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Dental tissue engineering: a new approach to dental tissue reconstruction

Elisa Battistella, Silvia Mele and Lia Rimondini
University of Eastern Piedmont "Amedeo Avogadro"
Novara, Italy

1. Introduction

Caries, trauma, erosion and periodontal disease are pathologies characterized by the damage and loss of dental tissues and sometimes loss of the whole tooth. These groups of diseases are very common and affect millions of people worldwide in both developing and industrialized countries.

Restorations of damaged tooth tissues and substitution of missing teeth with artificial prostheses represent the traditional therapeutic solutions. Although many sophisticated biomaterials are now available for restoration, their use is not yet completely satisfactory.

Implants, crowns, bridges and any kind of restorations are generally less functional, durable and aesthetic than intact teeth and they are aimed at repairing rather than regenerating tissues. In addition, the economical impact of such kind of therapeutic approaches is notable. The World Health Organization estimated that the dental treatment costs accounted for 5-10% of healthcare budgets in industrialized countries and adjunctive costs may be considered in terms of absences from work (Petersen, 2003).

In the last few decades the process of tooth mineralization and the role of molecular control of cellular behaviour during embryonic tooth development have received much attention. The knowledge gained in these fields has improved the general understanding about the formation of dental tissues and the whole tooth and set the basis for teeth regeneration.

The present chapter is divided as follows:

- the histology and histomorphological development of the dental hard tissues
- the re-mineralization strategies for enamel regeneration
- the potential application of stem cells to tissues regeneration

2. The histology and histomorphological development of the dental hard tissues

2.1 Enamel

Teeth are composed of several different tissues topographically assembled in order to guarantee the physiological functions. Teeth have to withstand physical and chemical processes. They are exposed to wear, compressive forces and chemical acidic attacks from

bacteria and foods. The function of external protection is made by enamel which covers the crown with a layer of 2 mm occlusally and more in correspondence with the cuspids and decreases cervically till only a few micrometers at the collar. Enamel is the most mineralized tissue in mammals. The mineral content is generally over 97% in weight and is mainly composed of various calcium phosphates (Fig.1) The mean Ca/P generally reported in normal enamel varies from 1.64 to 1.8 generally compatible with those of apatites. The stoichiometric form of hydroxyapatite $[\text{Ca}_6(\text{PO}_4)_6(\text{OH})_2]$ is rarely present in this mineralized tissue which is carbonated at various degrees ranging from 4 to 6 %. (Wilt, 2005).

The distribution of carbonate is generally homogeneous in normal teeth. On the contrary the hypomineralized enamel shows a higher content of carbonate. The hardness of enamel decreases in carbonated areas.

Fluorine is also detectable in the thickness of the enamel. There is a general agreement that the more fluoride is present, the more resistant the teeth are to acid attacks. Generally fluorine is distributed along a gradient and increases from the pulp to the outer surfaces. Some other elements are observed to be present in the enamel structures using techniques appropriate to detect elements at low concentration such as Secondary Ion Mass Spectrometry and X-ray Microanalysis (Jälevik et al., 2000; Chabala et al., 1988).

Elements such as F, Cl, Na, Mg, K and Sr are distributed in a gradient through the enamel layer from the dentine to the surface. Contents of Mg and K were reported to be slightly higher in hypomineralized areas, especially towards the surface. Also Na had a somewhat higher content toward the surface in defective enamel. Cl and Sr contents had no diversity in relation to the degree of mineralization (Jälevik et. al., 2000).

