydroxyapatite[65] or with patient's own mesenchymal stem cells[66] was effective in periodontal regeneration. A specific concentration of PRP however is required for periodontal regeneration around implant[67] or replanted teeth.[68] Beyond this concentration, an inhibition of cellular activities were recognised.[69] Furthermore, the relative proportion of PRP components, duration and timing of exposure should also be optimised.[69]

The third generation of periodontal regeneration strategies, following GBR/GTR and ERT, involves the use of enamel matrix derivatives (EMD, Emdogain®), that contains >90% amelogenin and <10% other protein.[70] A ≥1-year randomised controlled trials showed that EMD was superior to the conventional open flap debridement (OFD).[71] Additionally, a combination therapy of EMD and OFD was significantly resulted in better clinical outcome than OFD and PrefGel® in a 5-years randomised controlled study.[72] Also, a combination therapy of GTR and EMD showed better outcome than single therapies, but this effect was small as shown in Bayesian network meta-analysis study.[73] The use of EMD in periodontal regeneration was due to its stimulatory effect on the proliferation and differentiation of human periodontal ligament cells (HPDLCs).[74] From EMD, amelogenin in particular, was selectively taken by human periodontal ligament fibroblasts, HPDLFCs, internalised and processed into a 5 kDa peptide-tyrosine rich amelogenin peptide (TRAP, a specific amelogenin isoform).[75] Synthetically produced TRAP suppressed the osteogenic differentiation of bone precursor cells. Whereas, another synthetically produced amelogenin isoform, a leucine-rich amelogenin peptide (LRAP), enhanced terminal differentiation of bone-forming cells. This difference was related to the C-terminal; TRAP has unique C-terminal 12 amino acid sequence (TCT), but LRAP and its unique C-terminal 23 amino acid sequence (LCT). The differential effect of TRAP and LRAP can be employed to limit the pathological bone growth or to enhance bone formation as in the treatment of periodontal and orthopaedic diseases.[76] In addition to its action on HPDLFCs and bone precursor cells, EMD also acts as a proangiogenic factor in vitro and accordingly stimulate the blood vessel formation during periodontal regeneration.[77]

Tissue regeneration of the periodontium is no longer considered solely as an experimental approach, and significant progress has been made these past 10–15 years with respect to the development of biodegradable scaffold materials. Today's concepts of matrix-and scaffold-based tissue engineering involve the combination of a scaffold with cells and/or biomolecules that promote the repair and/or regeneration of such tissues. More recently, regenerative therapies have considered whole tissue architecture, the ultimate goal aimed at the creation of scaffolds that create a temporary 3D matrix upon which cells and tissues can grow exclusively in vitro and/or in vivo. The advances made by targeting particular families of growth factors and other signalling molecules at both the protein and gene levels has led to promising results. Much new data have been accumulated regarding the cell recruitment, attachment and chemotaxis, proliferation and differentiation, angiogenesis and extracellular matrix production of the regenerated tissue at the site of disease or damage. However, the results are still relatively unpredictable and vary greatly among different species and model systems and, in humans, depend on a host of other environmental factors which can play an important role in the successful outcome (or not) of periodontal therapy.

3.3. *Bioengineered teeth*

Tooth development, odontogenesis, is a complex process involving a series of reciprocal epithelial–mesenchymal interactions and coordination between the crown and the root with its associated periodontium.[78] Accordingly, cells dissociated from epithelium and mesenchymal tissues of prenatal or postnatal tooth germ were used to reconstitute a "bioengineered tooth germ" in vitro. Transplantation of bioengineered tooth germ into the oral environment or an organ culture has been then attempted to produce a whole tooth.[79] Implantation of biodegradable polyglycolic/polylactide scaffolds, having the shape of a tooth and seeded with cells isolated from dissociated postnatal porcine third molar tooth buds, into rat hosts for 20–30 weeks successfully produced recognisable tooth structures (dentine, well defined pulp chamber, putative Hertwig's root sheath epithelia, putative cementoblasts and dental organ with fully formed enamel). The size of bioengineered tooth however was very small and did not conform to the shape and size of the

scaffolds.[80] To understand the inductive potential of dissociated dental ectomesenchyme, the following combinations were used: (1) dissociated epithelial and mesenchymal cells (EC-MC), (2) dissociated epithelial cells and intact dental mesenchyme (EC-MT) and (3) intact dental epithelium with dissociated mesenchymal cells (ET-MC). As observed, the intactness of dental mesenchyme is essential for crown morphogenesis but not for epithelial histogenesis. Absence of intact dental mesenchyme, however, can be compensated by increasing the number of dissociated mesenchymal cells that are available for reassociation with intact dental epithelium.[78] Using prenatal tooth germ cells showed higher tendency for tooth formation with proper crown shape than postnatal tooth germ cells.[81] Again, the effect of the source and age of tooth bud on the innate regenerative capacity of the isolated cells as well as the effect of scaffolds on cell behaviour required more investigations. As seen, bioengineering of rat tooth occurred reliably in a shorter time than pig tooth i.e., 12 instead of 25 weeks. Furthermore, the 4-days postnatal (dpn) rat molar tooth bud cells exhibited the highest cell yield/tooth bud and viability when compared with 3–7 dpn cells.[25] As expected the natural scaffold e.g., collagen sponge showed higher degree of success in tooth production than synthetic scaffold materials e.g., PLGA mesh.[82]

Regardless of this achievement in tissue engineering of the whole tooth,[79] several challenges must be faced. For example, optimising the number and quality of dissociated tooth bud cells requires more investigation. However, due to the limited availability of autologous tooth bud cells, researching the possibility of using autogenic somatic stem cells of dental or non-dental origin (e.g., bone marrow stem cells or oral mucosa derived epithelial cells) as candidate sources for bioengineering of whole teeth is also required. Incorporation of growth factors and cytokines or even transplantation of a regenerated tooth rather than regenerated tooth bud requires further consideration. Understanding the events that are involved in engineering a specific type of teeth (incisors, canines, premolars or molars) is also crucial. Once getting the required type of tooth, controlling the anatomy and colour of bioengineered tooth is another area that requires investigation.[83] Reaching the continuity of the engineered tooth with the jaw bone by fully functional periodontium and highly vascularised pulp is also essential for the success of regeneration. Generally, the time required to regenerate a whole tooth is also an important factor which requires further consideration. Thus ''whole-tooth regeneration takes a village of scientists, clinicians and patients''.[84]

3.4. *Skin, oral mucosa, facial muscles and salivary glands*

Tissue engineering made extensive progress in the area of skin regeneration and recently several skin substitute products (epidermal, dermal or composite) are now commercially available. The pioneering work started by the observation of entire keratinising colonies from in vitro cultured epidermal keratinocytes.[85] The formation of keratinocytes sheets was then followed using autogenic or allogenic epidermal cells.[86] The keratinocytes sheet has the ability for renewal throughout the patient's lifetime[87] and can undergo organisation and differentiation after grafting.[88] The keratinocytes sheet, however, is too friable to handle and suture. Grafting fibroblasts-seeded decellularized dermis, obtained from cadaveric skin has been also attempted; the limited availability, reproducibility and safety of cadaver dermis, however, limited its use. Engineering bilayered skin or composite graft, consisting of epidermis and dermis[89] then followed. For this purpose, collagen was the most widely used scaffold since 1956.[90] An organotypic engineered skin was firstly employed to treat wounds in animals[91] and then in humans.[92] The first complete model of engineered skin using human cells was developed in 1991.[93] Recently, few composite allografts produced from decellularised collagen are commercially available e.g., Apligraf®.[94]

