Chapter 12
Oral and Maxillo-Facial

Kristina Arvidson, Michele Cottler-Fox, Sølve Hellem, and Kamal Mustafa

Abstract Regenerative medicine and dentistry are two rapidly growing fields of research with important clinical implications. Recent advances in cell biology, biotechnology, material science and tissue transplantation have been translated into new approaches to clinical repair and replacement of tissues and organs. In dentistry, a number of regenerative therapies and materials have been in clinical use for many years, to repair small and large defects involving multiple tissue types. Currently, various strategies are applied to stimulate healing of bone defects and to restore lost maxillofacial bone and periodontal support following traumatic insult, tumor ablation, disease or congenital deformities.

Bone tissue engineering is an emerging field using bone-forming cells seeded onto synthetic scaffolds to form hybrid constructs that can be used to regenerate tissues. There are numerous published case reports of the application of bone tissue engineering for oral and maxillofacial surgical reconstruction, periodontal tissue regeneration and sinus floor augmentation.

Mesenchymal stem cells (MSC) are currently the cells of choice for bone tissue engineering and can be isolated from many different tissues such as bone marrow, periosteum, and trabecular bone as well as from muscle, adipose tissue and synovial membrane. MSC have also been found among the cells derived from human umbilical cord: *in vivo*, these cells have demonstrated that they are capable of osteogenic differentiation, leading to bone formation and *in vitro* have shown adipogenic, chondrogenic, and osteogenic differentiation. Further, MSC have been identified in periodontal ligament, deciduous and permanent molar teeth. Recent research has shown that these cells have promising regenerative potential. Thus stem cell-based bone tissue engineering is a promising concept for reconstruction/ regeneration of craniofacial defects but much work remains before this approach becomes a routine part of clinical practice.

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G. Steinhoff (ed.), *Regenerative Medicine - from Protocol to Patient*,
DOI 10.1007/978-3-319-28293-0_12

Keywords Oral cavity • Teeth • Bone resorption • Endosseous cylindrical implant systems • Osseointegration of dental implants • Endosseous implantation • Periodontium • Periodontal disease • Periodontitis • Periodontal regeneration • Bone tissue engineering • Guided tissue regeneration (GTR) • Enamel matrix proteins (EMD • Bone defects • Bone grafting • Reconstruction of large bone defects • Segmental osteodistraction • Severe dento-alveolar trauma • Oral stem cells • Angiogenesis • Bone regeneration • Degradable aliphatic polyester scaffolds • Bone marrow osteoprogenitors • Aalveolar bone mesenchymal cells (BMSC) • Periodontal ligament (PDL) • Dental pulp tissue • Pulp-derived mesenchymal stem cells (PDSC) • Dentin • Deciduous tooth stem cells (SHED) • Embryonic tooth germ cells • Postnatal tooth germ cells • Stem cell-conditioned medium (CM)

12.1 Background

Modern dentistry is not limited to maintenance of dentition but has many subspecialties encompassing diagnosis and treatment of conditions affecting the oral and maxillofacial structures. In this relatively small area of the body, many different cells and tissue types occur in morphologically complex structures. Thus defects often involve multiple tissue types, including teeth and craniofacial bones, nerves and blood vessels, soft tissues such as mucosa, skin and muscles, salivary glands and specialized sensory organs.

The oral cavity plays an important role in daily living, including selection of nutritional intake through the complicated neural interactions of taste and smell. It is well documented in the scientific literature that teeth are important to both general health and quality of life through masticatory function, as well as to esthetics and speech. The oral cavity is important to general health and the quality of life because it is the initial organ of digestion: the first stage of the digestive process or mastication, the mechanical breaking up of solid food particles into smaller pieces by chewing and mixing them with saliva and its enzymes, occurs here. Natural dentition or a properly functioning substitute (fixed or removable prostheses) is of major importance to this function. The oral cavity is important to esthetics and speech because the physical appearance of the mouth, *i.e.* the teeth and lips, are essential to these functions and help in defining social and sexual attractiveness.

Over the past 50–60 years there have been major overall improvements in oral health, reflecting advances in dental research during this period. One of the most exciting developments is a change in traditional concepts of disease and its sequelae; from mechanical repair of damage to teeth and surrounding tissues caused by dental disease, to a more biologically-based approach to treatment options and the etiology of dental diseases. Advances in basic science using techniques from cellular and molecular biology have been translated into clinical practice. At the same time, clinical and epidemiological studies have improved methods of diagnosis, treatment and prevention of a wide range of oral health problems.

A striking development is the decrease in the number of edentulous people over the past 40 years. The elderly are retaining their natural dentition and the mean number of standing teeth is higher than a generation ago. Improvements in periodontal health and oral health care are obvious. Many children are caries free or without active caries and the caries rate in adults has decreased. Important contributing factors to caries prevention are water fluoridation and the widespread use of fluoride toothpaste, but it has also been shown that social, economic and geographic factors play important roles.

The focus of restorative care has shifted from 'black to white', as new tooth-colored resin-based materials have been widely adopted as alternatives to amalgam. The longevity and stability of the resin materials have also been improved. With increasing patient awareness of oral esthetics, not only are posterior composite restorations preferred to amalgam, but bleaching materials have also been introduced for a whiter, brighter smile.