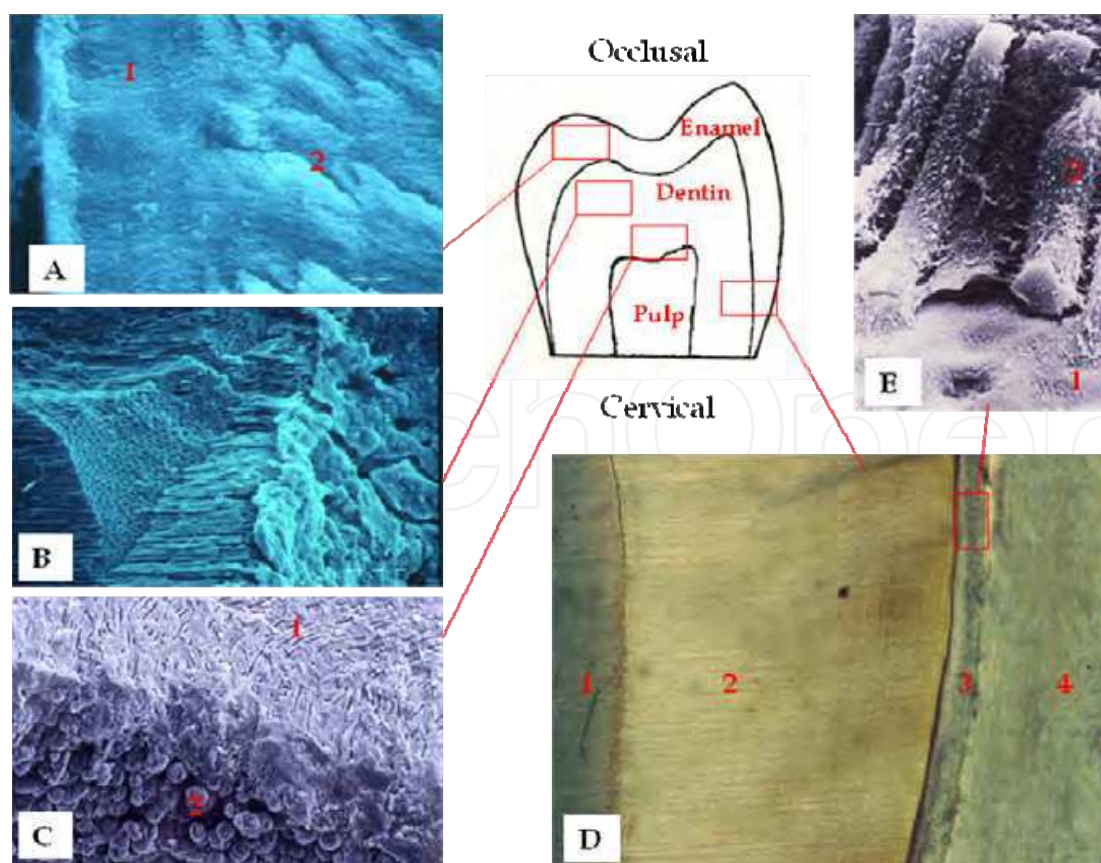


Fig. 1. Schematic representation of the crown of a tooth.

- A: Enamel: 1. Aprismatic enamel; 2. Prisms. Scanning Electron Microscopy of a specimen obtained by selective fracture. Original magnifications: 8000X
- B: Dentin: Dentinal tubules. Scanning Electron Microscopy of a specimen obtained by selective fracture. Original magnifications: 8000X
- C: Dentin: 1. Dentinal tubules and 2. Globular Dentin close to the pulp. Scanning Electron Microscopy of a specimen obtained by selective fracture. Original magnifications: 4000X
- D: Histological section of the hard tissues of a tooth before eruption embedded in resin and obtained by wear. Toluidine staining. Original magnifications: 20X. 1. Dentin; 2. Enamel 3. Ameloblasts; 4. Dental papilla
- E. Ameloblasts. Scanning Electron Microscopy. Original magnifications: 2000X. 1. Ameloblasts; 2. Enamel surface

Other trace elements such as Mg, Fe, Zn, Sr, Ba and Pb have been observed to be accumulated in enamel, using techniques such as laser ablation-inductively coupled plasma-mass spectrometry, in teeth of children raised in polluted areas or in consequence of particular nutrition regimes (Dolphin et al., 2005).

The teeth are made of the strongest and hardest tissue in vertebrates and they are perfectly adapted to their functions. They probably played an important role in the evolutionary success of vertebrates and later of both terrestrial (Luo, 2007) and marine mammals (Uhen, 2007).

The material and mechanical properties of the enamel can be attributed both to the gross morphology and internal architecture, as well as the nanoscale properties of the mineralized composite.

The mechanism of enamel formation and calcium phosphate deposition and crystallization into hydroxyapatite is not yet completely understood. As all mineralized tissues in living organisms it is a composite of natural polymers acting as a template for inorganic materials. The presence of natural polymers is essential to aid crystallization.

Enamel matrix is formed by the ameloblasts. The latter derive from the ectodermal epithelium when neural crest cells invade the underlying mesenchyme inducing the proliferation and formation of dental lamina and then the so called *enamel organs* or "dental papilla" (Järvinen et al., 2009). The ameloblasts are orderly disposed at the base of dental papilla which surround specialized mesenchymal cells, the odontoblasts, responsible for dentinal matrix secretion. The dentinal matrix contains a variety of molecular signals composed of structural polymers such as collagen type I, dentine sialoprotein, minerals, as well as many cytokines and signal molecules such as fibroblast growth factor and bone morphogenetic protein transforming growth factor (TGF) beta, Wnt, and Hedgehog (Thesleff et al., 2007; Tummers & Thesleff, 2009) which interfere with ameloblast cytofunctionality.

Dentin sialophosphoprotein seems to be particularly important in the early events of amelogenesis and it is strongly involved in the formation of the dentino-enamel junction and the adjacent "aprismatic" enamel. Overexpression of dentin sialoprotein, studied in trans-genic animals results in an increased rate of enamel mineralization, however, without alteration of morphology. In wild-type animals, the inclusion of dentin sialoprotein in the forming aprismatic enamel may improve the hardness and the mechanical properties of the mineralized tissue. In contrast, the over-expression of dentin phosphorprotein is associated

with hypomineralized enamels characterized by “pitted”, “chalky”, soft enamel, dis-homogeneous in thickness (Fig.2).