Due to the similarity between skin and oral mucosa, the development of engineered oral mucosa followed the same protocol i.e., started with the development of epithelial sheet,[95] then composite oral mucosal equivalent either by seeding oral keratinocytes on decellularised cadaveric human dermis (AlloDerm)[96] or three-dimensional cell seeded scaffold. Furthermore, both skin and mucosal substitutes can be used interchangeably.[97] Recently, the tissue engineered oral mucosa has been further improved for either intraoral or extraoral use.[98,99] To reduce the contraction that represented the major complication of tissue engineered oral mucosa, glutaraldehyde pretreatment of decellularised dermis and physical restraint of tissue engineered mucosa during the first phase of culture is required.[98] Due to its resiliency, suppleness and good tolerance, cross linked collagen membranes also found a great potential as mucosal grafts following surgical removal of precancerous or cancerous lesions or application of bone graft in oro-facial area.[100] The commercially available collagen products used for this purpose include Bio-Col or Mucograft.[101] Other polymers have been also employed to engineer oral mucosa e.g., silk fibroin that could reduce wound contraction;[102] nanofibrous elastin-like recombinant polymer collagen that was observed to improve the self-renewal potential of epithelial cells after grafting[103] and collagen-elastin (Matriderm®).[104] Moharamzadeh et al[105] have reviewed the synthetic oral mucosa developments in recent years and quite rightly report that despite being more physiologically relevant than monolayers; ultimately the basic structure of the connective tissue component and the reconstituted basement membrane in such biomimetic models enables only a simplistic representation of the native stromal microenvironment. Thus, most researchers in the field of oral tissue engineering are veering from the more traditional monolayer cell culture systems to the well-characterised and reproducible tissue-engineered oral mucosal models that mimic the native human oral mucosa and are more clinically relevant, perhaps more informative than the former systems. Currently, plastic compressed collagen has been extensively investigated as a potential scaffold for skin or mucosal grafting procedures.[106]

Facial muscles have unique anatomy and fibre composition compared to other skeletal muscles. Tissue engineering therefore holds promise for future treatment of patients with facial paralysis[107] and partial tongue resection.[108] Finding a 3-D scaffold that fulfils the demands of biocompatibility, elasticity and stability is a key issue for the clinical application of tissue engineered muscle.[109] Moreover, using the appropriate muscle progenitor cell that shows high proneness to

muscular differentiation while maintaining the same characteristics and contractility as the donor muscle, e.g., satellite cells, is another essential issue for engineering the facial muscles. In vivo, satellite cells respond to hypoxic, ischaemic muscle damage by differentiation into myotubes (immature muscle fibre) and maturation to muscle fibres.[110] In vitro, however, the efficiency of satellite cell differentiation is suboptimal; microRNA-1 and 206 however improved the human satellite cell differentiation by increasing the myogenic regulators.[111] Other cell population e.g., fibroblasts are also required to assist in the self-assembly of tissue engineered muscle.[112] Vascularisation and innervations of the muscle construct remains a major challenge in tissue engineering.[113] Applying an optimal electrical, chemotropic and mechanical stimulus is therefore essential for functional reconstruction of facial muscles.[114] Various tissue engineering strategies have been currently researched for regeneration of facial muscles. For example, in vivo implantation of a preformed tissue engineered muscle, made from neonatal rat myoblast seeded collagen constructs, into the face of rats was successful in regeneration of active myofibers, nerve fibres and blood vessels.[107] Implantation of myoblasts seeded collagen constructs was also effective in promoting volume preservation and/or tongue reconstruction.[108] Injection of platelet-rich plasma, growth factors and stem cell-based strategy has been also employed. The use of these biological therapies however requires a standardised, safe use in the clinic and careful understanding of the mechanisms involved in the survival, proliferation and differentiation of stem cells and in muscle regeneration as a whole.[115]

Treatment of salivary glands' hypofunction following irradiation in head and neck area is only limited to the administration of saliva substitutes and sialogogues that require frequent administration. Tissue engineering provides a biological substitute to impaired salivary glands. The main challenge however is to culture the human salivary gland cells as they are highly differentiated and difficult to expand in vitro. Using low-calcium system was found to be effective in enabling the human parotid gland acinar cells (PGAC) to continuously proliferate, maintain their phenotype and express both secretory (a- amylase) and function-related proteins (e.g., (aquaporin-3, aquaporin-5, and ZO-1)). Furthermore, these cells were able to form 3D cell aggregates, called post-confluence structures (PCSs), which were able to produce high level of function-related proteins than 2D cells. The use of 2D scaffold is however still possible to engineer salivary glands.[116] Primary human salivary gland cells seeded biodegradable polymer scaffolds showed the formation of post-confluence structure in vitro by enhancing E-cadherin expression[117] and acinar gland-like structure after 4 weeks of subcutaneous implantation in athymic mice. The gland-like structure also showed the production of human salivary α-amylase (acinar cell protein).[118] Selective functionalisation of degradable scaffold with chitosan and/or laminin-111 provide chemical signals that support proliferation of epithelial cells and promoted the apicobasal polarity, required for directional secretion by secretary cells.[119] Gene therapy is another potential approach for salivary gland regeneration. It relies on the using of a recombinant adenovirus to deliver a water-channel protein gene (aquaporin) to the surviving ductal epithelial cells. This gene is capable of transforming the ductal cells into acinar-like cells (i.e., saliva secreting cells) when integrated into their basement membranes.[120] Phase I clinical trial on the use of adenovirus containing human aquaporin is currently underway to treat patients with salivary glands hypofunction.[121]

Over the last three decades, there was a great progress in treating various burns and skin/mucosa-related disorders. This progress has been considered as a breakthrough due to the uniqueness and complexity of the tissue engineered. The presence of several products available for clinical use is testimony of the success of tissue engineering in this area of the body. The greatest challenge however is the complexity to mimic the host tissues. Therefore engineering a fully functional skin is one of the greatest challenges in tissue engineering due to the various compositions and functions of different layers of the skin. Furthermore, the development of an effective interface between epidermis and dermis in a full engineered skin is also challenging. Developing a product that can be used interchangeably for both skin and oral mucosa is also highly challenging as it is expected from the same product to behave differently under different situations i.e., has hair when used for skin regeneration but not for mucosa. Other limiting factors would be convenience of use, clinical efficacy and patient safety of the end-product. Engineering a fully functional skin replacement will therefore require the development of new scientific strategies and further thorough investigations to meet the patients' need with best effective and cheap replacement. Using the recent technology of "ultra-rapid plastic compression" could offer a great prospect for the development of asymmetric meso-scaled lamellar (multilayered) structures of compacted, aligned collagen fibrils that vary in density across the layers. These hierarchical structures could mimic the stratification, mechanical properties and complexity of the natural tissues.

