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Application of Tissue Engineering in Tooth: A Review on Recent Trends and Advances

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Abstract. Tooth loss has endangered human health for thousands of years, and people can apply dentures or dental implants to restore tooth loss today. Tissue engineering provides a novel way to regenerate a new functional tooth in vivo or vitro to help patients regain masticatory function and appearance. In this summarize review, we will discuss some promising seed cells in dental tissue engineering, the scaffolds that can be used to regenerate teeth, and some growth factors which can promote the development of tooth. Although significant progresses have been made nowadays, some challenges still remain. Hence, tissue engineering could be a choice to replace missing tooth in the future when the obstacles are solved.

1.Introduction

Tissue engineering is a combination of life science and engineering in order to produce or restore a functional and healthy tissue even an organ. By the differentiation potential of seed cells, people can obtain tissues that are needed with the help of scaffold in vivo or vitro. Nowadays, tissue engineering has made great progresses in many fields, such as bone regeneration and tooth regeneration. Tooth loss has a great impact on patient's health and appearance, resulting in disorder of patient's life and mentality. Tissue engineering may be a promising clinical approach to cure tooth loss which is always an unavoidable case in dentistry. Today dentures or dental implants are used as tooth replacement therapies. However, both of these two therapies have some defects. Dentures usually bring discomfort to wearers. And lack of compliance will result in bone resorption. Implants are so preferred as many clinicians insist that although performing endodontic treatments may prolong the life of a natural tooth yet root canal–treated teeth will probably desiccate and crack, and in the end implant is the ultimate solution anyway [1]. Despite the fact that dental implants are fairly useful, they are not as perfect as living teeth. So it would be every dentist's dream to restore a living, esthetic and functional tooth. Fortunately, researchers are able to regenerate tooth roots, dental pulp and tooth crowns with dentin and enamel successfully in proper condition. But there are still a lot of unsettled problems. A study had produce teeth containing enamel, dentin and dental pulp in immunocompromised mice. The teeth regenerated in biodegradable polymer scaffolds, however, develop in a disoriented way and fail to reach the planned size nor the shape of the scaffold [2-4]. Additionally, it's a challenge to gain suitable source of seed cells, mostly mesenchymal stem cells (MSCs). Of course, embryonic stem cells (ESCs) and induced pluripotent stem cells (IPSCs) seem perfect as seed cells in tooth regeneration. In addition, the choice of scaffold's materials is also a complicated problem. Every material's characteristics differ from each other, so researchers have to consider a scaffold's mechanical properties, biocompatibility, safety, scaffold architecture and biodegradability comprehensively[5]. So there are still massive problems to be considered, although major strides have been made in tooth tissue engineering.

In this paper, we will give a contemporary and systematic review of tissue engineering and particularly in the area of tooth regeneration, mainly focusing on stem cells, grafting materials and scaffolds and growth factors which will be needed in this technique. In the end, an example of the application of tooth regeneration is discussed, and summary of this review is presented.

2. Stem cells applied in tooth tissue engineering

It is essential to review the process of tooth development from the very beginning to choose suitable stem cells used in tooth tissue engineering. There is a series of complicated reaction between mesenchymal cells and epithelial organ when tooth develops, including cell differentiation, morphogenesis, tissue mineralization and eruption of teeth. Therefore, the stem cells should be able to present the processes above. And we will discuss some potential stem cells for tooth tissue engineering.

2.1.Dental pulp stem cells (DPSCs)

The phenomenon of dentinal repair in the postnatal organism implies that there may be some stem cells in pulp tissue. Fortunately, a team had successfully separated a clonogenic proliferating population of cells from adult human dental pulp, where only sporadic, but densely calcified nodules are produced, and adipocytes were not formed [6]. When they transplanted these dental pulp stem cells (DPSCs) into immunocompromised mice, DPSCs produced a dentin-like structure lined with human odontoblast-like cells surrounding a pulp-like interstitial tissue [6]. And DPSCs represent considerable self-renewal capability and multi-lineage differentiation given the fact that DPSCs could form ectopic dentin and associated pulp tissue in vivo and differentiate into adipocytes and neural-like cells [7]. Also, they are easy accessible, do not cause ethical concerns and can be stored for at least two years. In addition, a chemical medium containing a 1.25% human serum component helps to isolate a morphologically and phenotypically uniform population of DPSCs from dental pulp tissue[8]. However, an experience aimed to study the in-vivo performance and behavior of DPSCs, which were seeded onto 3-D scaffold materials, finally did not acquire the expected dentin-like tissue but resembled to connective tissue after subcutaneous implantation in nude mice for 6 or 12 weeks [9].

