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Dental Applications of Natural-Origin Polymers in Hard and Soft Tissue Engineering

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Abstract: Clinical advances in the treatment of dentoalveolar defects continue to evolve with the introduction of new innovations in regenerative medicine and tissue bioengineering. Recent developments in tissue engineering are aimed at safely and effectively regenerating a damaged or necrotic area by replenishing its cells and increasing surrounding gene expression. Various techniques have successfully given rise to porous scaffolds being used by clinicians to treat the defect and initiate the repair process. Tissue reconstruction using bioengineered scaffolds is advantageous over traditional autografting, since it prevents the instigation of pain and donor site morbidity while ultimately creating both the environment and machinery needed to induce cell proliferation, migration, and reattachment within the affected area. This review article aims to describe and review the available literature regarding the regenerative capacity of natural polymers used for the treatment of dentoalveolar defects. The repair mechanisms, advantages of protein and polysaccharide derivatives, and the potential of stem cell therapy are discussed.

Bone resorption is a progressive process that complicates restoration of edentulous and partially edentulous areas. Bone loss compromises the periodontal health of teeth, complicates placement of dental implants and in advanced cases may cause remarkable changes in facial morphology.

Tissue regeneration in the form of grafting using bone substitutes is used extensively for patient treatment. Every year in the United States, approximately 500,000 bone grafting procedures are performed in dental and medical offices. The market for bone substitutes used in these procedures is valued near 1 billion dollars.
In 2012, the market for biomaterials used exclusively in dental procedures was valued at 222 million dollars, and this number continues to grow.6

With the advent of tissue engineering, specific materials and scaffolds have been produced to effectively deliver synthetic and biological agents capable of tissue regeneration.7-17 Scaffolds must effectively facilitate the early stages of regeneration before transitioning to a natural mode of self-repair.18-26 In addition to delivering regenerative biomaterials, scaffolds seal off the affected area and protect it from infection, restore the extracellular matrix (ECM), and induce proliferation and differentiation of cells composing the four primary types of dental tissue: bone, cementum, connective tissue, and gingival epithelium.27-28 Therefore, scaffolds can be used in various dental applications and preprosthetic surgery including guided tissue regeneration and treatment of periodontal disease,29 osteogenic repair of the alveolar process and bone augmentation procedures to increase volume of bone and restore bony defects prior to endosseous implant surgery, craniofacial bone defect repair,30 socket preservation procedures, and in the treatment of ailing/failing dental implants.31

In the last decade, the use of bioprinted scaffolds composed of natural polymers and stem cells has remarkably allowed for repair of an array of defects while mimicking the human ECM.32 The ECM contains molecules that contribute to the strength and flexibility of the periodontium.33 The presence of growth factors in the matrix is important in the process of regeneration, and the combination of abundant biomolecules and signaling mechanisms within the ECM assists in the differentiation of progenitor cell populations, replenishing the mature cells native to a healthy bone and gingival tissue.34-35 Strong adhesion of cells to ECM proteins holds functional necessity, is vital to tissue integrity, and is a significant goal in restoring the chemical nature of the environment to be restored and augmented.36

The focus of this manuscript is to review materials used in 3D bioprinting of scaffolds, including polysaccharide derivatives, protein derivatives, and stem cells, that can restore the necessary interaction between the cells and matrix in preprosthetic surgery requiring tissue engineering. Nearly 100 papers in journals from both the United States and abroad dating from 1972 until 2015 were used to compile
information regarding the application of each polymer in the regeneration of dentoalveolar and maxillofacial tissues. Papers focusing on biomaterial characteristics and the clinical effects of tissue engineering were analyzed alike and cited to write the manuscript on this multidisciplinary topic.