3.5. *Bone and temporomandibular joints*

Application of autogenic periosteal cells-seeded polymer fleeces to augment the floor of the maxillary sinus before implants insertion showed encouraging results from both radiographical and histological examinations.[122] For irregular defects, injectable composites [e.g., β-TCP/alginate[123] and CPC-chitosan[124]] could be useful for stem cell-based bone engineering. Autogenic growth factors-rich plasma in combination with inorganic bone (Bio-Oss®) has been also employed clinically in sinus floor elevation; this treatment was effective in forming new vascularised bone.[125]

Temporomandibular joint (TMJ) is one of the most difficult tissues to treat due to the limited blood supply and hence limited capacity for self-repair. Patients suffering from TMJ disorders often experience pain during normal activities e.g., eating and speaking accordingly they have low quality of life (Fig. 3) showed diseased vs. normal TMJ. The articular cartilage of TMJ has a surface layer of fibrocartilaginous and deep layer of hyaline-like hypertrophic zone with a thin intermediate proliferative zone. For regeneration of this unique cartilage, cell therapy comes first and injectable smart hydrogels could be employed to transfer cells.[126] As known, the autogenic cells are the gold standard cell source used for tissue regeneration,

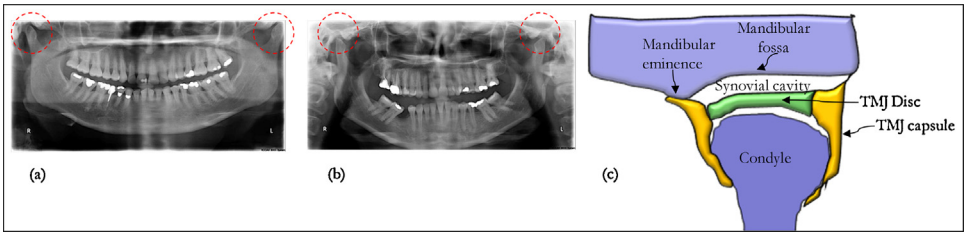

Fig. 3 – Panoramic X-ray showing normal TMJ, indicated by red circles (a) versus diseased TMJ (b). Close view of TMJ (c). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

but it would be very difficult to harvest cells from the diseased TMJ. Accordingly, finding another cell source would be an essential in such case e.g., human umbilical cord derived mesenchymal-like stem cells (HUCM)[127] or primary costal chondrocytes (CCs)[128] or hyaline cartilage cells from anywhere in the body[129] may be an alternative to TMJ condylar cartilage.

Since bone and cartilage require different competing conditions for their regeneration, growing a biphasic osteochondral construct in vitro is therefore very challenging. Ultra rapid tissue engineering techniques coupled with gradient-based scaffolding and a single cell population provide a potentially promising approach for future biological joint replacement. In such condition, hyper-hydrated collagen gels, for example, seeded with hMSCs preconditioned in an osteogenic media at one end but preconditioned in a chondrogenic media at the other end. The development of distinct bone-like and cartilage-like areas and mimicking a primordial joint-like structure has been demonstrated after 7 days of an in vitro culture.[130] The same concept of fabricating gradient-based scaffolding was also applied to poly(D,L-lactic-co-glycolic acid) microspheres. The gradation in such case was obtained by having growth factors instead of cells with different potentials e.g., cartilage-promoting TGF-1 at the cartilaginous end but bone-promoting BMP-2 growth factors at the bony end of the construct. In such case, a newly formed osteochondral tissue was observed in a small mandibular condyle osteochondral defect in New Zealand rabbits after 6 weeks of implantation.[131]

Regarding the TMJ disc, acellular porcine-derived ECM was effective as inductive template for reconstruction of TMJ disc when implanted in vivo for 6 months and it has been assumed that this bioscaffold represents an off-the-shelf solution for engineering of TMJ disc.[132] Regarding the cellular component, adipose stem cells (ADSCs) could be a potential cell source for TMJ engineering.[133] Furthermore, platelet-derived growth factor (PDGF) could be an effective for engineering of TMJ disc. PDGF within an optimal concentration of $\geq$5 ng/ml significantly increased the proliferation rat of the TMJ-disc derived cells, collagen and hyaluronic acid synthesis. It also upregulated RNA levels of type I and II collagens, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs).[134] Basic fibroblast growth factor (bFGF),[135] transforming growth factor-β1 (TGF-β1) and insulin-like growth factor-1 (IGF-1)[136] have been also investigated for potential application in TMJ disc regeneration. All these growth factors have been shown to induce bone marrow mesenchymal stem cell differentiation into fibroblast-like cells, which could synthesise TMJ disc matrix of GAG and type I collagen.

The approaches employed to overcome the challenge of TMJ engineering have been varied from cell injection therapy to the use of synthetic or natural scaffolds as well as relying to some extent on biological modulators; each with varying degree of success. The critical outcome of the success of all engineered TMJ replacements, however, will not only be measured by the restoration of function; the prevention of fibrous or ossified adhesions, the main complications of many surgical interventions, is also considered as a key factor in the success in clinical applications. Therefore, in designing TMJ replacement, incorporation of signalling molecules that allow for rapid and convenient tissue replacement but also prevent adhesions or ossification of the replaced tissue would be very challenging. Furthermore, engineering the osteochondral interface with its complex structure and its cartilaginous component with its zones of different structures and organisation is very challenging. To engineer such spatial complexity, designing scaffolds recapitulating the gradients in the regulatory signals between different cell types through understanding of the molecular cross talk between cells at the interface is required.

4. Concluding remarks and outlook

Tissue engineering provides a new era for therapeutic medicine; it is progressing very rapidly and extends to involve all tissues in our body. Three decades ago, tissue engineering was an idea and today it has become a potential therapy for several conditions. For a more regenerative breakthrough to develop and lead to off-the-shelf bioproducts to replace a variety of lost tissues and organs, a thorough understanding of embryonic development and stem cell biology are required. Regenerating oral tissues, in particular, is very challenging and requires recapitulation of the biological development of several tissues and interfaces.[137] The progress in this field is taking several routes including; cell biology, the development of novel scaffolds/fabrication methods/characterisation techniques. Stem cell therapy and engineering of irreversibly damaged tissues becomes less fictional and is actually progressing towards a reality. Since most of the current or emerging paradigms in tissue engineering have limited and variable outcomes; a true and biological tissue regeneration in not yet achievable. Translating tissue engineering research and development into clinical practice still drives much of science and technology in this field.[61]

