Role of stem cells in tooth bioengineering

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ABSTRACT

The creation of teeth in the laboratory depends upon the manipulation of stem cells and requires a synergy of all cellular and molecular events that finally lead to the formation of tooth-specific hard tissues, dentin, and enamel. This review focuses on the different sources of stem cells that have been used for making teeth in vitro. The search was performed from 1970 to 2012 and was limited to English language papers. The keywords searched on medline were 'stem cells and dentistry,' 'stem cells and odontoblast,' 'stem cells and dentin,' and 'stem cells and ameloblasts.'

Keywords: Ameloblasts, dentin, odontoblast, stem cells.

INTRODUCTION

Loss of tooth is a common and frequent situation that can result from numerous pathologies such as periodontal and carious diseases, fractures, injuries, or even genetic alterations. Recent efforts made in the field of biomaterials have led to the development of dental implants that can be inserted in the maxillary or mandibular bone to replace the missing teeth. However, implants are still not completely satisfactory and their successful use greatly depends on their osteointegration.¹ Furthermore, dental implant technology is dependent on bone volume. To overcome these difficulties, new ideas and approaches have emerged recently from the quickly developing fields of stem cell technology and tissue engineering.

Stem cells in regenerative dentistry

A stem cell is defined as an unspecialized cell that can continuously produce unaltered daughters and has the ability to generate cells with different and more restricted properties. Stem cells can divide either symmetrically or asymmetrically. Asymmetric divisions keep the number of stem cells unaltered and are responsible for the generation of cells with different properties. These cells can either multiply (progenitors or transit amplifying cells) or be committed to terminal differentiation.

Progenitors and transit amplifying cells have a limited lifespan and therefore can only reconstitute a tissue for a short period of time when transplanted. In contrast, stem cells are self-renewing and thus can generate any tissue for a lifetime. This is a key property for a successful therapy. During recent years, stem cells have been used extensively in many medical disciplines for the repair and regeneration of defective tissues and organs. The aim of regenerative dentistry is to stepwise re-create in vitro all the mechanisms and processes that nature uses during initiation and morphogenesis of a given organ. However, the knowledge in stem cell technology is increasing quickly in all disciplines and dictates the need for new strategic approaches in all fields, including reparative dentistry.

Stem niches and other stem cell sources for the development of teeth in vitro or ex vivo

Two different stem cell niches have been suggested in the teeth: the cervical loop of rodent incisor for epithelial stem cells (EpSC)^{2,3} and a perivascular niche in the adult dental pulp for mesenchymal stem cells (MSC).⁴ In rodent incisors, the proliferation of EpSC, which is located at the cervical loop area, is governed by signals from the surrounding mesenchyme. Fibroblast growth factor (FGF) signaling mainly FGF-3 and FGF-10 is of particular importance since it is linked to the Notch pathway.⁵ Molecules such as

Bone Morphogenic Protein (BMPs), Activin, and Follistatin are also expressed inside the stem cell niche and are known to regulate its maintenance and functionality through a complex integrative network.^{3,5,6} In the dental pulp, MSCs are thought to reside in a perivascular niche,³ but little is known on the exact location and molecular regulation of this niche. The Ephrin (Eph) receptor tyrosine kinase family of guidance molecules appears to be involved in the maintenance of the human dental pulp perivascular niche. Eph-B and its ligand Eph-B were shown to inhibit MSC migration and attachment via the mitogen-activated protein kinase (MAPK) pathway through unidirectional and bidirectional signaling, respectively.⁷

MESENCHYMAL STEM CELLS

Mesenchymal stem cell can give rise to mature cell types that have characteristic morphologies and specialized functions. First described in the bone marrow,⁸ MSCs have been extensively characterized *in vitro* by the expression of markers such as STRO-1, CD146, or CD44.⁹ STRO-1 is a cell surface antigen used to identify osteogenic precursors in the bone marrow, CD146 a pericyte marker, and CD44 a MSC marker. Mesenchymal stem cells possess a high self-renewal capacity and the potential to differentiate into mesodermal lineages thus forming cartilage, bone, adipose tissue, skeletal muscle, and the stroma of connective tissues. Mesenchymal progenitors have been assessed for tooth engineering purposes, such as progenitors derived from teeth and bone marrow.

