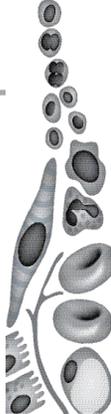


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Regenerated teeth: the future of tooth replacement?

“We hope that the rapid scientific and technological advancement will provide new information and solutions that will allow regenerated teeth to become a routine treatment for individuals with missing teeth.”



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Despite the considerable progress of dental treatment and tooth decay prevention, elderly people frequently encounter the problem of tooth loss, affecting their quality of life. Restorative prosthetics in the form of implants provide a common solution to this problem. Dental implant technology for tooth replacement was used in several ancient civilizations. Indeed, dental implants dating from 2500 BC were found in Egypt, and tooth replacement was documented from the Mayan culture in 600 AD [1]

Dental implants are still used today for the functional replacement of missing teeth. However, despite their long history, there are several limitations in functionality and longevity of the implants. Indeed, dental implants cannot represent the ideal solution for tooth replacement since the physiology and plasticity of naturally formed teeth is not respected. The tooth interacts actively with the alveolar bone through the periodontal ligament (PDL). The mechanical stress during mastication is supported and modulated by this highly specialized tissue that occupies the space between the tooth root and the alveolar bone. PDL is not formed around dental implants, making the bone tissue vulnerable when excessive forces are applied during mastication [2]. Alternative strategies are being tested to create a functional biocompatible type of replacement for dental implants and efforts are being made to regenerate parts or even the entire tooth organ.

A new concept: brand new teeth made in the laboratory

The rapid progress made in stem cell, material and molecular biology sciences over the last 20 years has allowed scientists working on teeth to imagine alternative and innovative strategies for tooth replacement [3,4]. Recently, scientists have implemented a new concept of tooth replacement, where new whole teeth

could be generated experimentally using stem cells (referred to as BioTeeth, meaning living teeth) [3]. Regenerated tooth (RegTooth) is a more appropriate term, since it is possible to distinguish between naturally and experimentally formed teeth. The rationale for the generation of a new whole tooth is simple and consists of recreating and mimicking the molecular and cellular events that occur during the initiation of odontogenesis. This procedure might be a better alternative to the use of dental implants for tooth replacement, since it involves the regrowth and eruption of new teeth in the mouth of the patients, after experimental manipulation *in vitro*. However, a regenerated tooth has several challenges that need to be solved prior to any clinical trial/application. The reactivation of the odontogenic program using stem cells is not obvious and does not guarantee the success of new tooth formation in an adult mouth. It is possible to regenerate several human dental tissues (e.g., dentin and PDL) after experimental manipulation [3–5]. However, the regenerated tissues were not identical to their naturally formed counterparts. With the continuous progress of science it might be possible in the future to regenerate more complex dental structures (e.g., enamel) or even the entire tooth. Scientists working in that field are confronted daily with new challenges and limitations that might postpone the generation of brand new teeth in the laboratory for many years.

Generation of specialized dental structures *in vivo*

Teeth are formed from specific embryonic cells, grow and finally erupt into the oral cavity. Through a series of epithelial–mesenchymal interactions, cells of the oral epithelium and cranial neural crest-derived mesenchymal cells (CNCCs) give rise to complex mineralized structures that form the tooth organ [6]. CNCCs

form the dental follicle and dental pulp, while the oral epithelium gives rise to the inner dental epithelium. Subsequently, dental pulp cells differentiate into odontoblasts and inner dental epithelium into ameloblasts. Odontoblasts are responsible for dentin matrix synthesis, whilst ameloblasts produce the enamel matrix. Once the mineralization of the crown is completed the tooth starts to erupt in the oral cavity, while the root continues to develop. Hertwig's epithelium root sheet, a derivative from the outer dental epithelium and the inner dental epithelium, initiates radicular dentin formation and determines the root shape. Root development will be accomplished together with the organization of innervation, vascularisation and root anchoring to the surrounding alveolar bone. This latest process will be accomplished mainly by the relationship of three main tissues in the periodontium: cementum, alveolar bone and PDL. PDL contains a great variety of cells and extracellular matrix. The cellular components include osteoblasts, fibroblasts, cementoblasts, osteoclasts, cementoclasts, endothelial cells and epithelial rests of Malassez [4,6].

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Partial dental tissue regeneration could be achieved more easily and rapidly than whole-tooth regeneration. In fact, methods that enhance tertiary dentin repair and regeneration of the entire dental pulp tissue are being evaluated with variable degrees of success [7–9]. In addition, periodontal tissue regeneration is progressing rapidly with the application of biodegradable scaffolds and growth factors [10]. By contrast, few studies exist on the regeneration of enamel, which is the hardest human tissue and represents the visible part of the teeth, protecting them from abrasion and bacterial attack.