Recent advances in tissue engineering suggest that significant changes in the more traditional areas of clinical dentistry are beginning to occur. Thus, there has been a recent surge in guided tissue engineering methods to establish new therapies to manage periodontal diseases beyond the traditional approaches based solely upon infection control.[138] Periodontal diseases are some of the most common oral diseases worldwide, after caries, and have been found to have a role in more general systemic diseases such as diabetes and cardiovascular disease. The need for more reproducible oral tissue replacement therapies is therefore considerable. To date, the regeneration of small or medium-sized periodontal defects using in vitro engineered cell-scaffold constructs is technically feasible, and some of the current products available on the market offer alternatives for selected clinical scenarios. These include Emdogain, Orthoss and BioOss. However, the predictable reconstruction of the innate organisation and function of whole teeth as well as their periodontal structures remains challenging. Future possibilities depend on an improved fundamental understanding of cellular and molecular mechanisms involved in the regeneration of all periodontal tissues, the differentiation potential of stem cells, and the biocompatibility stem cells and materials with host tissues. Major bone reconstructions because of trauma, cancer, or augmentation for dental implants are current examples of how tissue engineering can be also be used for craniofacial applications.[139]

The addition of various protein factors onto implant/material surfaces is also a current approach being widely investigated. While the addition of these growth factors is an exciting perspective, many questions still remain unanswered with respect to application mechanisms of these proteins and the control of their release pattern, increasing the time that they are bioactive and maximising their biological regenerative potential. However, this approach has some consequences such as the high cost of preparation, and protein concentration is crucial to reduce any toxicity/side effects; considerations that must be factored for this approach to become affordable and clinically safe.

The most recent advances in restorative dentistry involve the development, techniques and materials to regenerate the whole tooth complex in a biological manner. Tissue engineering-based approaches certainly have the potential to achieve this and the future research drive seems to be diverting from a metal-based implant to a biological, cell-based one. Thus, the absolute minimum requirement for tooth regeneration of this type is the successful formation of a heterogenous and dynamic array of tissues including roots, the periodontal ligament, nerve and vascular tissues, as well as the essential dentine-pulp complex. Perhaps the least important anatomical structures are the mineralised tissues of the crown as current synthetic tooth crowns function more than adequately, as well as being matched for size, shape, colour and occlusion.[140]

Despite some limited progress and minor successes, there remain distinct and important challenges in the development of reproducible and clinically safe approaches for oral tissue repair and regeneration. Clearly, there is a convincing body of evidence which confirms the need for this type of treatment, and public health data worldwide indicates a more than adequate patient resource. The future of these therapies involving more biological approaches and the use of dental tissue stem cells is promising and advancing. As more and more information is collated and knowledge acquired with respect to dental stem cells and tissues, there may well be a significant interest of their application and wider potential to treat disorders beyond the craniofacial region of the body.

REFERENCES

1. Zaky SH, Cancedda R. Engineering craniofacial structures: facing the challenge. *Journal of Dental Research* 2009;**88**:1077–91.
2. Bonassar LJ, Vacanti CA. Tissue engineering: the first decade and beyond. *Journal of Cellular Biochemistry Supplement* 1998;**30–31**:297–303.
3. Langer R. Tissue engineering: a new field and its challenges. *Pharmaceutical Research* 1997;**14**:840–1.
4. Ravichandran R, Venugopal JR, Sundarrajan S, Mukherjee S, Sridhar R, Ramakrishna S. Minimally invasive injectable short nanofibers of poly(glycerol sebacate) for cardiac tissue engineering. *Nanotechnology* 2012;**23**:385102.
5. Park H, Choi B, Hu J, Lee M. Injectable chitosan hyaluronic acid hydrogels for cartilage tissue engineering. *Acta Biomaterialia* 2013;**9**:4779–86.
6. Amini AA, Nair LS. Injectable hydrogels for bone and cartilage repair. *Biomedical Materials* 2012;**7**:024105.
7. Ravichandran R, Venugopal JR, Sundarrajan S, Mukherjee S, Ramakrishna S. Minimally invasive cell-seeded biomaterial systems for injectable/epicardial implantation in ischemic heart disease. *International Journal of Nanomedicine* 2012;**7**:5969–94.
8. Robey PG. Stem cells near the century mark. *The Journal of Clinical Investigation* 2000;**105**:1489–91.
9. Miron RJ, Zhang YF. Osteoinduction: a review of old concepts with new standards. *Journal of Dental Research* 2012;**91**:736–44.
10. Tai YY, Chen RS, Lin Y, Ling TY, Chen MH. FGF-9 accelerates epithelial invagination for ectodermal organogenesis in real time bioengineered organ manipulation. *Cell Communication and Signaling* 2012;**10**:1–10.
11. Mathieu S, Jeanneau C, Sheibat-Othman N, Kalaji N, Fessi H, About I. Usefulness of controlled release of growth factors in investigating the early events of dentin-pulp regeneration. *Journal of Endodontics* 2013;**39**:228–35.
12. Bento LW, Zhang Z, Imai A, Nor F, Dong Z, Shi S, *et al.* Endothelial differentiation of SHED requires MEK1/ERK signaling. *Journal of Dental Research* 2013;**92**:51–7.
13. Lee JS, Wikesjo UM, Park JC, Jang YJ, Pippig SD, Bastone P, *et al.* Maturation of periodontal tissues following implantation of rhGDF-5/beta-TCP in one-wall intra-bony defects in dogs: 24-week histological observations. *Journal of Clinical Periodontology* 2012;**39**:466–74.
14. Nakashima M, Reddi AH. The application of bone morphogenetic proteins to dental tissue engineering. *Nature Biotechnology* 2003;**21**:1025–32.
15. Berlanga J, Fernandez JI, Lopez E, Lopez PA, Del Rio A, Valenzuela C, *et al.* Heberprot-P: a novel product for treating advanced diabetic foot ulcer. *MEDICC Review* 2013;**15**:11–5.
16. Langer R, Vacanti J. Tissue engineering. *Science* 1993;**260**:920–6.
17. Rose FRAJ, Oreffo ROC. Bone tissue engineering: hops vs. hype. *Biochemical and Biophysical Research Communications* 2002;**292**:1–7.