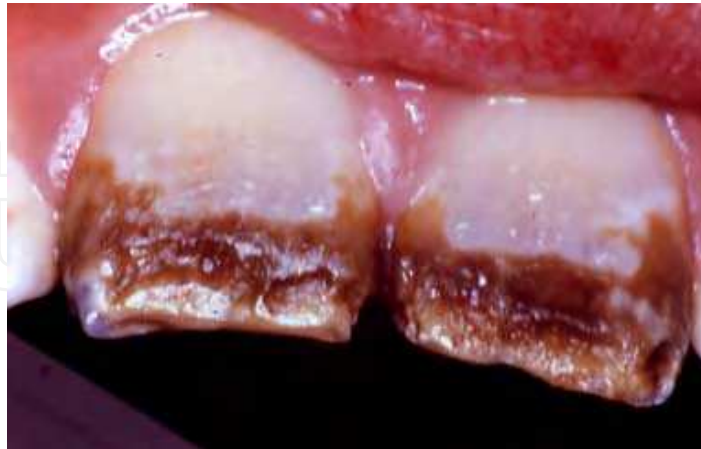


Fig. 2. Hypomineralized teeth.

The secretory surfaces of the ameloblast are called “Tomes processes”. During matrix deposition, they retract toward the apical epithelial surface, leaving behind a self assembly nanosphere of the matrix which rapidly arranges into ribbons (Fincham et al., 1999; Moradian-Oldak et al., 2006; Du et al., 2005).

Nanosized crystallites of carbonated apatite form in the channels created by these ribbons of matrix so that long polycrystalline rods of carbonated apatite are formed; the matrix proteins are degraded by some metalloproteinase (i.e. MPP20) (Li et al., 1999; Tan et al., 1998). The enamel matrix in fact disappears during enamel maturation. The degraded matrix is replaced by rapid infilling with more carbonated apatite crystallites. In the final stages the bulk of enamel is formed by a pack of crystals called “enamel prisms” surrounded by an amorphous lattice. “Aprismatic enamel” is found at the surface and at the dentin-enamel junction (Fig.1).

The matrix of enamel is particularly interesting. It is mainly constituted by a group of hydrophobic proteins called “amelogenins”. The most important seems to be a 180 amino acid long water soluble protein which is degraded from carboxyl-terminals during enamel maturation leaving its residual degradation peptides in the enamel matrix. Amelogenin aminoacid sequences are highly conserved among most species. The human amelogenin gene is expressed primarily (90%) from a single gene on the X chromosome, with minor expression on the Y chromosome.

Amelogenin interacts with carbonated apatite, *in vitro*, to limit crystallite growth, just as it might act to channel mineral crystallite accumulation into rods *in vivo*. Other hydrophilic proteins such as “enamelin” have been discovered in the enamel matrix but their function is still unknown.

The University of Helsinki offers a tutorial on teeth morphogenesis and a data-base of genes and signals molecules involved, at the web-site <http://bite-it.helsinki.fi/>

Over 300 genes have been associated with the patterning and morphogenesis of the teeth. Any alteration to these may lead to dental defects (Paine et al., 2001; Thesleff, 2006).

Defects of enamel mineralization are relatively common and vary from slight to very severe features. They are related to some break in the enamel formation process related or not to

genetic alteration involving the amino acid sequence of amelogenins or to an alteration of the protease responsible for enamel matrix degradation (Paine et al., 2000; Gibson et al., 2001). Other histological alterations are related to a disrupting of some signal sequences at the very beginning of the dental papillae formation or any disturbance in matrix deposition. For instance an excessive fluoride uptake causes detrimental effects on ameloblast activity and diabetes early induced in animals provokes deformities of the mineralized structures of the calvaria and maxilla and teeth prone to wear (Atar et al., 2007).

2.2 Dentin

In contrast to enamel, dentin is soft, flexible and tough, and able to absorb energy and resist fracture.

It is less mineralized than enamel and it is a sort of sponge crossed by channels of 1 micron wide radially departing from the pulp. The channels called "dentin tubules" are occupied by a part of the cytoplasm of the odontoblasts whose cytoplasm body underlies the dentin-pulp interface. The tubules are also occupied by the dentinal fluids. The dentin is formed by mineralization of the dentinal matrix mainly composed of collagen type I and some specific proteins previously mentioned. The deposition of dentin occurs from the pulp front over the life of the teeth. Sometimes the immature dentin appears like globules which are fusing during the maturation of the tissue (Fig.1).