Polysaccharide derivatives

Chitosan

Chitosan is a biopolymer obtained from chitin, a nitrogen-containing polysaccharide derived from glucose. Chitosan is biocompatible, flexible, and antibacterial and can accelerate wound healing. It can be shaped into various structures, including microspheres, paste, sponges, and porous scaffolds, and therefore, it has the potential to be used in tissue engineering. Lahiji et al noted that chitosan can be successfully used as a template for bone defect restorations, due to its ability to support viable osteoblasts. A study on the effects of chitosan on socket preservation after extraction showed that the bone densities in the middle and apical sections of the repaired socket were significantly greater in chitosan-treated sockets than the untreated, unfilled control group, concluding that it can be used for bone repair in the cases of bone loss. In another study, chitosan was used to restore bony defects in the upper tibia of rats. The results showed that compared to the control, in which chitosan powder was not used to fill the defect, bone repair was significantly expedited in the chitosan group at 1 week and 4 weeks post-surgery. Further attesting to chitosan potential in tissue engineering, Muzzarelli et al demonstrated that chitosan ascorbates produced in the gel form are fit for the regeneration of periodontal ligament (PDL) tissues. They also demonstrated that the chitosan was gradually reabsorbed in hosts with a satisfactory clinical recovery of periodontal defects, where both tooth mobility and pocket depths were significantly reduced.

Because of its biocompatibility and bioactivity, chitosan can be combined with several inorganic materials to make 3D microstructures to be used as scaffolds. Examples of these inorganic materials include bioactive glass, hydroxyapatite, alginate, collagen, and recombinant
vectors. Bioactive glass, as the name implies, has excellent bioactivity and when added to chitosan membranes would promote guided bone regeneration. Mota et al showed that membranes containing chitosan/bioactive glass nanoparticles, in contrast to pure chitosan membranes, were able to stimulate the deposition of an apatite layer, which highlights the advantages of a composite scaffold.

Another inorganic material that can be combined with chitosan is hydroxyapatite, which is known for its biocompatibility and its osteoconductive potential. Kong et al developed a homogenous nanohydroxyapatite/chitosan porous scaffold that possessed better biocompatibility than a pure chitosan scaffold, while also allowing a higher proliferation of cells. In agreement with Kong et al's findings, Zhang et al concluded that a nanohydroxyapatite/chitosan scaffold had better cytocompatibility than a pure chitosan scaffold.

Other materials have been used to enhance the strength of chitosan-based scaffolds. Li et al used alginate to construct a chitosan/alginate composite with significantly enhanced mechanical strength. The chitosan/alginate scaffold allowed seeded osteoblasts to attach and proliferate well in culture without the use of an osteogenic medium and promoted quicker deposition of minerals compared to pure chitosan.

Zhang et al used chitosan/collagen scaffolds to create a porous structure that could be loaded with plasmids and adenoviral vectors encoding human transforming growth factor-β1 (TGF-β1). Human PDL cells were seeded within the scaffold's pores and implanted into athymic mice. It was found that no inflammatory reaction or extrusions occurred, confirming the biocompatibility of this TGF-β1 encoding scaffold. This scaffold also showed the highest proliferation rate as well as upregulated expression of both type I and type III collagen. Moreover, tagged human PDL cells caused surrounding tissues to grow within the porous scaffolds. The study concluded that the chitosan/collagen composite scaffold encoding TGF-β1 is a candidate that could work well for periodontal tissue regeneration.
Cellulose

Cellulose membranes were the first biomaterial employed as surgical barriers for guided tissue regeneration, followed by expanded polytetrafluoroethylene (ePTFE) and some other biodegradable polymers.\textsuperscript{53-55} Cellulose and ePTFE have been introduced with the commercial names of Millipore filter and Gore-Tex as non-resorbable biomaterials for cell-occlusive barriers used for tissue regeneration.\textsuperscript{55}

As a relevant form of cellulose in tissue engineering, microbial cellulose (MC) is a polysaccharide that has been developed from \textit{Acetobacter xylinum}.\textsuperscript{56} In addition to its use in the treatment of periodontal defects, guided-tissue regeneration, and preprosthetic surgery, MC membranes offer many other applications in regenerative medicine, including their use as a graft for dura mater surrounding brain tissues.\textsuperscript{56} MC has been previously used in wound-healing, confirming its promise as a product in the biomaterials field.\textsuperscript{56} MC membranes have been employed as a scaffold material to increase cell attachment.\textsuperscript{57} Wan et al\textsuperscript{58} synthesized a MC membrane coated with hydroxyapatite and found that mesenchymal stem cells (MSC) attached and proliferated onto the MC membrane with a low inflammatory response.\textsuperscript{58}