18. Nussenbaum B, Krebsbach PH. The role of gene therapy for craniofacial and dental tissue engineering. *Advanced Drug Delivery Reviews* 2006;**58**:577–91.
19. Zhang QZ, Nguyen AL, Yu WH, Le AD. Human oral mucosa and gingiva: a unique reservoir for mesenchymal stem cells. *Journal of Dental Research* 2012;**91**:1011–8.
20. Wang L, Zhao Y, Shi S. Interplay between mesenchymal stem cells and lymphocytes: implications for immunotherapy and tissue regeneration. *Journal of Dental Research* 2012;**91**:1003–10.
21. Vacanti JP, Langer R, Upton J, Marler JJ. Transplantation of cells in matrices for tissue regeneration. *Advanced Drug Delivery Reviews* 1998;**33**:165–82.
22. Gupte MJ, Ma PX. Nanofibrous scaffolds for dental and craniofacial applications. *Journal of Dental Research* 2012;**91**:227–34.
23. Tomlinson MJ, Tomlinson S, Yang XB, Kirkham J. Cell separation: terminology and practical considerations. *Journal of Tissue Engineering* 2012;**4**:1–14.
24. Herring SW, Ochareon P. Bone – special problems of the craniofacial region. *Orthodontics and Craniofacial Research* 2005;**8**:174–82.
25. Duailibi TM, Duailibi SE, Young CS, Bartlett JD, Vacanti JP, *et al.* Bioengineered teeth from cultured rat tooth bud cells. *Journal of Dental Research* 2004;**83**:523–8.
26. Huang GT. Pulp and dentin tissue engineering and regeneration: current progress. *Regenerative Medicine* 2009;**4**:697–707.
27. Periodontal and Dental Implant Education and Information. http://wwwsangerddscom/patient-educationhtml.
28. Aghazadeh A, Rutger Persson G, Renvert S. A single-centre randomized controlled clinical trial on the adjunct treatment of intra-bony defects with autogenous bone or a xenograft: results after 12 months. *Journal of Clinical Periodontology* 2012;**39**.
29. Tecles O, Laurent P, Aubut V, About I. Human tooth culture: a study model for reparative dentinogenesis and direct pulp capping materials biocompatibility. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 2008;**85**:180–7.
30. Malhotra N, Mala K. Regenerative endodontics as a tissue engineering approach: past, current and future. *Australian Endodontic Journal* 2012 Dec;**38**:137–48. doi: 101111/j1747-4477201200355x.
31. Laurent P, Camps J, About I. Biodentine™ induces TGF-b1 release from human pulp cells and early dental pulp mineralization. *International Endodontic Journal* 2012;**45**:439–48.
32. Neslihan TP, Tapsin S, Demirel S, Yalvac ME, Akyuz S, Yarat A, *et al.* Isolation and characterization of dental pulp stem cells from a patient with Papillonân Lefevre syndrome. *Journal of Endodontics* 2013;**39**:31–8.
33. Na S, Zhang H, Huang F, Wang W, Ding Y, Li D, *et al.* Regeneration of dental pulp/dentine complex with a three-dimensional and scaffold-free stem-cell sheet-derived pellet. *Journal of Tissue Engineering and Regenerative Medicine* 2013. [Epub ahead of print].
34. Ji K, Liu Y, Lu W, Yang F, Yu J, Wang X, *et al.* Periodontal tissue engineering with stem cells from the periodontal ligament of human retained deciduous teeth. *Journal of Periodontal Research* 2013;**48**:105–16.
35. Ma L, Makino Y, Yamaza H, Akiyama K, Hoshino Y, Song G, *et al.* Cryopreserved dental pulp tissues of exfoliated deciduous teeth is a feasible stem cell resource for regenerative medicine. *PLoS ONE* 2012;**7**:e51777.
36. Galler KM, Hartgerink JD, Cavender AC, Schmalz G, D'Souza RN. A customized self-assembling peptide hydrogel for dental pulp tissue engineering. *Tissue Engineering Part A* 2012;**181**:176–84.
37. Galler KM, Cavender AC, Koeklue U, Suggs LJ, Schmalz G, D'Souza RN. Bioengineering of dental stem cells in a PEGylated fibrin gel. *Regenerative Medicine* 2011;**6**:191–200.
38. Wang Y, Zhao Y, Jia W, Yang J, Ge L. Preliminary study on dental pulp stem cell-mediated pulp regeneration in canine immature permanent teeth. *Journal of Endodontics* 2013;**39**:195–201.
39. Fransson H. On the repair of the dentine barrier. *Swedish Dental Journal Supplement* 2012;**226**:9–84.
40. Orhan EO, Maden M, Senguuven B. Odontoblast-like cell numbers and reparative dentine thickness after direct pulp capping with platelet-rich plasma and enamel matrix derivative: a histomorphometric evaluation. *International Endodontic Journal* 2012;**45**:317–25.
41. Kozlovsky A, Aboodi G, Moses O, Tal H, Artzi Z, Weinreb M, *et al.* Bio-degradation of a resorbable collagen membrane (Bio-Gide®) applied in a double-layer technique in rats. *Clinical Oral Implants Research* 2009;**20**:1116–23.
42. Oortgiesen DAW, Plachokova AS, Geenen C, Meijer GJ, Walboomers XF, van den Beucken JJJP, *et al.* Alkaline phosphatase immobilization onto Bio-Gide® and Bio-Oss® for periodontal and bone regeneration. *Journal of Clinical Periodontology* 2012;**39**(6):546–55.
43. Moses O, Pitaru S, Artzi Z, Nemcovsky CE. Healing of dehiscence-type defects in implants placed together with different barrier membranes: a comparative clinical study. *Clinical Oral Implants Research* 2005;**16**:210–9.
44. Retzepi M, Donos N. Guided bone regeneration: biological principle and therapeutic applications. *Clinical Oral Implants Research* 2010;**21**:567–76.
45. Gielkens PFM, Schortinghuis J, De Jong JR, Raghoebar GM, Stegenga B, Bos RRM. Vivosorb®, Bio-Gide®, and Gore-Tex® as barrier membranes in rat mandibular defects: an evaluation by microradiography and micro-CT. *Clinical Oral Implants Research* 2008;**19**:516–21.
46. Duskova M, Leamerova E, Sosna B, Gojis O. Technical strategies guided tissue regeneration, barrier membranes and reconstruction of the cleft maxillary alveolus. *The Journal of Craniofacial Surgery* 2006;**7**:1153–60.
47. Bunyaratavej P, Wang H-L. Collagen membranes a review. *Journal of Periodontology* 2001;**72**:215–29.
48. Verissimo DM, Leitao RF, Ribeiro RA, Figueiro SD, Sombra AS, Goes JC, *et al.* Polyanionic collagen membranes for guided tissue regeneration: Effect of progressive glutaraldehyde cross-linking on biocompatibility and degradation. *Acta Biomaterialia* 2010;**6**:4011–8. doi: 101016/jactbio201004012.
49. Song J-H, Kim H-E, Kim H-W. Collagen-apatite nanocomposite membranes for guided bone regeneration. 7. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 2007;**83B**:248–57.
50. Yadav VS, Narula SC, Sharma RK, Tewari S, Yadav R. Clinical evaluation of guided tissue regeneration combined with autogenous bone or autogenous bone mixed with bioactive glass in intrabony defects. *Journal of Oral Science* 2011;**53**:481–8.
51. Kaigler D, Avila G, Wisner-Lynch L, Nevins ML, Nevins M, Rasperini G, *et al.* Platelet-derived growth factor applications in periodontal and peri-implant bone regeneration. *Expert Opinion on Biological Therapy* 2011;**11**:375–85.
52. Miron RJ, Saulacic N, Buser D, Iizuka T, Sculean A. Osteoblast proliferation and differentiation on a barrier membrane in combination with BMP2 and TGFbeta1. *Clinical Oral Investigations* 2013;**17**:981–8.
53. Hong KS, Kim EC, Bang SH, Chung CH, Lee YI, Hyun JK, *et al.* Bone regeneration by bioactive hybrid membrane