3. The remineralization strategies for enamel regeneration

Enamel is a definitive tissue. It has no chance to grow, heal or regenerate after eruption even if it may be subjected to absorption and desorption of molecules and ions at the surfaces. Even though enamel is highly mineralized it loses substance under acid conditions. Therefore to preserve its integrity and aesthetic it is important to remineralize enamel already at the early stages of acid etching.

For this purpose, various approaches have been attempted. They include application of inorganic and amorphous materials or nanostructured crystalline forms. Inorganic materials in combination with proteins or polymers are also reported. Physical transformations of enamel substances as occurs after laser treatment have also been proposed. Irradiations of tooth enamel ArF excimer Laser alter the structure and chemical composition and increase the Ca/P ratio and decrease the amount of carbonate and proteins (Feuerstein et al., 2005).

The results obtained in terms of "remineralization" are dependent on both the models and the technologies used to characterize the treated enamel.

The depth of demineralization of the enamel influences the degree of remineralization achievable (Lynch et al., 2007).

Supersaturated solutions containing stoichiometric hydroxyapatite are observed to be very effective to remineralize, *in vitro*, superficial lesions but less efficient than less crystalline calcium phosphate to treat deep demineralization (Lynch et al., 2007).

Fluoride compounds are traditionally claimed as remineralizing agents for damaged enamel both *in vitro* (Gaengler et al., 2009) and *in vivo*. A high concentration of fluoride seems to be more effective in *in vitro* models. *In vivo* clinical experimentations of different modalities of application (ionophoresis using 2% sodium fluoride solution, 1.23% acidulated phosphate fluoride gel application, and 5% sodium fluoride varnish application) seem to be equally

effective in reducing the dimension of demineralised areas and increase microhardness (Lee et al., 2009).

Glass ionomers applied on etched enamel have been observed to be transformed *in vitro* into enamel-like material when studied with SEM and EDAX analysis (Van Duinen et al., 2004) as well as other compounds containing fluoride such as TiF and aminated Fluoride (Am.F). Carbon dioxide Laser irradiation is observed to enhance the formation of fluoride precipitates from TiF and Am-F onto demineralized enamel (Wiegand et al., 2009).

Particular attention has recently been focused on the role of some milk proteins which are postulated to stabilise calcium phosphate. Remineralization of enamel subsurface lesions was observed *in vitro* using transverse microradiography and electron microprobe after application of a solution of casein phosphopeptide stabilised amorphous calcium phosphate and amorphous calcium fluoride phosphate solutions (Ranjitkar et al., 2009; Willershausen et al., 2009; Yengopal et al., 2009, Walker et al., 2009; Elsayad et al. 2009). The remineralization, consistent with hydroxyapatite and fluorapatite, was pH dependent with a maximum at pH 5.5. Fluoridate materials obtained better results than non fluoridated (Cochrane et al., 2008). Proteins contained in milk and its derivatives are also suggested to be effective in reducing the demineralising effect of acid. In particular proteose-peptone fractions 3 and 5 characterized by gel electrophoresis were found to be sufficient to reduce the extent of demineralisation of enamel by acid buffer solutions in *in vitro* models (Gremby et al., 2001).

Phosphate and calcium solution alone is also observed to be effective to reduce subsurface demineralization of enamel obtained with immersion of teeth in demineralising solution. The subsurface demineralization in enamel was reduced by 95% by increasing the calcium concentration of the demineralizing solution from 7 to 21 mmol/l. A similar reduction (87%) was observed by increasing the phosphate concentration. However, the amount of phosphate needed was approximately 20 times more than that of calcium (Tanaka et al., 2000).

Different modalities of calcium intake such as chewing gum with added calcium have recently been observed to promote white remineralization of enamel (Cai et al., 2009).

The application of 20 nm carbonate hydroxyapatite nanocrystals was recently described as a remineralizing technique for enamel (Huang et al., 2009; Nakashima et al., 2009) and dentin (Rimondini et al., 2007).

Hydroxyapatite nanocrystals have been synthesized with a nearly stoichiometric in bulk Ca/P molar ratio of about 1.6-1.7 and containing $4 \pm 1\text{wt}\%$ of carbonate ions replacing prevalently phosphate groups. CHA nanocrystals have been synthesized both at about 100 nm and 20 nm in size and used to remineralize *in vitro* the surface of enamel previously etched with 37% of ortho-phosphoric acid. After treatment for 10 minutes by aqueous slurry of both 20 nm and 100 nm sized synthetic CHA nanocrystals, the surface of the demineralized enamel appears covered by the CHA phase arranged thick and homogeneous apatitic layer (Roveri et al., 2009) which rebuilt the lost mineral tissue even if prismatic enamel structures cannot be built (Fig.3). The surface Ca/P molar ratio determined by XPS analysis for demineralized enamel slabs before and after *in vitro* remineralization by application of fluoride or CHA showed an increase of Ca/P molar ratio after fluoride application and the maintenance of the apatite stoichiometric one (Ca/P = 1.7) when CHA nanocrystals were used. These results suggest that the use of CHA nanocrystals leads to

remineralization by means of the addition of a layer of apatite similar to those of natural tissues whereas fluoride act more by changing the chemical composition of the enamel.