In vivo studies using MC demonstrated that MC samples did not cause any macroscopic inflammatory responses after 1 and 3 weeks of implantation,\textsuperscript{56} confirming findings from in vitro experiments.\textsuperscript{58} MC has also been used as a physical barrier in periodontal regeneration, to separate incised oral epithelial cells and gingival connective tissue from the treated root surface, allowing PDL cells to proliferate inside the treated area, leading to tissue regeneration.\textsuperscript{56,59}

Another form of cellulose used in tissue engineering is cellulose hydrogels. They have been shown to be biocompatible with connective tissues. In vitro experiments on cellulose hydrogels revealed that this material has potential to enhance osteoblast cell attachments and proliferation for bone tissue engineering.\textsuperscript{60}
Protein derivatives

Collagen

Collagen, an extremely abundant protein and defining component of connective tissue throughout the body, is a potent biomaterial with a vast potential for tissue regeneration. Examples of collagen’s beneficial role during tissue repair include surrounding tissue compatibility, biodegradability, induction of epithelial regeneration, and its low immunogenicity and cytotoxicity. More specifically, due to its low immunogenicity, collagen fosters the growth of a native ECM, is chemotactic for fibroblasts, and induces a cascade by which the internal matrix is further developed.

Collagen has a strong osteoinductive effect, stimulating osteoblast differentiation and proliferation, which would lead to bone growth. Collagen also increases gene expression for morphogenic proteins, such as BMP. To engineer skeletal tissues, a scaffold should contain a significant number of progenitor cells in addition to bioactive agents that can draw in cells with a restorative functionality to the site of damage. Collagen plays a crucial role in facilitating this biological process because it acts as an anchor for the attachment of proteoglycans and glycosaminoglycans, improving the overall mechanical strength and stability of the regenerated tissue. While collagen’s inductive role in BMP expression has demonstrated a strong, notable regeneration of the peri-implant and periodontal defects, previous in vivo studies demonstrate that duration of BMP availability and activity in scaffolds is limited.

The rehabilitative potential of collagen in tissue engineering lies primarily in its use for delivering plasmids and adenoviral vectors. For example, a study examining the combination of adenovirus encoding PDGF-B (AdPDGF-B) with a collagen gel noted an increase in the area of cementum, filling of alveolar defects, and an increase in bone volume. However, the target tissue and type of collagen in the scaffold are important factors to consider because different types of collagen may provide different degrees of biocompatibility, immunogenicity, degradation rates, and different mechanical properties of the scaffold. For example, types I and III collagen are...
known to make up the PDL,\textsuperscript{74} and type IV collagen makes up the basement membrane of junctional epithelium and the epithelial rests of the PDL.\textsuperscript{72}

\textit{Fibrin}

As a natural protein, fibrin is synthesized from fibrinogen, which may be autologously harvested from the human body.\textsuperscript{73} Polymerized fibrin is a main constituent of blood clots and has an important function in the later wound-healing processes.\textsuperscript{74} Due to its natural ability to enhance cellular interaction and follow scaffold remodeling compared to the synthetic scaffolds, fibrin has found a well-established application in various areas of research in regenerative dentistry.\textsuperscript{75} Fibrin provides a biocompatible carrier for biomolecules and presents a substrate for adhesion of tissue-forming cells such as endothelial cells, and fibroblasts, which are engaged in angiogenesis and are responsible for remodeling of the new regenerated tissues.\textsuperscript{76}

Fibrin provides suitable locations for cell-substrate binding, and therefore it mimics the native tissue\textsuperscript{77} and allows for remodeling by cell-associated proteolytic activity.\textsuperscript{78} Fibrin gel has been produced recently with a shape of three-layered human PDL cell sheets and transplanted with a human dentin block subcutaneously into the back of a rat. The results of histological evaluations revealed that the human PDL cells/dentin blocks stimulated a new cementum-like hard tissue on the surfaces of more than 60\% of the samples after 6 weeks of implantation.\textsuperscript{79}

Fibrin glue has also been used with periosteal cell/matrix hybrids to form new bone at heterotopic sites in nude mice. The periosteal cells were mixed with fibrin glue in a syringe, and then the cell/matrix-fibrin glue was injected on the dorsum of athymic nude mice. After 3 months, histological evaluations confirmed the ability of fibrin glue carrier to cause new bone formation.\textsuperscript{80}