Stem cells from human exfoliated deciduous teeth

The isolation of post-natal stem cells from an easily accessible source is indispensable for tissue engineering and clinical applications. Recent findings demonstrated the isolation of mesenchymal progenitors from the pulp of human deciduous incisors.¹⁰ These cells were named stem cells from human exfoliated deciduous teeth (SHED) and exhibited a high plasticity since they could differentiate into neurons, adipocytes, osteoblasts, and odontoblasts. *In vivo* SHED cells can induce bone or dentin formation.

Dental pulp stem cells

After a dental injury, dental pulp is involved in a process called reparative dentinogenesis, where cells elaborate and

deposit a new dentin matrix for the repair of the injured site.¹¹ It has been shown that adult dental pulp contains precursors capable of forming odontoblasts under appropriate signals. These signals are the calcium hydroxide or calcium phosphate materials, which constitute pulp-capping materials used by dentists. Dental pulp progenitors have not been clearly identified but some data suggest that pericytes, which are able to differentiate into osteoblasts, could also differentiate into odontoblasts. Tooth repair is a lifetime process thus suggesting that MSC might exist in the adult dental pulp. The *in vivo* therapeutic targeting of these adult stem cells remains to be explored.

Stem cells from the apical part of the papilla

Stem cells from the apical part of the papilla (SCAP) have been isolated and their potential to differentiate into odontoblasts was compared with that of the periodontal ligament stem cells (PDLSC).¹² Stem cells from the apical part of the papilla exhibit a higher proliferative rate and appear more effective than PDLSC for tooth formation. Importantly, SCAP are easily accessible since they can be isolated from human third molars.

Dental follicle stem cells

Dental follicle stem cells (DFSC) have been isolated from the follicle of the human third molars and express the stem cell markers Notch 1, STRO-1, and nestin.¹³ These cells can be maintained in culture for at least 15 passages. STRO-1 positive DFSC can differentiate into cementoblasts *in vitro* and are able to form cementum *in vivo*. Immortalized dental follicle cells are able to re-create a new periodontal ligament (PDL) after *in vivo* implantation.

Periodontal ligament stem cells

The PDL is a specialized tissue located between the cementum and the alveolar bone and has as a role the maintenance and support of the teeth. Its continuous regeneration is thought to involve mesenchymal progenitors arising from the dental follicle. Periodontal ligament contains STRO-1 positive cells that maintain certain plasticity since they can adopt adipogenic, osteogenic, and chondrogenic phenotypes *in vitro*.¹⁴ It is thus obvious that PDL itself contains progenitors, which can be activated to self-renew and regenerate other tissues such as cementum and alveolar bone.

Bone marrow-derived mesenchymal stem cells

Bone marrow-derived mesenchymal stem cells (BMSC) have been tested for their ability to re-create periodontal tissue. These cells are able to form *in vivo* cementum, PDL, and alveolar bone after implantation into defective periodontal tissues. Thus, the bone marrow provides an alternative source of MSC for the treatment of periodontal diseases.¹⁵ Bone marrow-derived MSC share numerous characteristics with dental pulp stem cells (DPSC) and are both able to form bone-like or tooth-like structures. However, BMSC display a lower odontogenic potential than DPSC, indicating that MSCs from different embryonic origins are not equivalent. Indeed, DPSC derive from neural crest cells, whereas BMSC originate from the mesoderm. Furthermore, the comparison of the osteogenic and adipogenic potential of MSC from different origins shows that, even if cells carry common genetic markers, they are not equivalent and are already committed toward a specific differentiation pathway. Commitment could arise from the conditioning of stem cells by their specific microenvironment or stem cell niche. Mesenchymal stem cells can also be obtained from several other sources such as synovial membrane and periosteum. As these cell populations display distinctive biological properties depending upon their tissue of origin, it remains to be explored which source might be used for an optimal tooth development for clinical application.

EPITHELIUM-ORIGINATED DENTAL STEM CELLS

Although, significant progress has been made with MSC, there is no information available for dental EpSC in humans. The major problem is that dental epithelial cells such as ameloblasts and ameloblast precursors are eliminated soon after tooth eruption. Therefore, epithelial cells that could be stimulated *in vivo* to form enamel are not present in the human adult teeth. Stem cell technology appears to be the only possibility to re-create an enamel surface.

Epithelial stem cells from the labial cervical loop of rodent incisor

The rodent incisor is a unique model for studying dental EpSC since, in contrast to human incisors or other vertebrates, this tooth grows throughout life. An EpSC niche which is located in the apical part of the rodent incisor epithelium (cervical loop area) is responsible for a continuous

enamel matrix production.² In this highly proliferative area, undifferentiated epithelial cells migrate toward the anterior part of the incisor and give rise to ameloblasts.