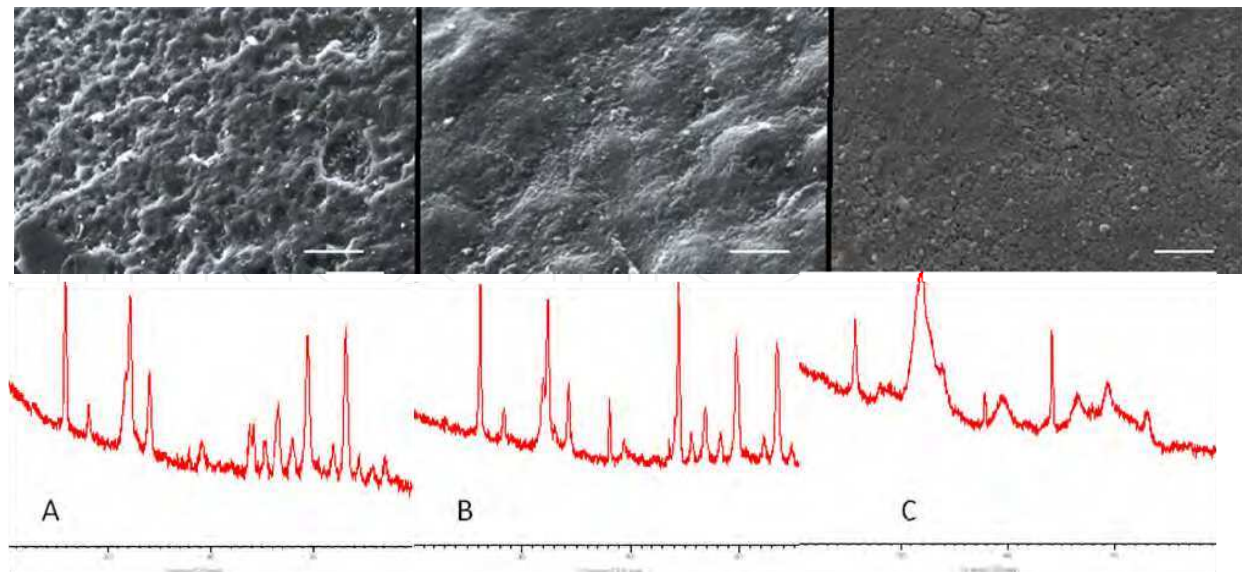


Fig. 3. A. Scanning electron microscopic graph and XRD spectrum of enamel surface demineralised with 37% orthophosphoric acid and brushed *in vitro* 2 times (1 min) a day for 15 days without any past application. Pits obtained by chemical attack and disruption by mechanical wear may be observed. Original magnification 5000X Bar=10 μ m.

B. Scanning electron microscopic graph and XRD spectrum of enamel surface demineralised with 37% orthophosphoric acid and brushed *in vitro* 2 times (1 min) a day for 15 days with fluoridated toothpastes. Pits are covered by a layer. The underneath surface is still perceptible. Original magnification 5000X Bar=10 μ m.

C. Scanning electron microscopic graph and XRD spectrum of enamel surface demineralised with 37% orthophosphoric acid and brushed *in vitro* 2 times (1 min) a day for 15 days with a paste containing a mix of 100nm and 20 nm CHA. The surface is completely covered by a globular layer. The XRD spectrum are compatible with those of CHA. Original magnification 5000X Bar=10 μ m.

In both cases the prism form of crystallization is not obtainable. To reach this goal the use of the described biomimetic carbonate-hydroxyapatite nanocrystals together with a suitable scaffold is suggested as being the promising strategy (Kirkam et al., 2007).