containing FGF2 within rat calvarium. *Journal of Biomedical Materials Research Part A* 2010;**94**:1187–94.
54. Guimaraes. Mdo C, Passanezi E, Sant'Ana AC, Grechi SL, Taba Junior M. Digital subtraction radiographic analysis of the combination of bioabsorbable membrane and bovine morphogenetic protein pool in human periodontal infrabony defects. *Journal of Applied Oral Science* 2010;**18**:379–84.
55. Jung RE, Windisch SI, Eggenschwiler AM, Thoma DS, Weber FE, Hammerle CH. A randomized-controlled clinical trial evaluating clinical and radiological outcomes after 3 and 5 years of dental implants placed in bone regenerated by means of GBR techniques with or without the addition of BMP-2. *Clinical Oral Implants Research* 2009;**20**:660–6.
56. Scantlebury T, Ambruster J. The development of guided regeneration: making the impossible possible and the unpredictable predictable. *Journal of Evidence Based Dental Practice* 2012;**12**:101–17.
57. Zarkesh N, Nowzari H, Morrison JL, Slots J. Tetracycline-coated polytetrafluoroethylene barrier membranes in the treatment of intraosseous periodontal lesions. *Journal of Periodontology* 1999;**70**:1008–16.
58. Chen YT, Hung SL, Lin LW, Chi LY, Ling LJ. Attachment of periodontal ligament cells to chlorhexidine-loaded guided tissue regeneration membranes. *Journal of Periodontology* 2003;**74**:1652–9.
59. Chou AH, LeGeros RZ, Chen Z, Li Y. Antibacterial effect of zinc phosphate mineralized guided bone regeneration membranes. *Implant Dentistry* 2007;**16**:89–100.
60. Martins-Junior PA, Alcantara CE, Resende RR, Ferreira AJ. Carbon nanotubes: directions and perspectives in oral regenerative medicine. *Journal of Dental Research* 2013;**92**:575–83.
61. Chen F-M, Zhang J, Zhang M, An Y, Chen F, Wu Z-F. A review on endogenous regenerative technology in periodontal regenerative medicine. *Biomaterials* 2010;**31**:7892–927.
62. Chen F-M, Sun H-H, Lu H, Yu Q. Stem cell-delivery therapeutics for periodontal tissue regeneration. *Biomaterials* 2012;**33**:6320–44.
63. Fawzy E-SKM, Paris S, Becker ST, Neuschl M, De Buhr W, *et al.* Periodontal regeneration employing gingival margin-derived stem/progenitor cells: an animal study. *Journal of Clinical Periodontology* 2012;**39**:861–70.
64. McGuire MK, Scheyer ET. A randomized, double-blind, placebo-controlled study to determine the safety and efficacy of cultured and expanded autologous fibroblast injections for the treatment of interdental papillary insufficiency associated with the papilla priming procedure. *Journal of Periodontology* 2007;**78**:4–17.
65. Yamamiya K, Okuda K, Kawase T, Hata K, Wolff LF, Yoshie H. Tissue-engineered cultured periosteum used with platelet-rich plasma and hydroxyapatite in treating human osseous defects. *Journal of Periodontology* 2008;**79**:811–8.
66. Yamada Y, Ueda M, Hibi H, Baba S. A novel approach to periodontal tissue regeneration with mesenchymal stem cells and platelet-rich plasma using tissue engineering technology: a clinical case report. *International Journal of Periodontics and Restorative Dentistry* 2006;**26**:363–9.
67. Birang R, Torabi A, Shahabooei M, Rismanchian M. Effect of plasma-rich in platelet-derived growth factors on peri-implant bone healing: an experimental study in canines. *Dental Research Journal (Isfahan)* 2012;**9**:93–9.
68. Reichert. da Silva Assuncao L, Colenci R, Ferreira do-Amaral CC, Sonoda CK, Mogami Bomfim SR, *et al.* Periodontal tissue engineering after tooth replantation. *Journal of Periodontology* 2011;**82**:758–66.
69. de Oliva MA, Maximiano WM, de Castro LM, da Silva Jr PE, Fernandes RR, Ciancaglini P, *et al.* Treatment with a growth factor-protein mixture inhibits formation of mineralized nodules in osteogenic cell cultures grown on titanium. *Journal of Histochemistry and Cytochemistry* 2009;**57**:265–76.
70. Parrish LC, Miyamoto T, Fong N, Mattson JS, Cerutis DR. Non-bioabsorbable vs. bioabsorbable membrane: assessment of their clinical efficacy in guided tissue regeneration technique. A systematic review. *Journal of Oral Science* 2009;**51**:383–400.
71. Koop R, Merheb J, Quirynen M. Periodontal regeneration with enamel matrix derivative in reconstructive periodontal therapy: a systematic review. *Journal of Periodontology* 2012;**83**:707–20.
72. Bhutda G, Deo V. Five years clinical results following treatment of human intra-bony defects with an enamel matrix derivative: a randomized controlled trial. *Acta Odontologica Scandinavica* 2013;**71**:764–70.
73. Tu YK, Needleman I, Chambrone L, Lu HK, Faggion Jr CM. A Bayesian network meta-analysis on comparisons of enamel matrix derivatives, guided tissue regeneration and their combination therapies. *Journal of Clinical Periodontology* 2012;**39**:303–14.
74. Zhang FQ, Meng HX, Han J, Liu KN. Effects of emdogain on human periodontal ligament cells in vitro. *Beijing Da Xue Xue Bao* 2012;**44**:6–10.
75. Lees JD, Robinson C, Shore RC, Paine ML, Brookes SJ. Cellular uptake and processing of enamel matrix derivative by human periodontal ligament fibroblasts. *Archives of Oral Biology* 2012. pii:S0003-9969(12):00262-2.
76. Amin HD, Olsen I, Knowles JC, Donos N. Differential effect of amelogenin peptides on osteogenic differentiation in vitro: identification of possible new drugs for bone repair and regeneration. *Tissue Engineering Part A* 2012;**18**:1193–202.
77. Kasaj A, Meister J, Lehmann K, Stratul SI, Schlee M, Stein JM, *et al.* The influence of enamel matrix derivative on the angiogenic activity of primary endothelial cells. *Journal of Periodontal Research* 2012;**47**:479–87.
78. Hu B, Nadiri A, Kuchler-Bopp S, Perrin-Schmitt F, Peters H, Lesot H. Tissue engineering of tooth crown, root, and periodontium. *Tissue Engineering* 2006;**12**:2069–75.
79. Ikeda E, Morita R, Nakao K, Ishida K, Nakamura T, Takano-Yamamoto T, *et al.* Fully functional bioengineered tooth replacement as an organ replacement therapy. *Proceedings of the National Academy of Sciences of the United States of America* 2009;**106**:13475–80.
80. Young CS, Terada S, Vacanti JP, Honda M, Bartlett JD, Yelick PC. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. *Journal of Dental Research* 2002;**81**:695–700.
81. Honda MJ, Fong H, Iwatsuki S, Sumita Y, Sarikaya M. Tooth-forming potential in embryonic and postnatal tooth bud cells. *Medical Molecular Morphology* 2008;**41**:183–92.
82. Sumita Y, Honda MJ, Ohara T, Tsuchiya S, Sagara H, Kagami H, *et al.* Performance of collagen sponge as a 3-D scaffold for tooth-tissue engineering. *Biomaterials* 2006;**27**:3238–48.
83. Bluteau G, Luder H-U, De Bari C, Mitsiadis TA. Stem cells for tooth engineering. *European Cells and Materials* 2008;**16**:1–9.
84. Snead ML. Whole-tooth regeneration: it takes a village of scientists, clinicians, and patients. *Journal of Dental Education* 2008;**72**:903–11.
85. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell* 1975;**6**:331–43.
86. Green H, Kehinde O, Thomas J. Growth of cultured human epidermal-cells into multiple epithelia suitable for grafting. *Proceedings of the National Academy of Sciences of the United States of America* 1979;**76**:5665–8.