Although, these findings are important for understanding the mechanisms of stem cell homing, renewal, and differentiation, this source of dental EpSC cannot be used for treatment in humans since it would require the introduction of rodent cells in the human mouth.

Dental EpSC can be isolated from post-natal teeth but exhibit complex problems that strongly limit their clinical application in humans. Other sources are thus required. Ideally, these sources should be easily accessible, available from adult individuals, and the derived cells must have the potential for enamel matrix production. The use of non-dental EpSC will only be possible with the transfer of genes, creating an potential to non-dental epithelia prior to any association with mesenchymal cells. This is certainly one of the most exciting goals of the next decade in tooth engineering.

Association of epithelial and mesenchymal stem cells and its application

Since, teeth are formed from two different tissues, building a tooth logically requires the association of odontogenic mesenchymal and epithelial cells. The recombination of dissociated dental epithelial and mesenchymal tissues leads to tooth formation both *in vitro* and *in vivo*. Numerous attempts have been made in order to form teeth *in vivo* with very promising results. Single cell suspensions obtained from rat, pig, or mice tooth germs have been seeded onto the surface of selected biomaterials (e.g. collagen-coated polyglycolic acid, calcium phosphate material, and collagen sponges) and successfully re-implanted into the omentum of immunocompromised animals.^{16,17} All these reports describe the presence of both dentin and enamel. This indicates that the recombined cells could re-organize themselves and form individual layers and, furthermore, that they can differentiate properly into odontoblasts and ameloblasts. In most of these studies, the cells were directly seeded onto biomaterials without any additional *in vitro* procedure. In studies including *in vitro* steps before the *in vivo* transplantation, the results could be influenced by several critical parameters such as the presence or absence of serum, the type of serum, the composition of culture media, the cell density, and the ratio between epithelial and mesenchymal cells. For these reasons, a definitive and universal protocol for tooth formation does not exist so far.

Making entire teeth with enamel and dentin structures *in vivo* is a reality and not a utopia. However, these bioengineered teeth have been produced in ectopic sites and

Figure 1 A tooth created in vitro after co-culture of isolated epithelial and mesenchymal stem cells.

are still missing some essential elements such as the complete root and periodontal tissues that allow correct anchoring into the alveolar bone. Recently, a new approach has been proposed for growing teeth in the mouse mandible. In this study, epithelial and mesenchymal cells were sequentially seeded into a collagen gel drop and then implanted into the tooth cavity of adult mice. With this technique, the presence of all dental structures such as odontoblasts, ameloblasts, dental pulp, blood vessels, crown, PDL, root, and alveolar bone could be observed.¹⁸ Thus, the implantation of these tooth germs in the mandible allowed their development, maturation, and eruption indicating that stem cells could be used in the future for the replacement of missing teeth in humans (Figure 1). Despite the outstanding advances in tooth bioengineering, such a technology cannot be applied to human restorative dentistry for one simple reason: the epithelial and mesenchymal cells used for tooth reconstruction are of dental origin and have been given by a donor. The challenge that remains is to find out new and easily accessible sources of both epithelial and MSCs that can be reprogrammed for an odontogenic potential and then associated to form a fully functional tooth. One alternative could be the use of genetically modified cells expressing specific genes (e.g. transgenes and siRNA) or with a specifically deleted gene (e.g. knock-in and knock-out). Ideally, this approach should provide a non-limited source of cells and introduce new genetic information to reprogram a non-dental cell to acquire odontogenic properties. For example, p53-deficient mice were used to establish dental epithelial clonal cell lines subsequently associated with mesenchymal cells to bioengineer teeth in vivo.¹⁹ These cell lines demonstrated heterogeneous outcomes in terms of regeneration depending on their differentiation state. This technique provides us with an unlimited source

of epithelial cells and shows the potential of genetically modified cells that can be used for tooth engineering.

CONCLUSION

Thorough understanding of the cellular and molecular events involved in development, repair, and regeneration of teeth is necessary. Identification of several types of epithelial and MSCs in the tooth and the knowledge of molecules involved in stem cell fate is a significant achievement. In vitro and in vivo experiments using these cells have provided promising results by the regeneration of a complete tooth with all dental structures including cells and extracellular matrix deposition. The engineering of tridimensional matrices with a composition similar to that of the organs to reconstruct, and the addition of growth factors such as FGF, BMP, or platelet-derived growth factor (PDGF) might facilitate the transplantation and the differentiation of stem cells.

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