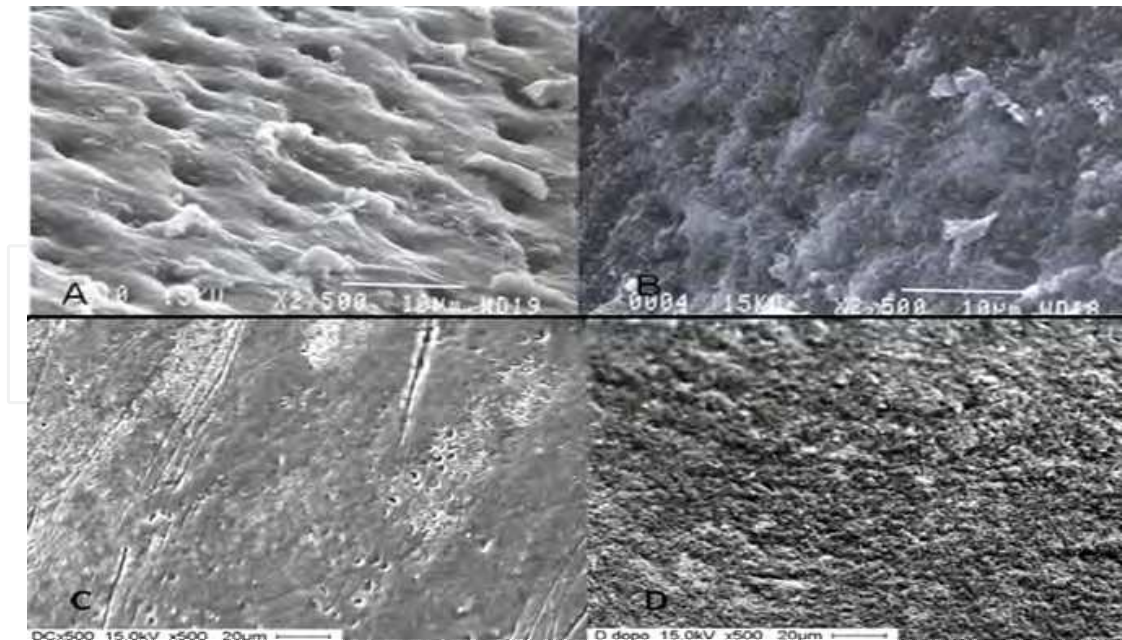


Fig. 4. Scanning electron microscope of *in vitro* demineralized dentin (A) and remineralized dentin with CHA (B); Scanning electron microscope of replica model obtained *in vivo* of demineralized dentin (C) and remineralized dentin with CHA (D).

An interesting application of nanocrystals of CHA could also be to obtain remineralization of dentin in order to reduce dentin hypersensitivity or improve mineralization of the dentinal tissues, for instance in the case of abutment preparation. The application of CHA nanocrystals closes the patent tubules of the exposed dentin *in vitro* (Fig. 4, A-B) and also *in vivo* as observable with a replica model technique (Fig. 4, C-D).

4. The application of stem cells in dental tissue regeneration

As already described, the traditional approach in the treatment of oral diseases often involves replacing teeth with artificial components. This approach is relatively successful. Biomaterials used may be not fully compliant with native tissues and often require multi-steps surgery and delayed healing (Hacking & Khademhosseini, 2009).

Tooth regeneration offers new and innovative approaches to difficulties encountered in oral and dental surgery. The aim of regenerative medicine is to simulate natural processes *in vitro* in order to re-create a tissue or an organ. This approach can involve cells, biomaterials and molecular factors; in this context, stem cells offer a great potential for tissue repair and regeneration. Stem cells are unspecialized cells capable of renewing themselves and under certain physiologic or experimental conditions, they can be addressed to more specialized committed lineages.

There are two main kinds of stem cells: adult stem cells (ASC) and embryonic stem cells (ESC). The first derive from adult tissue whereas the second derive from blastocysts. Both of them are largely used in the research field of tissue regeneration for their capability of self-renewal, their differentiation potential and their low immunogenicity.

4.1 Endodontic regeneration

Pulp and dentin tissue engineering may be an interesting alternative to traditional methods to treat compromised teeth endodontically. Sharpe and Young (Sharpe & Young, 2005) introduced the concept of using stem cells for dental tissue engineering and demonstrated that it is possible to engineer murine teeth by using adult stem cells of non-dental or dental origin.

Dental pulp contains highly proliferative cells that can be activated upon injury and undergo proliferation and differentiation toward osteoblastic phenotypes to provide for dentin repair. The first isolation of such kind of cells was reported in 2000; Gronthos and al. isolated a clonogenic population of cells from adult human dental pulp and assessed their differentiation potential (Gronthos et al., 2000). These cells, called dental pulp stem cells (DPSCs), had the capacity to form dense calcified nodules *in vitro*; moreover, STRO-1 + cells extracted from dental pulp of adult rat can differentiate toward adipogenic, neurogenic, myogenic and chondrogenic lineages.

In the same work, it was demonstrated that DPSCs can generate a dentin/pulp-like structure *in vivo*. DPSCs were transplanted in conjunction with HA/TPC powder, a scaffold "odonto-conductive", into immunocompromised mice and after 6 weeks a collagenous matrix was deposited. Besides, transplanted DPSCs expressed dentin matrix components and the gene DSPP, which encode for dentin sialoprotein and dentin phosphoprotein.