12.2 State of the Art

12.2.1 Loss of Permanent Teeth

One of the most common challenges for the dental clinician today, however, is rehabilitation following loss of the permanent teeth and the surrounding structures. Maintenance of good oral function is significant for general wellbeing, nutritional status and general health (Buhlin et al. 2002, 2003; Sheiham and Steele 2001; Nowjack-Raymer and Sheiham 2003). Loss of all the teeth or even of one tooth is a dramatic life event. For many people replacing missing teeth with complete dentures is unsatisfactory: not only are oral factors such as pain, taste perception and chewing capacity adversely affected, but the patient may also undergo marked psychological changes such as reduced self-image and loss of confidence in social situations (Trulsson et al. 2002).

Bone resorption is a common sequela to tooth extraction, but both the rate and the total amount of resorption may vary between individuals. While the causes of this variation are still unclear, it is recognised that resorption of residual ridges after loss of all the teeth is a complex biophysical process. Successful replacement of the dentition with complete removable dentures that merely rest on the mucosa presents a challenge, not only for dentists but for the wearer: in order to eat, drink, or talk whilst wearing dentures, patients must master amazing adaptations of the oral musculature (Fig. 12.1).

The concept of treating edentulism by osseointegration of dental implants was first proposed in the 1960's by two independent groups: Professor Schroeder at the University of Berne, Switzerland and Professor Brånemark at the University of Gothenburg, Sweden. Their data were based on treatment protocols using endosseous, root analogue, titanium implants. These investigators were the first to document

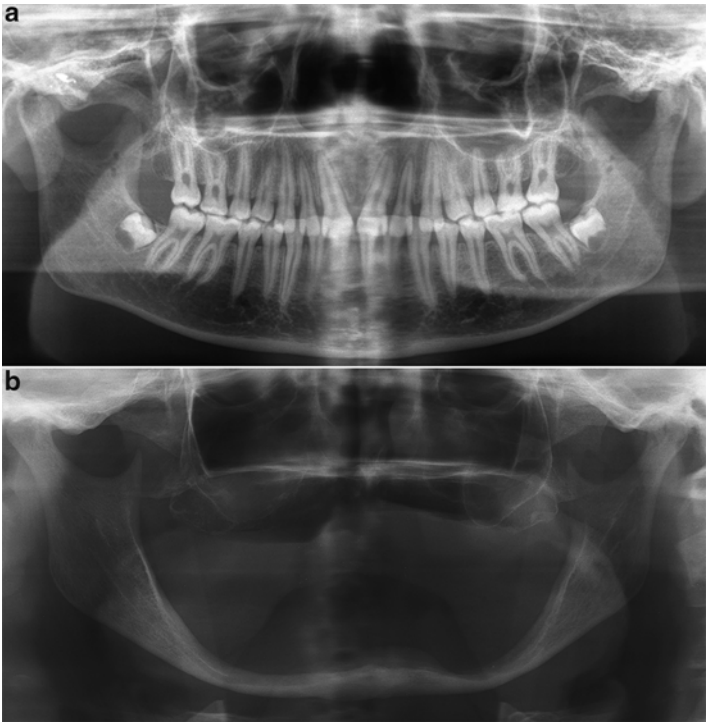

Fig. 12.1 Radiograph of fully dentate jaws with no signs of bone loss or other defects (**a**). Advanced resorption of the mandible in an edentulous patient (**b**)

the fundamental requirements for osseointegration and the interaction between the titanium surface and bone (Brånemark et al. 1969, 1977; Schroeder et al. 1981). They also addressed the primary biomechanical requirements for dental implant design. Both research teams obtained excellent results through the integration of basic biological and biomechanical knowledge and the initiation and application of clinical research projects.

Most of the endosseous cylindrical implant systems subsequently developed, both for submerged and non-submerged implant procedures, followed the guidelines for successful osseointegration by Adell et al. (1981), *i.e.* a 3- to 6-month unloaded healing period. It was argued that implants required an undisturbed healing time for successful tissue integration and that premature loading might prevent direct bone apposition and lead to fibrous tissue encapsulation. Improved understanding of the osseointegration process, bone resorption and re-modelling and the interaction between bone and metal surfaces has resulted in recent departures from the traditional conservative approach established some 40 years ago. The importance of the surface characteristics and choice of the implant material in determining the quality of bone anchorage was recognized early (Albrektsson et al. 1981; Buser et al. 1991; Johansson 1991). Various surface treatments have been successfully used to achieve more rapid and more stable bone integration *i.e.* bone-metal anchorage

(Buser et al. 1999; Albrektsson et al. 2000; Arvidson 1998; Arvidson et al. 1998, 2008; Fischer et al. 2008, for recent reviews see, Esposito et al. 2004, 2007a, b; Wennerberg and Albrektsson 2009).