Fibrin enhances the mitogenic response of the periosteum during the bone-healing process by trapping and activating platelets that cause the secretion of growth factors, such as platelet-derived growth factor, transforming growth factor, and insulin-like growth factor I.\textsuperscript{81,82} Choukroun et al\textsuperscript{83} developed a fibrin matrix known as a
platelet-rich fibrin (PRF) in order to hold platelet cytokines and cells before releasing them after a predetermined time. Gassling et al. showed that PRF is an excellent scaffold material for proliferation of human periosteal cells, with a great potential for use in tissue engineering; however, the imperative property of PRF is a profit not only due to its biocompatibility, but also because of its lower costs.

Numerous researchers have confirmed fibrin as an appropriate scaffold for adherence, colonization, and proliferation of human mesenchymal stem cells (HMSC). Therefore, using fibrin as an autologous scaffold for periosteal cell or stem cell transplantation and for bone tissue engineering is a promising application; however, its fast biodegradability may be a disadvantage for its use as a shape-specific scaffold in tissue engineering. Thus, optimizing fibrin composition is an area requiring further research to acquire a scaffold system that provides the best shape integrity.

**Stem cells**

Preclinical investigations revealed that stem cell delivery can be used to create consistent and efficient outcomes in managing dental and orofacial defects, so that some clinical trials employing in vitro expanded stem cells have begun or are in progress. The first clinical alveolar bone reconstruction was conducted in 2009 using dental pulp stem cells (DPSCs). This work proposed that a dental pulp stem cells/collagen sponge biocomplex can entirely repair human mandible defects and demonstrated that this cell population could be employed for the regeneration of organs. In 2011, there was an approved clinical study for periodontal regenerative therapy using cell sheet technology. The results of this work indicated that the use of autologous PDL cell sheets and β-tricalcium phosphate (β-TCP) could restore the defects of alveolar bone and PDL tissue.

Due to the lack of native stem cells, tissue regeneration does not naturally occur in bone defects. For this reason, exogenous regenerative materials including in vitro manipulated stem cells are required to encourage the host cell niche and help tissue repair. Stem cells and/or progenitor cells are typically cultured ex vivo and used to treat patients as biological agents. A promising source of stem cells needs to have high proliferation ability, ability to
differentiate into intended types of cells, low antigenicity, and easy manipulation into expanded processing. PDL stem cells (PDLSCs), stem cells from apical papilla (SCAP), stem cells from human exfoliated deciduous teeth (SHED), and bone marrow mesenchymal stem cells (BMMSCs) are the common potential cell sources being employed in dental tissue engineering. These cells are isolated from the dental tissue, bone marrow, and adipose, and then they are proliferated and implanted into the periodontal defect using either biomaterial-free or biomaterial-based scaffolds. Otherwise, the cells may be allogeneic from a cell bank. Recent reports have shown that MSC have been used for the regeneration of PDL, cementum, and alveolar bone in vivo.

BMSCs can be used practically in periodontal tissue engineering. In one study, biodegradable collagen as the biomaterial scaffold supported BMSCs during implantation. The results of another study on the implantation of cultured human PDL stem cells into the produced periodontal defects in rats demonstrated that the stem cells were adhered to both the alveolar bone and cementum surfaces, and there was an indication of the development of a PDL-like structure. Stem cell therapy will need to consider the costs, provide help to patients, satisfy regulatory agencies, meet enhanced stem cell supplies, be covered by medical insurance, and prove lucrative for pharmaceutical companies to get to the final stages of clinical applications.

Summary

The applications of natural polymers have proven to be extremely potent in preprosthetic surgery and in periodontal tissue regeneration. In every substance, the biocompatibility and induction of cell migration and attachment are essential for a successful repair. Although minor differences exist between the polysaccharide and protein derivatives, the overall goal is that scaffold implantation and cellular activity guides regeneration of all components of the periodontium. These biological agents target the soft tissue and underlying hard tissue, and the addition of a growth factor can further stimulate vascularization and osteoprogenitor differentiation, for example, to repair the resorbed bone. The mimicry of a native matrix
allows for normal biochemical processes. A leading candidate for alternative treatment, stem cell therapy, was also examined. In vitro testing has shown that stem cells hold great promise for the future of regenerative dentistry. All the natural origin polymers covered in this mini review (Table 1) are the most widely recognized treatment agents, and we have demonstrated comprehensively, with the support of material characteristics and clinical applications (Table 2), that the potential for each can induce repair of periodontal and craniofacial defects.