87. Phillips TJ. Cultured skin grafts, past, present, future. *Archives of Dermatology* 1988;**124**:1035–8.
88. Faure M, Mauduit G, Schmitt D, Kanitakis J, Demidem A, Thivolet J. Growth and differentiation of human epidermal cultures used as autografts and allografts in humans. *British Journal of Dermatology* 1987;**116**:161–70.
89. Boyce ST, Goretsky MJ, Greenhalgh DG, Kagan RJ, Rieman MT, Warden GD. Comparative-assessment of cultured skin substitutes and native skin autograft for treatment of full-thickness burns. *Annals of Surgery* 1995;**222**:743–52.
90. Ehrmann RL, Gey GO. The growth of cells on a transparent gel of reconstituted rat-tail collagen. *Journal of the National Cancer Institute* 1956;**16**:1375–403.
91. Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T. Living tissue formed in vitro and accepted as skin-equivalent tissue of full thickness. *Science* 1981;**211**:1052–4.
92. Madden MR, Finkelstein JL, Staianocoico L, Goodwin CW, Shires GT, Nolan EE, *et al.* Grafting of cultured allogenic epidermis on 2nd-degree and 3rd-degree burn wounds on 26 patients. *Journal of Trauma-Injury Infection and Critical Care* 1986;**26**:955–62.
93. Parenteau NL, Nolte CM, Bilbo P, Rosenberg M, Wilkins LM, Johnson EW, *et al.* Epidermis generated in vitro: practical considerations and applications. *Journal of Cellular Biochemistry* 1991;**45**:245–51.
94. Falanga V, Sabolinski M. A bilayered living skin construct (APLIGRAF (R)) accelerates complete closure of hard-to-heal venous ulcers. *Wound Repair and Regeneration* 1999;**7**:201–7.
95. Sasaki R, Yamato M, Takagi R, Ohki T, Matsumine H, Okano T, *et al.* Punch and spindle-shaped biopsies for collecting oral mucosal tissue for the fabrication of transplantable autologous epithelial cell sheets. *Journal of Biomedical Materials Research Part A* 2012;**100A**:2849–54.
96. Bayar GR. Ex vivo produced oral mucosa equivalent preliminary report: a technical note. *Turkish Journal of Medical Sciences* 2011;**41**:109–15.
97. Bornstein MM, Reichart PA, Buser D, Bosshardt DD. Tissue response and wound healing after placement of two types of bioengineered grafts containing vital cells in submucosal maxillary pouches: an experimental pilot study in rabbits. *International Journal of Oral and Maxillofacial Implants* 2011;**26**:768–75.
98. Patterson JM, Bullock AJ, MacNeil S, Chapple CR. Methods to reduce the contraction of tissue-engineered buccal mucosa for use in substitution urethroplasty. *European Urology* 2011;**60**:856–61.
99. Sotozono C, Inatomi T, Nakamura T, Koizumi N, Yokoi N, Ueta M, *et al.* Visual improvement after cultivated oral mucosal epithelial transplantation. *Ophthalmology* 2013;**120**:193–200.
100. Rastogi S, Modi M, Sathian B. The efficacy of collagen membrane as a biodegradable wound dressing material for surgical defects of oral mucosa: a prospective study. *Journal of Oral and Maxillofacial Surgery* 2009;**67**:1600–6.
101. Herford AS, Akin L, Cicciu M, Maiorana C, Boyne PJ. Use of a porcine collagen matrix as an alternative to autogenous tissue for grafting oral soft tissue defects. *Journal of Oral and Maxillofacial Surgery* 2010;**68**:1463–70.
102. Ge Z, Yang Q, Xiang X, Liu KZ. Assessment of silk fibroin for the repair of buccal mucosa in a rat model. *International Journal of Oral and Maxillofacial Surgery* 2012;**41**:673–80.
103. Kinikoglu B, Rodriguez-Cabello JC, Damour O, Hasirci V. The influence of elastin-like recombinant polymer on the self-renewing potential of a 3D tissue equivalent derived from human lamina propria fibroblasts and oral epithelial cells. *Biomaterials* 2011;**32**:5756–64.
104. Golinski PA, Groger S, Herrmann JM, Bernd A, Meyle J. Oral mucosa model based on a collagen-elastin matrix. *Journal of Periodontal Research* 2011;**46**:704–11.
105. Moharamzadeh K, Colley H, Murdoch C, Hearnden V, Chai WL, Brook IM, *et al.* Tissue-engineered oral mucosa. *Journal of Dental Research* 2012;**91**:642–50.
106. Abou Neel EA, Bozec L, Knowles JC, Syed O, Mudera V, Day R, *et al.* Collagen – emerging collagen based therapies hit the patient. *Advanced Drug Delivery Reviews* 2013;**65**:429–56.
107. Huang G, Li L, Wen Y. Functional reconstruction with tissue engineered myoblast in facial muscle of rat. *Hua Xi Kou Qiang Yi Xue Za Zhi* 2003;**21**:432–4.
108. Kim J, Hadlock T, Cheney M, Varvares MJM. Muscle tissue engineering for partial glossectomy defects. *Archives of Facial Plastic Surgery* 2003;**5**:403–7.
109. Klumpp D, Horch RE, Bitto F, Boos AM, Kneser U, Beier JP. Skeletal muscle tissue engineering – current concepts and future perspectives. *Handchirurgie Mikrochirurgie Plastische Chirurgie* 2010;**42**:354–9.
110. Koning M, Werker PM, van Luyn MJ, Harmsen MC. Hypoxia promotes proliferation of human myogenic satellite cells: a potential benefactor in tissue engineering of skeletal muscle. *Tissue Engineering Part A* 2011;**17**:1747–58.
111. Koning M, Werker PM, van der Schaft DW, Bank RA, Harmsen MC. MicroRNA-1 and microRNA-206 improve differentiation potential of human satellite cells: a novel approach for tissue engineering of skeletal muscle. *Tissue Engineering Part A* 2012;**18**:889–98.
112. Li M, Dickinson CE, Finkelstein EB, Neville CM, Sundback CA. The role of fibroblasts in self-assembled skeletal muscle. *Tissue Engineering Part A* 2011;**17**:2641–50.
113. Bueno EM, Diaz-Siso JR, Sisk GC, Chandawarkar A, Kiwanuka H, Lamparello B, *et al.* Vascularized composite allotransplantation and tissue engineering. *Journal of Craniofacial Surgery* 2013;**24**:256–63.
114. Koning M, Harmsen MC, van Luyn MJ, Werker PM. Current opportunities and challenges in skeletal muscle tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine* 2009;**3**:407–15.
115. Longo UG, Loppini M, Berton A, Spiezia F, Maffulli N, Denaro V. Tissue engineered strategies for skeletal muscle injury. *Stem Cells International* 2012;**2012**:1–9.
116. Chan YH, Huang TW, Young TH, Lou PJ. Human salivary gland acinar cells spontaneously form three-dimensional structures and change the protein expression patterns. *Journal of Cellular Physiology* 2011;**226**:3076–85.
117. Chan YH, Huang TW, Chou YS, Hsu SH, Su WF, Lou PJ, *et al.* Formation of post-confluence structure in human parotid gland acinar cells on PLGA through regulation of E-cadherin. *Biomaterials* 2012;**33**:464–72.
118. Joraku A, Sullivan CA, Yoo JJ, Atala A. Tissue engineering of functional salivary gland tissue. *The Laryngoscope* 2005;**115**:244–8.
119. Cantara SI, Soscia DA, Sequeira SJ, Jean-Gilles RP, Castracane J, Larsen M. Selective functionalization of nanofiber scaffolds to regulate salivary gland epithelial cell proliferation and polarity. *Biomaterials* 2012;**33**:8372–82.
120. Baum B, Zheng C, Alevizos I, Cotrim AP, Liu S, McCullagh L, *et al.* Development of a gene transfer-based treatment for radiation-induced salivary hypofunction. *Oral Oncology* 2010;**46**:4–8.
121. Nguyen TT, Mui B, Mehrabzadeh M, Chea Y, Chaudhry Z, Chaudhry K, *et al.* Regeneration of tissues of the oral complex: current clinical trends and research advances. *Journal Canadian Dental Association Journal de l Association Dentaire Canadienne* 2013;**79**:1–9.
122. Schmelzeisen R, Schimming R, Sittinger M. Making bone: implant insertion into tissue-engineered bone for