Successful endosseous implantation in the alveolar ridge requires sufficient quality and quantity of bone at the recipient site. Several surgical techniques have been described to augment bone before or in combination with dental implant installation (for a review see Hammerle and Jung 2003). More recently, in a relatively limited RCT study, Jung et al. (2009) demonstrated that implants installed in defective bone sites grafted with demineralised bovine mineral with or without a growth factor (rhBMP-2) had excellent clinical and radiological outcomes after 5 years.

12.2.2 Loss of Periodontal Tissues

The main function of the periodontium is to attach the tooth to the alveolar bone and to maintain the surface integrity of the masticatory mucosa. Epidemiological studies have shown that infections are the main cause of destruction of bone as a supporting tissue of the teeth. The etiology has, however, been shown to be multi-causal. Periodontal disease, especially the most severe forms, is no longer regarded as a simple infection, but rather as the result of a complicated interaction with systemic factors or disorders. In the most severe cases the outcome can be the loss of most or all teeth (Fig. 12.2).

An important goal of periodontal therapy is to achieve a reduction in the depth of the periodontal pocket in order to prevent further disease progression. In patients with moderate periodontitis, *i.e.* pocket depths ≤6 mm, this goal can be accomplished by non-surgical therapy, whereas in severe cases, particularly in the presence of intrabony defects and furcations (Fig. 12.3), the treatment must be supplemented with periodontal surgery. There is increasing use of regenerative procedures to restore lost periodontal support.

Periodontal regeneration has been defined as the process by which the architecture and function of the periodontal tissues are completely renewed (The American Academy of Periodontology 1992) and includes the formation of a new connective tissue attachment, cementum and supporting bone (Ellegaard et al. 1973, 1974; Karring et al. 1993). Regenerative periodontal therapy comprises procedures which are specially designed to restore, by reattachment or new attachment, those parts of the supporting apparatus which have been lost due to periodontitis, *i.e.* gingiva, periodontal ligament, root cementum and alveolar bone. For true regeneration, the root surface must therefore be repopulated by epithelial cells and cells derived from the gingival connective tissue, bone and periodontal ligament. Guided tissue regeneration (GTR) is a treatment modality intended to promote regeneration of periodontal tissue lost through periodontitis. Animal studies have confirmed that in intra-bony defects, this treatment results in true regeneration, albeit with some limitations (Laurell et al. 2006). GTR has also been used in implant rehabilitation, using different techniques and membrane materials (for a review see Hammerle and Jung 2003).

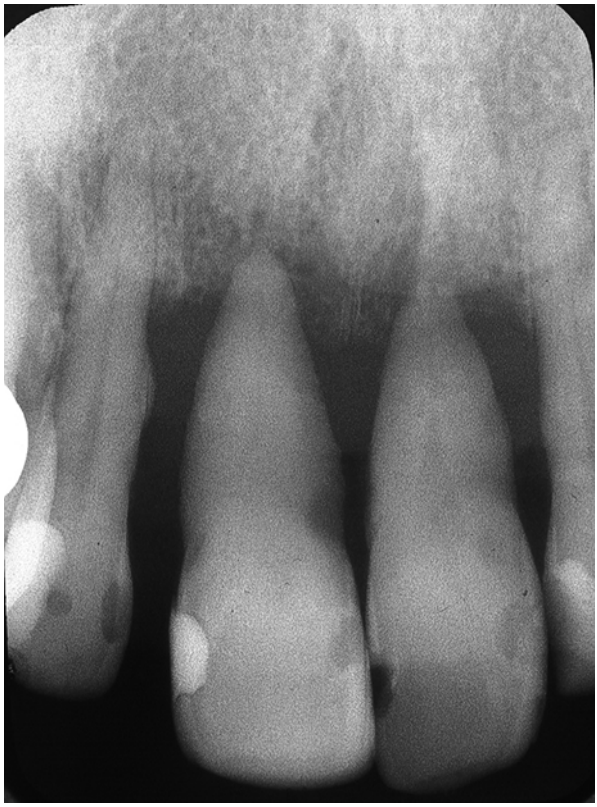

Fig. 12.2 Radiograph of very severe bone loss around the maxillary anterior teeth