2.2.Dental follicle stem cells (DFSCs)

Dental follicle, consisting of mesenchymal cells is surrounding the developing tooth germ, and dental follicle progenitors create periodontal components, such as cementum, periodontal ligament (PDL), and alveolar bone, during the formation of the tooth root [10, 11]. Dental follicle stem cells (DFSCs), as clonogenic cells, also have the ability of multilineage differentiation and could differentiate into cementoblasts, neural-like cells, membrane-like structures and periodontal ligament(PDL)-like tissue[11-14]. And a novel method for biological tooth root regeneration was reported, which apply tert-butylhydroquinone (tBHQ) treatment of scaffolds, derived from treated dentin matrix (TDM) that is seeded with allogeneic dental follicle stem cells (TDM/aDFC) to prevent osteoclastic resorption and produce a tooth root [15]. So DFSCs seem to be a promising choice to restore a tooth root.

2.3.Stem cells from human exfoliated deciduous Teeth (SHEDs)

An accessible approach to separate human postnatal stem cells of high quality was provided by Miura and her team, that is to isolate stem cells from human exfoliated deciduous teeth (SHED)[16]. According to the report, SHED were identified to be a population of highly clonogenic proliferating cells which are able to differentiate into various cell of different types including neural cells, adipocytes, and odontoblasts, similar to DPSCs. Moreover, SHEDs exhibit higher proliferation rate compared to DPSCs and MSCs originated from bone marrow (BMMSCs) among other kinds of MSCs (SHED > DPSCs > BMMSCs)[17].

2.4.Periodontal ligament stem cells (PDLSCs)

The periodontal ligament is a connective tissue derived from dental follicle and neural crest cells and serves important function such as support of the tooth and protection of tooth in a healthy tooth,[10]. Seo and colleagues found that stem cells that have the potential to produce in-vivo cementum/PDL-like tissue are contained in PDL[18]. PDLSCs could as well differentiate into osteoblasts, chondrocytes and adipocytes [19]. A recent study successfully enhanced periodontal regeneration by PDLSC-conditioned medium (CM) transplantation, and also reported that obvious enhancement in periodontal regeneration due to the suppression of the inflammatory response through TNF-α production due to PDLSC-CM[20].

2.5.Induced pluripotent stem cells (iPSCs)

Dr. Shinya Yamanaka's team provided the possibility of creating autologous cell of different kinds from cells simply obtained from individuals without any pain via generating induced pluripotent stem cells (iPSCs) [21]. Autologous cells are extremely difficult to obtain because of its limited sources and additional harm or damage to individuals during the process of acquisition. Therefore, iPSCs may be the most accessible stem cells. Currently, the combination of CRISPR/Cas9 and iPSC technology has enabled the study of underlying inherited diseases at the molecule and cellular level [22]. Maybe genome editing can help iPSCs perform perfectly in dental tissue engineering and especially in whole-tooth tissue engineering.

3. Scaffolds in tooth engineering

Suitable scaffolds in tooth engineering should meet the requirements including mechanical properties, biocompatibility, scaffold architecture, biodegradability[5]. For this part, some widely applied biomaterials will be discussed.

3.1.Collagen

Collagen is the most commonly existing protein that is distributed in soft and hard connective tissues such as bone, cartilage, and skin, which is also the most prevalent structural protein of the dental pulp and overlying dentin matrix [9, 23]. Collagen offers many advantages including biocompatibility, biodegradability and bioactivity as it naturally serves to cell's adhesion, migration and growth. Regarding to its excellent features, collagen has been investigated in depth in many dental tissue regeneration studies. Pulpal tissue-like matrix formation was produced by collagen scaffolds seeded DPSCs in vivo for 6 weeks[24]. A recent study designed and fabricated a 3D printer aiming to assay the collagen printing, and successfully printed a porous mesh of fibrillar collagen [25]. With the help of 3D printing, biocompatible 3D plotted scaffolds composed of collagen can be produced in a lower cost.