Strategies for building the RegTooth

There are two main approaches in constructing a new whole tooth. The first implies the *in vivo* implantation of tooth germ cells that were previously generated from various populations of stem cells or dental progenitor cells and grown *in vitro* for some time. Organotypic culture is the most appropriate of the techniques for the development of the teeth *in vitro*. The other approach consists of implanting into the jaw tooth-shaped

polymer scaffolds that are filled with *in vitro* expanded stem cells or dental progenitor cell populations. Ideally, this implant should reproduce the 3D structure required for the transplanted cells to support their differentiation and avoid xenograft rejection [3].

One of the challenges in regenerative dentistry is to find cells that can replace the clonogenic CNCCs. The identification and characterization of human adult stem cells (or progenitor cells) of dental origin will contribute to regenerate successfully tooth primordia. Dental stem cells could be removed from a patient, expanded and put back into the same individual when tooth regeneration becomes necessary, thereby removing the need for immunosuppression [3]. These cells can be isolated from either primary or permanent teeth. Dental stem cells can be extracted from the apical papilla of shed primary teeth, exfoliated deciduous teeth and the dental pulp (DPSCs). These stem cells have the potential to differentiate into various cell types, such as chondrocytes, adipocytes, osteoblasts, myocytes, neuronal cells and cardiomyocytes [3,4]. The composition of the culture medium in which DPSCs are grown can dictate their differentiation into odontoblasts, osteoblasts and chondroblasts. However, adult stem cells are present at low frequency (e.g., roughly one stem cell per 100,000 bone marrow cells), making both isolation and expansion of DPSCs problematic [4]. Recently, dental pulp cells were reprogrammed into induced pluripotent stem (iPS) cells [11].

Limited information is available regarding the regenerative potential of dental epithelial stem cells (DESCs), which could give rise to ameloblasts. Ameloblasts are not present in erupted teeth. Undifferentiated wisdom teeth present a potential source of human dental epithelium [12]. The Epithelial Rests of Malassez (ERM) constitute another source of DESCs [PAPAGERAKIS P, UNPUBLISHED DATA]. ERM are localized in the periodontal ligament of adult teeth and exhibit stem cell properties. ERM can differentiate into enamel-secreting cells when co-cultured with DPSCs [PAPAGERAKIS P, UNPUBLISHED DATA] [13]. Thus, DESCs isolated from ERM can provide a viable source of ameloblast progenitors for enamel regeneration. Alternatively, epithelial stem cells of nondental origin (e.g., hairs and skin) could be used for the formation of enamel.

Therefore, dental mesenchymal stem cells that interact *in vitro* with dental epithelial stem cells (after recombination) might be able to form the various mesenchymal and epithelial cell populations in a regenerated tooth. A study in rodents

has shown that bone marrow cells have stem cell properties and can substitute for CNCCs during tooth regeneration [14]. However, this experimental approach was partly successful since a truncated ‘tooth’ (i.e., absence of roots) was obtained, which resembled an odontoma (a dental pathological condition) more than a normal tooth.

Tooth-like structures have also been produced using heterogeneous dental cell populations in biodegradable polymer scaffolds. Disaggregated and reaggregated dental epithelial and mesenchymal cells are able to interact and recapitulate odontogenesis and formation of tooth-specific structures in animal models. However, these implants exhibited a mosaic of organized and disorganized enamel, dentin and pulp tissues, and did not embrace the size and shape of the scaffolds [15].

Challenges of dental tissue regeneration

■ Enamel regeneration: structure, color & time

Enamel formation (amelogenesis) is the result of a series of complex, dynamic and programmed cellular, chemical and physiological events [16]. These events allow categorization of enamel formation in three distinct stages (i.e., the secretory, transition and maturation stages). The secretory stage is characterized by active protein synthesis and secretion by the ameloblasts. Ameloblasts also deposit enamel crystals at oblique angles while they are moving in the direction of the future cusps to accommodate expansion of the enamel surface. The maturation stage is characterized by removal of enamel organic materials and growth of hydroxyapatite crystals in thickness, as well as by regulated movement of ions into and out of the enamel matrix [17,18]. Ions arrive to ameloblasts from the blood vessels after traversing a distance of 50–100 μm (i.e., 2–3 cell layers). Stem cells destined to form enamel must reproduce these three stages and the cellular movement that occurs during enamel apposition. Organic material removal, vascularization and extensive ion transport must also be achieved in the regenerated enamel.