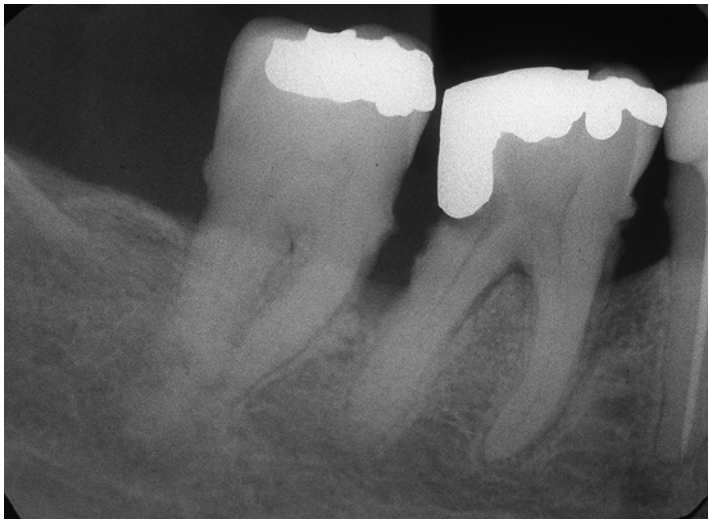

Fig. 12.3 Radiograph of vertical bone loss around a mandibular molar

The most commonly used clinical methods for regeneration of the periodontal attachment apparatus are GTR (Sculean et al. 2008) and a derivative of enamel matrix proteins (EMD). GTR, using bioabsorbable barriers made of e.g. polylactide acetyltributyl citrate or polydioxanon, has shown stable clinical results in both short and long term studies (Eickholz et al. 2004). EMD are acidic extracts of extracellular enamel matrix, and include a heterogeneous mixture of polypeptides encoded by several genes (Bosshardt 2008). It is unclear which of the enamel matrix proteins induces the regeneration, and the underlying molecular mechanisms have yet to be determined.

The use of bioactive molecules to induce local bone formation is an active field of research. Bioactive agents are used alone or together with grafting or GTR for treatment of intra-osseous and furcation defects (Trombelli and Farina 2008). A variety of growth factors have also been tested for local bone regeneration (for a recent systematic review see Jung et al. 2008).

At present, regimes that encounter bone formation and attachment formation and hold the promise of significantly increasing bone density and volume and leads to the formation of a new functional periodontal attachment, have yet to come available. In this context, the concept of tissue engineering has emerged as a valid approach to the current therapies for periodontal tissue regeneration and attracting considerable attention. It has been shown that the use of MSCs in Platelet-rich plasma gel could be useful for periodontal tissue regeneration (Yamada et al. 2006). However, not much has been reported on the application of tissue engineering for regeneration of periodontal tissues.

12.2.3 Loss of Bone

Bone defects in the oral and maxillo-facial region may arise following surgical treatment of tumors, cysts and other pathological conditions as well as traumatic insults to the facial and dento-alveolar structures. As such defects often involve structures of different origins, the reconstructive procedures are very demanding. Maxillofacial tumors and cysts may arise from both soft and hard tissues and may be of odontogenic or nonodontogenic origin. Lesions located within the jaws thus include odontogenic cysts and tumors, nonodontogenic cysts and benign tumors and malignant, nonodontogenic neoplasms. Benign cysts and tumors occur frequently, are clinically and radiologically well-delineated and treated by curettage or enucleation, whereas highly proliferative lesions are treated by resection. Malignant primary neoplasms of the jaws are rare, the most common being osteosarcoma. Much less common are chondrosarcoma, plasmocytoma and Ewing's sarcoma. Some of these tumors may require extensive surgical treatment and reconstruction. Further examples of pathological conditions of the jaws requiring treatment by extensive bone resection are osteoradionecrosis or extensive, proliferative benign lesions which have proved resistant to other therapies.

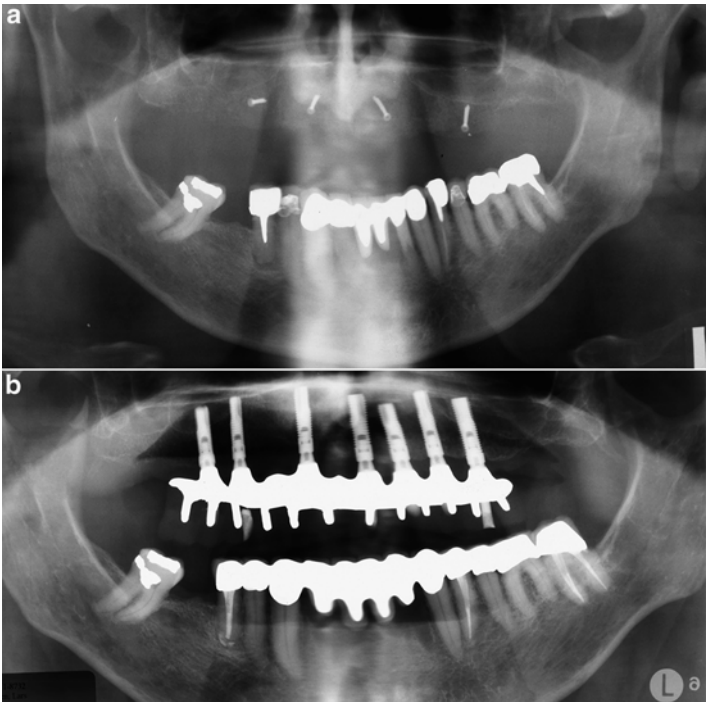

Fig. 12.4 Edentulous maxilla with extensive resorption of the alveolar crest. Reconstructed with free cortical onlay blocks from the iliac crest, fixed with miniscrews (**a**). After a healing period, a full arch maxillary bridge was retained on seven osseointegrated implants (**b**)