An osteo-dentin like matrix was observed to be formed only two weeks after subcutaneous implantation in rabbits, when poly (lactic-co-glycolic acid) polymeric porous scaffolds grafted with dental pulp stem cells were engineered (El-Backly et al., 2008). In this study, they observed that cells formed mineralised-like structures even without the addition of any differentiation chemicals. These cells may undergo differentiation into hard tissue forming cells when provided with an appropriate substrate.

Other cells known as SHED, stem cells isolated from dental pulp of human exfoliated deciduous teeth, have been observed to be capable of regenerating pulp and dentin if proper biochemical stimuli are provided. SHED exhibited a high plasticity, since they are able to differentiate *in vitro* into neurons, adipocytes, osteoblasts and odontoblast (Miura et al., 2003).

In 2008 Cordeiro et al. (Cordeiro et al., 2008) seeded SHED onto dentine slices and implanted them subcutaneously in immunodeficient mice. After 14-28 days, they observed a new dental pulp-like tissue, whose cellularity and architecture are very similar to that of physiologic pulp. SHED seeded on tooth slices/scaffolds were capable of differentiating into odontoblast-like cells and showed characteristics of dentin-secreting odontoblast. Moreover, in the same work it was demonstrated that SHED co-implanted with human endothelial cells HDMEC improve the new-formed tissue organization, microvascular network and oxygen and nutrient influx.

4.2 Periodontal regeneration

The periodontum is a complex organ consisting of epithelial tissue and connective tissue, both soft and mineralized. The periodontum comprises the alveolar bone, gingiva, cementum and periodontal ligament. Several diseases affecting periodontal integrity can lead to tooth loss.

The loss of numerous teeth can entail difficulties in eating and talking, moreover a significant loss of surrounding bone can imply limitations to future options of surgical

intervention. In particular periodontal ligament regeneration is a major concern in periodontology and an ambitious aim in implantology.

Periodontal ligament itself contains progenitor cells; recently human stem cells have been isolated from periodontal ligament. They express stemness markers such as STRO-1, CD146 and they are able to form alizarin red-positive nodules and cementoblastic/osteoblastic markers (alkaline phosphatase, osteocalcin and bone sialoprotein) *in vitro*. These cells have been called periodontal ligament stem cells (PDLSC). *In vitro* expanded PDLSCs were transplanted subcutaneously into the dorsal surfaces of immunocompromised mice using hydroxyapatite tricalcium phosphate (HA/TCP) as a carrier. These cells showed the capacity to differentiate into cementoblasts and to form cementum/PDL-like structures. (Seo et al., 2004).

Moreover, PDLSCs showed the capacity to form collagen fibres connecting to the cementum-like tissue; these fibres are similar to Sharpey's fibres and they suggest the potential to regenerate PDL attachment.

A recent study of a preclinical model made in miniature pigs (Liu et al., 2008) showed that autologous PDLSCs are capable of forming bone, cementum and periodontal ligament if they are transplanted onto HA/TCP carrier into surgical, created periodontal defects.

Not only PDL derived cells have periodontal regeneration capability. Precursor cells (PCs) from human dental follicles of wisdom teeth have been isolated and characterized for their periodontal regeneration potential. These cells formed *in vitro* a membranous structure that can be compared to PDL, consisting of a connective-like matrix and a mesothelium-like cellular structure with nuclei of granular calcification. *In vivo* differentiation potential of PCs was assessed by their transplantation in conjunction with hydroxyapatite powder into immunocompromised mice, but no sign of cementum or bone formation was found in histological sections of transplants (Morsczeck et al., 2005).

More recently, in 2007 Yokoi et al. demonstrated that immortalized mouse dental follicle cells are able to re-generate *in vivo* a PDL-like tissue (Yokoi et al., 2007).

Also non-dental derived stem cells have been tested in order to obtain periodontal regeneration. In a work of Tobita M. (Tobita et al., 2008), ASCs (Adipose-derived Stem Cells) isolated from rat and mixed with platelet-rich plasma (PRP) were implanted into the periodontal tissue defect generated in the test rat. A partial alveolar bone regeneration and a periodontal ligament-like structure was observed 8 weeks after implantation.

Even bone marrow could represent an alternative source of MSCs for the treatment of periodontal disease (Kawaguchi et al., 2004). Its potential in periodontal regeneration was assessed *in vivo*, when bone marrow derived MSCs isolated from beagle dogs were mixed with atelocollagen and transplanted into experimental periodontal defects.

Transplanted stem cells have been able to differentiate into cementoblasts, osteoblasts, osteocytes, fibroblasts and four weeks after transplantation the defects were almost regenerated with periodontal tissue. (Hasegawa et al., 2006).