maxillary sinus floor augmentation-a preliminary report. *Journal of Cranio-Maxillo-Facial Surgery* 2003;**31**:34–9.
123. Matsuno T, Hashimoto Y, Adachi S, Omata K, Yoshitaka Y, Ozeki Y, *et al*. Preparation of injectable 3D-formed beta-tricalcium phosphate bead/alginate composite for bone tissue engineering. *Dental Materials Journal* 2008;**27**:827–34.
124. Moreau JL, Xu HH. Mesenchymal stem cell proliferation and differentiation on an injectable calcium phosphate-chitosan composite scaffold. *Biomaterials* 2009;**30**:2675–82.
125. Anitua E, Prado R, Orive G. Bilateral sinus elevation evaluating plasma rich in growth factors technology: a report of five cases. *Clinical Implant Dentistry and Related Research* 2012;**14**:51–60.
126. Vinatier C, Gauthier O, Fatimi A, Merceron C, Masson M, Moreau A, *et al*. An injectable cellulose-based hydrogel for the transfer of autologous nasal chondrocytes in articular cartilage defects. *Biotechnology and Bioengineering* 2009;**102**:1259–67.
127. Bailey MM, Wang L, Bode CJ, Mitchell KE, Detamore MS. A comparison of human umbilical cord matrix stem cells and temporomandibular joint condylar chondrocytes for tissue engineering temporomandibular joint condylar cartilage. *Tissue Engineering* 2007;**13**:2003–10.
128. Anderson DE, Athanasiou KA. A comparison of primary and passaged chondrocytes for use in engineering the temporomandibular joint. *Archives of Oral Biology* 2009;**54**:138–45.
129. Wang L, Lazebnik M, Detamore MS. Hyaline cartilage cells outperform mandibular condylar cartilage cells in a TMJ fibrocartilage tissue engineering application. *Osteoarthritis and Cartilage* 2009;**17**:346–53.
130. Brady MA, Sivananthan S, Mudera V, Liu Q, Wiltfang J, Warnke PH. The primordium of a biological joint replacement: coupling of two stem cell pathways in biphasic ultrarapid compressed gel niches. *Journal of Cranio-Maxillo-Facial Surgery* 2011;**39**:380–6.
131. Dormer NH, Busaidy K, Berkland CJ, Detamore MS. Osteochondral interface regeneration of rabbit mandibular condyle with bioactive signal gradients. *Journal of Oral and Maxillofacial Surgery* 2011;**69**:e50–7.
132. Brown BN, Chung WL, Almarza AJ, Pavlick MD, Reppas SN, Ochs MW, *et al*. Inductive, scaffold-based, regenerative medicine approach to reconstruction of the temporomandibular joint disk. *Journal of Oral and Maxillofacial Surgery* 2012;**70**:2656–68.
133. Maenpaa K, Ella V, Mauno J, Kellomaki M, Suuronen R, Ylikomi T, *et al*. Use of adipose stem cells and polylactide discs for tissue engineering of the temporomandibular joint disc. *Journal of the Royal Society Interface* 2010;**7**:177–88.
134. Hanaoka K, Tanaka E, Takata T, Miyauchi M, Aoyama J, Kawai N, *et al*. Platelet-derived growth factor enhances proliferation and matrix synthesis of temporomandibular joint disc-derived cells. *Angle Orthodontist* 2006;**76**:486–92.
135. Su X, Bao G, Kang H. Effects of basic fibroblast growth factor on bone marrow mesenchymal stem cell differentiation into temporomandibular joint disc cells. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi* 2012;**29**:732–6.
136. Kang H, Bi YD, Li ZQ, Qi MY, Peng EM. Effect of transforming growth factor beta(1) and insulin-like growth factor-I on extracelluar matrix synthesis of self-assembled constructs of goat temporomandibular joint disc. *Zhonghua Kou Qiang Yi Xue Za Zhi* 2011;**46**:541–6.
137. Scheller EL, Krebsbach PH, Kohn DH. Tissue engineering: state of the art in oral rehabilitation. *Journal of Oral Rehabilitation* 2009;**36**:368–89.
138. Chen FM, Jin Y. Periodontal tissue engineering and regeneration: current approaches and expanding opportunities. *Tissue Engineering Part B Reviews* 2010;**16**:219–55. doi: 101089/tenTEB20090562 2010.
139. Xu HH, Weir MD, Simon CG. Injectable and strong nano-apatite scaffolds for cell/growth factor delivery and bone regeneration. *Dental Materials* 2008;**24**:1212–22. doi: 101016/jdental200802001.
140. Volponi AA, Pang Y, Sharpe PT. Stem cell-based biological tooth repair and regeneration. *Trends in Cell Biology* 2010;**20–206**:715–22.