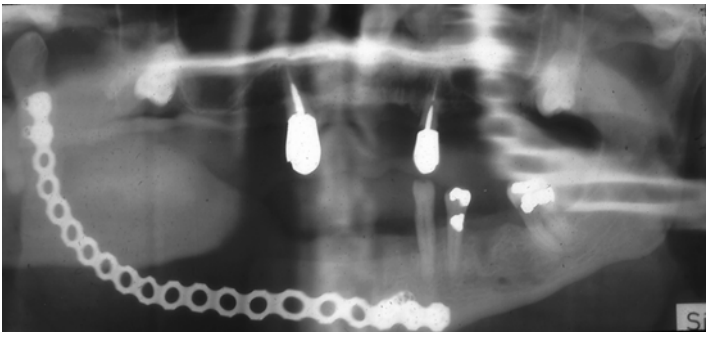

Fig. 12.5 Mandibular defect after tumor resection. Treated with a free revascularized forearm soft tissue graft and a bridging reconstruction plate

Despite progress in the field of reconstruction as a result of new surgical techniques, improved biomaterials and advances in cell biology, autologous bone grafting remains the "gold standard", especially for the reconstruction of large bone defects (Chiapasco et al. 2008; Raveh et al. 1987) (Figs. 12.4 and 12.5).

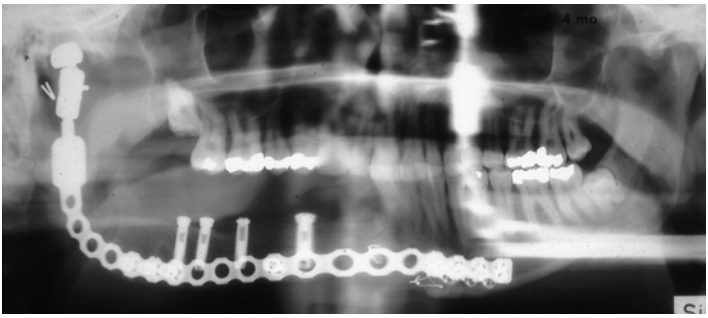

Fig. 12.6 Hemi-mandibular defect after tumor resection. Treated with a temporo-mandibular joint prosthesis, a bridging reconstruction plate with free iliac crest graft and osseointegrated dental implants

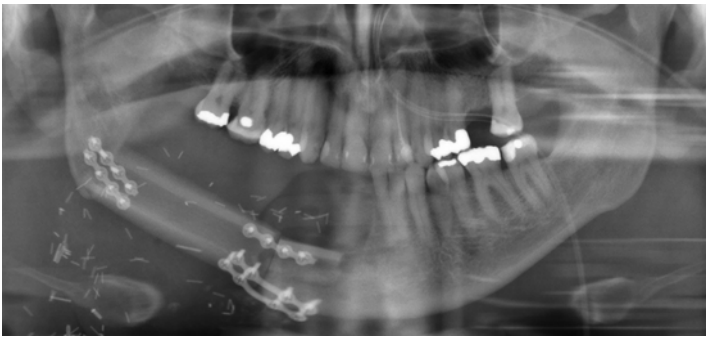

Fig. 12.7 Lateral mandibular defect after tumor resection. Treated with a free revascularized compound fibular graft

Free, nonvascularized autologous transplants are function for bridging of defects and as volume fillers by inducing bone growth. In some cases, however, the prognosis may be guarded, due to the risk of inadequate vascular regeneration and impaired tissue repair following hypoxia. Of vital importance to success are adequate microvascularity of the recipient tissues and optimal fixation of the grafts, in order to prevent infection and loss of osteogenic cells. Segmental osteodistraction may have potential as a treatment solution. In cases of compromised tissue healing or composite tissue defects, the treatment of choice is the use of revascularized hard and soft tissue free flaps (Torroni et al. 2007; Smolka and Iizuka 2005; Emerick and Teknos 2007; Chepeha et al. 2008; Chiapasco et al. 2006). Despite the above risk factors, good functional and esthetic outcomes have been reported (Chiapasco et al. 2008; Louis et al. 2008) (Figs. 12.6 and 12.7).

For reconstruction of minor and single tissue defects, a wide range of autografts, allografts, xenografts and synthetic substitutes has been extensively used in recent years, in some instances showing outcomes comparable with autologous grafts (Hallman and Thor 2008; Hellem et al. 2003). In a review article Kretlow et al. (2009)

presented an excellent summary of newer materials and methods in bone and soft tissue regeneration. Compared with autologous transplants, the disadvantages of allografts, xenografts and synthetic biomaterials include lack of osteoinductive properties and relatively varying osteoconduction. Varying, and to some degree uncontrolled resorption rates may represent a challenge in a clinical situation when assessing the amount and progression of tissue regeneration. The risks of bacterial, viral or prion transmission from allo- and xenografts as well as immunologic reactions are minimal and dependent on the method used for tissue preservation (Kretlow et al. 2009).

Bone and soft tissue defects due to traumatic high velocity insults may be extensive, and involve several areas of tissue loss and progressive necrosis, demanding extensive surgery. These defects often have to be reconstructed by two stage surgery, using revascularized free or pedicled compound flaps or osteodistraction (Bertele et al. 2005; Pereira et al. 2007). Defects due to non-optimal repositioning of fractures in the periorbital and naso-ethmoidal regions remain a challenge for the surgeon. The midface and mandibular regions, however, may be reconstructed using osteodistraction devices.