3.2.Fibrin

Fibrin formed from fibrinogen is a fibrous and tough protein that plays an important role in the clotting process of blood. There are many advantages of fibrinogen, including widespread distribution in the blood, low price and good biocompatibility. Even though fibrinogen performs well in immune response, its mechanical properties are rather poor, accompanying with rapid shrinkage and degradation. In a case report, platelet rich fibrin (PRF) obtained from patient himself was used to revitalize the patient's necrotic infected immature tooth, and the mechanism that can explain this successful case is probably that the PRF caused proliferation of human Dental Pulp Cells and increased the protein expression activity of osteoprotegerin (OPG) and alkaline phosphatase (ALP)[26]. However, there is unsettle discussion on the absence of consistent therapeutic effects of autologous PRF regarding tissue regeneration, and this might because the growth factors, cytokines, producing methods and applying protocols of PRF are different [27].

3.3.Alginate

Alginate (Alg) is a natural polysaccharide refined from red algae, and it has the advantages of biocompatibility and no toxicity. However, Alg also offers undesirable feature of weak mechanical properties and uncontrollable degradation. A way to enhance the mechanical strength of Alg is to increase calcium density. Calcium density inside the structure of Alg can be increased using a cross-linking agent such as calcium chloride which can increase covalent cross linking[5, 28]. Recently, some studies reported that 3D-printed alginate/gelatin hydrogel (Alg-Gel) scaffold performed better than Alg-Gel scaffold in stem cells' growth and proliferation [29, 30].

3.4.Poly (Ethylene Glycol)

Poly ethylene glycol (PEG) is a polyether compound with great biocompatibility, mild degradation and low immunogenicity in vivo. The molecular weight and concentration of polymer determine its mechanical properties. When PEG hydrogels are loaded with fibrin gels, the composite hydrogel will support rapid vascularized tissue ingrowth, which renders it a good scaffold candidate for the growth of DPSCs and PDLSCs for porous PEG hydrogel offers mechanical support while fibrin is attributed to the formation of vascularized tissue [31].

4. Growth factors (GFs)

Any group of proteins that plays a great role in promoting cellular growth, cellular differentiation, cell division, proliferation and/or healing can be called a growth factor. Growth factor is an important concept in tissue engineering.

4.1.Basic fibroblast growth factor (bFGF)

Among the family of Fibroblast Growth Factor (FGF), FGF2, also called basic fibroblast growth factor (bFGF), is one kind of fibroblast growth factors that involves in cellular proliferation, cell survival, and cell differentiation activities.[32, 33]. In the field of stem cells, bFGF plays an important role in enhancing stemness and control ing differentiation of many stem cells including iPSCs and DPSCs [34-38]. A recent investigation showed apparent stimulation and promotion effect due to SDF-1 and bFGF in periodontal ligament reconstruction, and laid the foundation for connecting periodontal membrane regeneration with stem cell tissue engineering [39]. And when FGF2 is bounded to the fibrin network noncovalently it can provide certain specific bioactivities, the example of which can be seen in the enhancement effect in endothelial cell proliferation[40]. However, there remains controversy of the effect of bFGF on differentiation. Further investigation of the role of bFGF in certain cell types and in different environments is therefore needed[41].

4.2.Platelet derived growth factor (PDGF)

Platelet-derived growth factor (PDGF), as one specific kind among many multifunctional polypeptides, consisting of A, B, C, and D polypeptide chains which form homo- or heterodimeric molecules [42], has positive influence in proliferation inducement of undifferentiated mesenchymal cells. PDGF can also be seen during the process of embryo development, in the development of the neural crest cell, in the patterning of limbs, in mesoderm induction and in mesenchymal-epithelial interactions during the development of organs[43].

5. Summary and outlook

In dental tissue engineering, tremendous progresses have been made, by applying the stem cells such as DPSCs, DFSCs, SHEDs, PDLSCs and iPSCs. Dental hard tissue and soft tissue was successfully regenerated in vivo or vitro. Some scaffold materials including natural and synthetic have been prove promising in tissue engineering. And we discuss some GFs promoting the development of tooth in this article. However, there are still some problems needed to be conquered. For example, the control of the size and shape of the bioengineered tooth is a fundamental requirement because it has direct effect to tooth function in mouth. If a full functional tooth could be regenerated in an economic and convenient way in future, it would be a significant improvement in dentistry.

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