Besides their involvement in crystal formation, ions also contribute to the color of the teeth [19]. Enamel in humans is characterized by a big diversity of colors (i.e., a rich spectrum of yellow, grey and white tonalities), a variety that can be often observed in teeth of the same individual. Tooth color variations are

also observed in other animal species, such as in mice, where the enamel of the incisors exhibits a deep yellow color, while molars have a white color. However, little information exists on how ions control color variability in human enamel adding another difficulty in the enamel regeneration process.

Time represents another great challenge of enamel regeneration. It is well known that the whole process of enamel formation in human permanent teeth may take more than 5 years and might be regulated by complex interactions of clock genes [20]. This long-term physiological procedure may be discouraging for individuals with missing enamel who look forward to immediate treatment outcomes.

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Therefore, regeneration of enamel requires production and secretion of the right amount of proteins, at the right place, at the right time. Otherwise, the effort to regenerate enamel will fail (as happens in several mutations affecting enamel formation) [21]. Thus, regeneration of human enamel is a very difficult – almost impossible – task at the present. Much more must be discovered before enamel regeneration becomes a routine procedure in dentistry. This also holds true for whole tooth regeneration.

■ The right shape & size of the RegTooth

It is imperative that the RegTooth, which will develop in the patient’s mouth after transplantation, acquires the correct morphology and size. Tooth shape is primarily determined during early odontogenesis. The formation of the various tooth shapes might be either the result of prepatterned CNCC, or a consequence of the response of CNCCs to signals originated by the oral epithelium. Tissue recombination and transplantation experiments indicated that signals from the oral epithelium influence CNCCs to adopt a dental identity [6].

Several secreted signaling molecules, such as BMPs, FGFs, Wnts and Shh, are expressed in the epithelium and function as morphogens that control the generation of diverse tooth shapes. For example, BMP4 expression is linked with the incisors’ shape, while FGF8 is linked with the shape of molars. BMP4 activates expression

of *Msx1* and *Msx2* in the mesenchyme of future incisors. Similarly, *Islet1* is expressed only in the epithelium of the incisors and its expression is regulated by BMP4. By contrast, FGF8 activates *Dlx1*, *Dlx2* and *Barx1* expression in the mesenchyme of future molars [6].

Furthermore, the shape of maxillary and mandibular teeth differs and is controlled by genes such as *Dlx*, *Barx1* and *Pitx1* [6]. For example, *Pitx1* deletion affects only the mandibular molars, which are smaller and have fewer cusps [22].

Alteration of the odontogenic signaling cascade might also lead to modification of tooth size. For example, smaller teeth were reported in mice after deletion of Wnt signaling [6].

■ Root & eruption of the RegTooth

Successful tooth regeneration requires the formation of roots with appropriate shape and length. For example, short roots could be problematic in retaining the regenerated teeth in place. Similarly, it is important that root-related structures (e.g., PDL) of the regenerated teeth remain functional for long periods, thereby avoiding pathological manifestations such as tooth ankylosis. The time and orientation of tooth eruption in adults also has to be controlled. Both proper root formation and tooth eruption are time-consuming processes, thus making the choice of a RegTooth for tooth replacement problematic.

Ideally, autologous tissues must be used for the implantation of regenerated teeth in a given patient, thus avoiding immunological rejection [3]. Gene therapy-based strategies must be applied for the regeneration of teeth in individuals with mutations that affect their dentition (e.g., amelogenesis imperfecta, ectodermal dysplasia) [6].

Conclusion

Tooth regeneration provides an attractive alternative to existing tooth restoration therapies. This concept relies on the *in vitro* recreation

of the genetic odontogenic program using stem cells. Numerous genes control embryonic tooth development and define the various dental territories (i.e., incisors, canines, premolars and molars) in the mouth, as well as the number, shape, size and color of the teeth. A stem cell strategy for tooth regeneration must combine stem cell populations with adequate signaling molecules. Cell-based therapies are in their infancy and many issues need to be addressed before any clinical application. The existing challenges include the need to determine consistent protocols to control the size, shape and color of teeth, as well as to considerably shorten the time of enamel and root formation and tooth eruption. Furthermore, the use of culture-expanded stem cell populations needs to take into account the possibility of genetic and epigenetic instability.

“Scientists ... are confronted daily with new challenges and limitations that might postpone the generation of brand new teeth in the laboratory for many years.”

Although the prospect of tooth regeneration using stem cells is very attractive, it is not likely that they will replace routine clinical dental practices in the near future. We hope that the rapid scientific and technological advancement will provide new information and solutions that will allow regenerated teeth to become a routine treatment for individuals with missing teeth.

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