Severe dento-alveolar trauma occurring in isolation or in combination with facial trauma, is often associated with loss of teeth and defects in the alveolar crest. Cases involving primary or secondary loss of teeth and bone tissue have to be reconstructed as a prerequisite for treatment with dental implants. In some cases replacement of lost mucosal/gingival soft tissue must also be addressed. Where functional and esthetic outcomes are priorities, the treatment of choice would be reconstruction of bone defects in the maxillary anterior alveolar crest, bone grafting and local osteodistraction (Lundgren and Sennerby 2008) or even non resorbable bone substitutes (Hallman et al. 2009; Hellem et al. 2003).

12.3 Future Directions

12.3.1 Oral Stem Cells in Regenerative Dentistry

Physiological bone tissue regeneration is a remarkable process that results in healing without scarring. It is a multi-faceted process, beginning with angiogenesis, followed by callus formation and eventually bone remodelling. Key contributing factors in this process are growth factors [VEGF, PDGF-BB, plGF, BMPs, basic Fibroblast Growth Factor (bFGF)] osteocytes and angiocytes of the surrounding bone tissue, adult mesenchymal and hematopoietic stem cells. However, the prognosis is uncertain in the presence of large defects (>1 cm) or conditions associated with healing impairment such as old age, diabetes or radiation therapy. Under such suboptimal conditions, the gold standard of autologous bone transplantation is however associated with disadvantages, such as the limited amount of bone which can be harvested, unpredictable donor bone turnover, donor site morbidity, and the added cost incurred by surgical procedures to harvest the bone as well as pain at the harvest site.

Currently, various strategies are applied to stimulate healing of bone defects and to restore lost maxillofacial bone and periodontal support following traumatic insult, tumor ablation, diseases or congenital deformities. Despite the fact that materials science and technology has markedly improved the field of bone regeneration, none of the currently available treatment regimes stimulates bone and attachment formation. They therefore lack the potential to increase bone density and volume significantly and to form a new, functional periodontal attachment. For this reason large defects/injuries still represent a major challenge for dentists and oral maxillofacial surgeons. The clinical challenges have stimulated interest in developing new therapies that involve regeneration of bone and periodontal ligament.

Bone marrow has been shown to contain a population of rare cells capable of differentiating into the cells that form various tissues. These cells, referred to as mesenchymal stem cells (MSC), are located within the bone marrow and, depending on the culture conditions chosen, have the potential to differentiate into fibroblastic, osteogenic, adipogenic or reticular cells (Friedenstein 1976; Bianco et al. 2001). The lack of immunogenicity of MSC heightens the potential of these cells for bone repair. Human bone marrow osteoprogenitors can be isolated and enriched from the CD34+ fraction using selective markers such as STRO-1 (Stewart et al. 1999). In recent years there has been increasing interest in the possibility of using adult MSC for regeneration of oral tissues, not only to enhance attachment around periodontally compromised teeth, but also to augment alveolar bone before and/or after placement of oral implants. Adult stem cells, previously thought to be limited in potential, have increasingly been shown to be able to differentiate into tissues of an entirely different germ layer, with potential clinical application in the treatment of a number of diseases. Cell based therapies are promising new therapeutic tools in regenerative medicine. By using mesenchymal stem cells (MSCs), good results have been reported for bone engineering in a number of clinical studies (Gomez-Barrena et al. (2011). Thus, stem cell-based bone tissue engineering is a promising concept for reconstruction/ regeneration of craniofacial defects, but much work remains before this approach becomes a routine part in clinical practice. The reconstruction of bone defects using stem cells seeded onto biodegradable carrier materials or scaffolds requires timely formation of functional blood vessels. After implantation, complex tissues are dependent on a functional vasculature, not only for cell survival, but also for tissue organization. Recently, the ability of MSC to support development of blood vessels as perivascular cells was reported (Pedersen et al. 2013, 2014). The data showed that generation of endothelial microvascular networks *in vitro* affected the angiogenic and osteogenic potential of tissue-engineered constructs.

One of the most extensively studied populations of multipotent stem cells has been mesenchymal stem cells (MSC) from bone marrow. It has been demonstrated that their precursors are typically associated with the blood vessels, and found in most of the human tissues (Crisan et al. 2009), thus making it theoretically possible to obtain MSC from an unlimited number of organs and tissues. It has been reported that from a small volume (0.1–3 ml) of marrow aspirate, alveolar bone mesenchymal cells (BMSC) can be expanded successfully 70 % of the time (Matsubara et al. 2005). Alveolar BMSC might be useful for regenerative medicine, because small marrow

aspirates from alveolar bone can be made with minimal pain. Furthermore, Matsubara et al. (2005) demonstrated a high osteogenic potential from alveolar BMSC. Although this raises few ethical issues, harvesting of cells from bone marrow is still an invasive procedure, and stem cell numbers decrease significantly with the age. The search for more readily accessible sources of pluripotent stem cells has led to investigation of other tissues, including mobilized peripheral blood, umbilical cord blood and more recently, fat (adipose) tissues, periodontal ligament (PDL), deciduous and permanent teeth.