4.3 Tooth regeneration

Humans are genetically programmed to replace their teeth once during childhood. Therefore, when adult teeth are lost or damaged, they cannot be regenerated or re-grown. However, with the advancement of stem cell biology and tissue engineering, regenerating the whole tooth has become a realistic and attractive option to replace a lost or damaged tooth. The loss of teeth because of caries, periodontal disease, or trauma is a relatively

common problem among older people. Several therapies such as artificial dentition, tooth transplantation, and dental implants are often necessary to recover lost masticatory function. However, at present, complete restoration therapy to compensate for complete tooth loss has not been achieved (Masaki et al., 2008).

Teeth and their surrounding structures (tooth/periodontal complexes) are typical of complicated organs and consist of both hard tissue (dentin, enamel, cementum, alveolar bone) and soft tissue (dental pulp, periodontal ligament and gingiva). Because of its complexity, tooth regeneration presents some limits which regard the traditional principles of tissue engineering, related to whole tooth regeneration with correct morphology. Tooth development is regulated by the interaction between the dental epithelium and the dental mesenchyme. For this reason two populations of stem cells need to be considered in the development of the tooth: epithelial (EpSC) and mesenchymal stem cells (MSC), EpSCs differentiate into ameloblasts whereas MSCs give rise to odontoblasts, cementoblasts, osteoblasts and fibroblasts of the periodontal ligament.

Numerous attempts have been made in order to regenerate teeth *in vivo* with an association of odontogenic, mesenchymal and epithelial cells with encouraging results.

Some experiments were done by Sharpe's group. They discovered that recombinations between an *in vitro* created mesenchyme (with non-dental stem cell from different sources) and embryonic oral epithelium stimulate an odontogenic response in the stem cells (Ohazama et al., 2004). When this engineered tissue was implanted into adult renal capsules it resulted in the development of tooth structures and associated bone.

Other studies demonstrated that cells isolated from both porcine (Young et al., 2005) and rat (Duailibi et al., 2004) tooth buds, seeded onto polyglycolide/poly-L-lactide (PGA/PLLA) scaffolds, lead to the formation of an ectopic bioengineered tooth with pulp, dentin and enamel tissues, even though in an anomalous special arrangement. In fact, the main limitation of the new formed tooth is the abnormal shape and the relative diminution of the dental tissues generated.

In a recent study (Nakao et al., 2007) a new approach was proposed for the formation of a bioengineered tooth in the mouse mandible. Mesenchymal and epithelial cells, before the transplantation, were sequentially seeded into adjacent regions within a collagen gel drop. The implantation of both early primordia and a tooth that had partially developed in a subrenal capsule was tested. With this method, a correct tooth structure comprising enamel, dentin, root, dental pulp and bone could be observed, showing penetration of blood vessels and nerve fibers.

Encouraging results have also been obtained by Duailibi et al. (Duailibi et al., 2008) using rat tooth bud cells implanted into the jaw of an adult rat for 12 weeks. This study demonstrated that stem cells could be a useful future tool in the replacement of missing or lost teeth.

Lesot et al. showed that also bone-marrow-derived cells can be reprogrammed to give rise to ameloblast-like cells, offering novel possibilities for tooth-tissue engineering and the study of the simultaneous differentiation of one bone marrow cell subpopulation into cells of two different embryonic lineages (Unda et al., 2006).

However the odontogenic potential of bone marrow and dental derived stem cells do not seem to be the same. A work of Yu et al. (Yu et al., 2007) showed that DPSC STRO-1 + co-cultured with apical bud cells (ABCs) possess more active odontogenic differentiation ability than STRO-1 + BMSCs/ABCs. Recombined DPSCs/ABCs were able to form a tooth shaped tissue with balanced amelogenesis and dentinogenesis, whereas BMSCs/ABCs

formed an atypical dentin-pulp complex and they were not able to form enamel. These data indicate that mesenchymal stem cells derived from different embryonic origins; in fact DPSCs derive from neural crest whereas BMSCs from mesoderm and they could not be equivalent in their differentiation pathway.

5. Conclusion

Remineralization of enamel represents a useful tool to counteract the loss of mineral tissue due to bacteria metabolism and foods and drinks ingestion. The technologies now available are based on the substitution of hydroxyapatite with fluor-hydroxyapatite in order to reduce solubility. The use of nanotechnology as well as the crystallization of hydroxyapatite starting from amorphous compound are effective strategies to gain mineralization even if they fail to reconstruct the histological and crystallographic integrity of enamel tissue.

The therapeutic potential of adult stem cells for regenerative purposes is well-accepted, but further studies are necessary in order to furnish experimental data useful in clinic. Recently it was hypothesized that bone-marrow-derived mesenchymal stem cells could be a source of carcinoma-associated fibroblasts (CAF), which has an important role in the growth of epithelial solid tumors (Mishra et al., 2009). In the regenerative medicine, using stem cells may comport some risks. In particular a thorough understanding is necessary of all the molecular events that control tissues development, repair and regeneration. The pathology and consequence involved should be considered and investigated in order to avoid any problems.

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Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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