**Table 1. Summarized material characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Chitosan</th>
<th>Cellulose</th>
<th>Collagen</th>
<th>Fibrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advantages</td>
<td>Biocompatible</td>
<td>Enhanced cell attachment</td>
<td>Low immunogenicity</td>
<td>Mimics native tissue</td>
</tr>
<tr>
<td></td>
<td>Antibacterial</td>
<td>No macroscopic inflammatory</td>
<td>Strong osteoconductivity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wound healing</td>
<td>effect</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Poor mechanical strength</td>
<td>Cytotoxic effects</td>
<td>Poor mechanical properties</td>
<td>Rapid biodegradability</td>
</tr>
<tr>
<td></td>
<td>Bioactive glass</td>
<td>Hydroxyapatite</td>
<td>Hydrogel</td>
<td>Collagen</td>
</tr>
<tr>
<td>Suggested</td>
<td>Hydroxyapatite</td>
<td>Chitosan</td>
<td>PLGA</td>
<td>Polyurethane</td>
</tr>
<tr>
<td>materials to form the potent composite</td>
<td>Alginate</td>
<td>Pectin</td>
<td>Hyaluronic Acid</td>
<td>Hydroxyapatite</td>
</tr>
<tr>
<td></td>
<td>Collagen</td>
<td>Hygrophil</td>
<td>Hyroxyapatite</td>
<td>Calcium Phosphate</td>
</tr>
</tbody>
</table>

**Table 2. Summarized clinical applications**

<table>
<thead>
<tr>
<th>Clinical mode of delivery</th>
<th>Chitosan</th>
<th>Cellulose</th>
<th>Collagen</th>
<th>Fibrin</th>
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<tbody>
<tr>
<td></td>
<td>Nanofiber membrane for GTR</td>
<td>Surgical barrier placement for GTR</td>
<td>Porous sponge implantation</td>
<td>Fibrin gel</td>
</tr>
<tr>
<td></td>
<td>Sponge</td>
<td>Injectable gels</td>
<td>3D scaffold</td>
<td>Fibrin glue</td>
</tr>
<tr>
<td></td>
<td>Electros spun nanofibers</td>
<td></td>
<td></td>
<td>Fibrin microbeads</td>
</tr>
<tr>
<td></td>
<td>Bioactive coating</td>
<td></td>
<td></td>
<td>3D scaffold</td>
</tr>
<tr>
<td>On-site advantages</td>
<td>One of the most potent curative materials used</td>
<td>Has a unique nanofibrillar composition that mimics a perfect matrix for regeneration</td>
<td>Extremely biocompatible</td>
<td>Instigates hemostasis and tissue bonding</td>
</tr>
<tr>
<td></td>
<td>Enhanced proliferation of human PDL cells</td>
<td>Allows for rapid healing</td>
<td>Controls preodontoblast rearrangement and their adhesion to pulp tissue</td>
<td>Minimizes blood loss</td>
</tr>
<tr>
<td></td>
<td>Enhanced angiogenesis</td>
<td>Serves as a barrier against infection</td>
<td>Widely used in maxillofacial and</td>
<td>Combats bacterial infection at the site of injury</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Successful use of fibrin-based</td>
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<td></td>
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<td>alveolar bone grafting</td>
<td>scaffold in regenerative endodontics observed as well as regeneration of peri-implant defects, socket preservation, and healing of sinus perforations</td>
</tr>
</tbody>
</table>

Complications

- Uncontrolled dissolution in some cases
- Inflammatory reaction noted in several in vivo studies for bone engineering
- Lack of durability under compression of maxillofacial bone
- Losses capacity to maintain space in humid environment
- Animal allograft of collagen could transmit disease
- Rapid shrinkage remains a serious concern for clinicians
- Requires use as a composite scaffold to overcome low mechanical strength

Study type

- In vitro
- In vivo
- Clinical trial

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