Although potential of BMSC for future clinical use in bone tissue engineering (BTE) is undoubted, recently, adipose tissue stem cells (ASC) have shown a great promise as an alternative to BMSC in BTE for several reasons (Sándor et al. 2013). Firstly, the stem cell yield and proliferation rate is much higher than that of BMSC. Secondly, harvesting procedure of ASC is less complicated and is associated with less morbidity and complications. Several recent studies indicate potential clinical use of ASC in BTE (Zuk et al. 2001; Sándor et al. 2013; 2014; Gotoh et al. 2014). Despite the above advantages, both *in vitro* and *in vivo* osteogenic ability of ASC seem to be inferior as compared to that of BMSC (Liao and Chen 2014).

The PDL is one of the tissues that has attracted interest as a source of stem cells and its potential for regeneration. It contains a heterogeneous cell population that can differentiate into cementoblasts or osteoblasts. Recent findings suggest that PDL cells have osteoblast-like properties. They have the capacity to form mineralized nodules *in vitro*, express bone-associated markers such as alkaline phosphatase and sialoprotein, and also respond to bone inductive factors such as parathyroid hormone, insulin -like growth factor 1, bone morphogenetic protein 2, and transforming growth factor β_1. Seo et al. (2004) showed that human PDL cells participate in periodontal tissue repair in immunocompromised rats, indicating that the PDL contains stem cells.

Dental pulp tissue is also a readily accessible source of pulp-derived mesenchymal stem cells (PDSC). PDSC express the endothelial and smooth muscle marker STRO-1 (Shi and Gronthos 2003) and display a pericyte phenotype, with expression of the pericyte-associated antigen 3G5 (Shi and Gronthos 2003). It is therefore assumed, but not yet confirmed, that the perivascular region in the pulp is the niche for PDSC and that pericytes give rise to dental pulp stem cells. Isolated dental pulp stem cells have been shown to be plastic-adherent and express the MSC markers STRO-1, CD90, CD29, CD44, CD166, CD105, CD106, CD146, CD13 and are also negative for CD14 and CD34 (Shi et al. 2005; Ikeda et al. 2006). *In vitro*, PDSC are capable of self-renewal, display plasticity and mutilineage potential (adipocytes, chondrocytes, osteoblasts, neural cell progenitors and myotubes) and can therefore be considered as stem cells (Gronthos et al. 2002).

For tissue engineering purposes, PDSC have shown potential for both dentin and bone production. From the pool of human dental pulp cells, odontoblasts capable of forming dentin-like structures can be differentiated when cultured under mineralization-enhancing conditions (About et al. 2000). Moreover, in immunocompromised mice, subcutaneously implanted cells derived from human dental pulp generate a dentin-pulp-like complex without lamellar bone (Shi et al. 2005).

Using a similar model, another research group has also shown that PDSC are able to generate vascularized bone tissue that *in vivo* was remodelled into a lamellar bone (Laino et al. 2005, 2006a, b; d'Aquino et al. 2007). Further, when implanted into immunocompromised rats, a distinguishable STRO-1 positive subpopulation of cells was found to produce woven bone efficiently and to remodel lamellar tissue (d'Aquino et al. 2007; Laino et al. 2006b). After implantation, PDSC expressed bone markers including osteocalcin, Runx-2, collagen I and alkaline phosphatase (d'Aquino et al. 2007). Furthermore, it might be possible for PDSC to contribute to the formation of new bone containing Haversian channels with appropriate vascularization *in vivo* (Huang et al. 2008; Pierdomenico et al. 2005; Shi et al. 2005; Young et al. 2002; d'Aquino et al. 2007; Laino et al. 2006b; Ikeda et al. 2006; Gronthos et al. 2000; About et al. 2000; Batouli et al. 2003; Cheng et al. 2008). Even when removed from their native location, dental pulp cells maintain the potential to contribute to the formation of both dentin and alveolar bone (Diep et al. 2009).

The transition from deciduous (baby) teeth to permanent (adult) teeth is a unique, dynamic process in which the development and eruption of the permanent teeth is co-ordinated with the resorption of the roots of deciduous teeth. In humans, it may take >7 years to complete the orderly replacement of 20 deciduous teeth. It was found that a naturally exfoliated human deciduous tooth contains a population of stem cells (SHED) and are thus available without surgical intervention (Laino et al. 2006b). These cells have been shown to be plastic-adherent, have great proliferative capacity and positive for MSCs markers STRO-1, CD29, CD106, CD146, while negative for CD14, CD34 (Shi et al. 2005). Further, they exhibited a high degree of plasticity with the capacity to differentiate into neurons, adipocytes, osteoblasts and odontoblasts (Miura et al. 2003; Huang et al. 2008). SHED are not only derived from a very accessible tissue resource but are also capable of providing enough cells for potential clinical application. Thus, exfoliated teeth may be an unexpected, unique resource for stem cell therapies including autologous stem cell transplantation and tissue engineering. These cells could aid the repair of damaged teeth and perhaps even treat neural injuries or degenerative diseases. Stem cells isolated from deciduous teeth (SHED) have several advantages. Although unlikely to have the differentiation and proliferative potential of ESC, deciduous tooth stem cells require no invasive harvesting procedure. Furthermore, there are no ethical issues, as in the normal course of events deciduous teeth exfoliate and are discarded.

12.3.2 Artificial Scaffolds in Regenerative Dentistry

In contrast to the conventional biomaterials approach, tissue engineering is based on an understanding of tissue formation and regeneration, and aims at inducing new functional tissues, rather than just implanting replacement parts. There are numerous published case reports of the application of bone tissue engineering for oral and maxillofacial surgical reconstruction, periodontal tissue regeneration and sinus

floor augmentation. Tissue engineering is the application of scientific principles to the design, construction, modification and growth of living tissues, using biomaterials, cells, and factors alone or in combination. Skeletal tissue engineering requires a scaffold conducive to cell attachment and maintenance of cell function, in combination with a rich source of osteoprogenitor cells and osteoinductive growth factors. Crucial to success is an understanding of how cells function and form a matrix, and the development of appropriate materials for fabrication of scaffolding designed to promote cell attachment and maintain cell function.

Recently, much effort has been devoted to synthesis methods and fabrication techniques used to design and select a scaffold with properties that most closely match those required for bone regeneration. Highly porous and degradable aliphatic polyester scaffolds with varying pore size and interconnected pores were fabricated by bulk copolymerization of poly(L-lactide) (PLLA), 1,5-dioxepan-2-one (DXO-co-LLA) and ϵ-caprolactone (CL-co-LLA) (Dånmark et al. 2010). The degradation rates of polyester scaffolds and loss of mechanical integrity were greatly increased in porous scaffolds made with hydrophilic co-monomers (Dånmark et al. 2011). By incorporating hydrophobic co-monomers with limited ability to crystalize instead of hydrophilic co-monomers, the mechanical stability was retained longer during degradation. It has been shown that these scaffolds are biocompatible and stimulate bone regeneration both *in vitro* and *in vivo* (Arvidson et al. 2011; Dånmark 2011; Idris 2010; Xue 2011; Xing 2012). These polyester scaffolding materials show great potential as bone tissue constructs (Suliman et al. 2015; Yassin et al. 2015). However, the scaffolds need to be optimized to control cell differentiation and growth as well as to achieve angiogenesis before they are ready for human use.

12.3.3 Paracrine Effects of Stem Cell-Derived Growth Factors

Tooth regeneration by cell transplantation is a meritorious approach. However, there are hurdles in the translation of cell-delivery-based tooth regeneration into therapeutics. The inaccessibility of autologous embryonic tooth germ cells for human applications, the limited availability of autologous postnatal tooth germ cells (e.g. third molars) and the low survival rates of the implanted cells may undermine the efficacy of the cell-based treatment. Furthermore, other factors such as the availability of autologous stem cells, the excessive costs of cell isolation, handling, storage, shipping and *ex vivo* manipulation, liability issues if contamination occurs, and potential for transmission of infectious disease are all potential drawbacks to cell transplantation (Inanc and Elcin 2011; Yildirim et al. 2011).

It has been reported that stem cells secrete multiple metabolites, growth factors, signaling molecules, and extracellular matrix proteins during *in vitro* culture that affect cellular behavior (Kinnaird et al. 2004; Barcelos et al. 2009; Cai et al. 2009; Perin and Silva 2009; Osugi et al. 2012). Stem cell-conditioned medium (CM) can be used, transplanted or injected with or without scaffolds to induce cell homing, migration, proliferation and differentiation (Ueda and Nishino 2010; Kim et al. 2009;

Yang et al. 2009). Therefore, the use of stem cell-conditioned medium as an alternative to transplanting stem cells might be a feasible approach for tissue engineering. The paracrine effects of the growth factors in CM on recruiting circulating progenitor/stem cells and/or endogenous adjacent cells to the treatment site is attracting considerable research attention at present. Although the molecular mechanisms that direct mobilization and homing of cells in response to the paracrine factors secreted by stem cells are not fully understood, cell homing represents a novel concept for regenerative dentistry and may offer a clinically useful approach (Kim et al. 2010).

The therapeutic effects of CM derived from stem cells derived from different sources have been demonstrated in experimental animal models (Cho et al. 2012; Osugi et al. 2012). It has been shown that conditioned medium derived from mesenchymal stem cells as well as SHED-conditioned medium is able to accelerate wound healing as well as that seen with stem cell transplantation, and thus may become a new therapeutic method for wound healing in the future (Tamari et al. 2011; Ueda and Nishino 2010). Thus conditioned medium might be used to create a highly inductive microenvironment, with many possible uses in regenerative dentistry. However, further studies are required to address the underlying mechanisms involved in organogenesis mediated by conditioned medium.

12.4 Conclusion

ASC, PDL, PDSC, SHED and BMSC stem cells appear to be appropriate candidates for tissue engineering involving restoration of dental and periodontal tissues, as well as bone, suggesting a potential future therapeutic role of these cells for craniofacial regeneration. Artificial scaffolds are currently underdevelopment and may, together with cells from these different sources, lead to improvements in tissue engineering of bone defects in the oral cavity. The use of paracrine factors to improve tissue regeneration is a very promising new concept. However, much work remains before this approach will be ready for routine clinical use.

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