REVIEW ARTICLE

Bone's smart envelope - The periosteum: Unleashing its regenerative potential for periodontal reconstruction

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Abstract

Prime aim of periodontal therapy is to set up a state of periodontal health with pocket elimination and attachment level gain, preferably by periodontal regeneration. Various tools/techniques have been proposed for this purpose. But, the quest still continues. In this context, the periosteum offers an attractive option for periodontal regeneration. The rationale for the use of periosteum lies in its anatomy and its physiologic functions during normalcy. Periosteum contains the desired stem cells and progenitor cells that are capable to produce periodontal tissues. Periosteum has high vasculo-proliferative and neuro-trophic activities. In addition, it is easier for the dentist to harvest the periosteum for clinical use. It can be harvested from adjacent to the surgery site in sufficient amounts. Even though it has many advantages, there is only limited research for exploiting the regenerative potential of periosteum for periodontal regeneration so far. Conversely, in the medical field, the periosteum is extensively used and proved to be promising. Hence, the aim of this paper is to discuss the regenerative potential of periosteum and various available tools and techniques to harness it for periodontal regeneration.

Keywords: Periosteum, periodontal regeneration, stem cells, tissue engineering

Introduction

A plethora of research has been done in the past and even today the search for the "holy grail" of periodontal regeneration continues. Many regenerative approaches have been applied in an attempt to regenerate the lost periodontium with varying degrees of success.[1-3] These approaches include surgical placement of bone grafts or biologic agents (growth factors or enamel matrix derivatives) or their combination with or without a barrier membrane or tissue engineering approaches including stem cell research or other pharmacological agents. While the notable regenerative ability of the periosteum was documented in reports more than a century ago, only in the past decade scientists have carried out mechanistic examinations of periosteum's regenerative potential. As of now, in the regenerative armamentarium of periodontology the periosteum has not got its deserved place.

Periosteum: Rationale for Its Use

Anatomy

From a structural outlook, periosteum is a composite biomaterial,[4] enveloping external surfaces of all the bones except in the areas of bone articulations and tendon attachments.[5] Periosteum is composed of three zones. Zone 1, also known as the cambium layer (closest to the bone) basically contains osteoblasts, osteoblast progenitor cells, and multipotent stem cells. Zone-2, also known as matrix layer basically contains fibroblasts, fibroblast progenitor cells, and dense vascular plexus. It is because of this matrix layer of the periosteum is highly vascular. Zone-3, also known as collagenous layer is the outermost layer of the periosteum, and it basically contains dense collagen fibers. Zone-2 and Zone-3 are together known as fibrous layer. So in broader terms, the periosteum consists of two main layers, an inner cambium layer and an outer fibrous layer [Figure 1].[4,6,7] More than 90% of the cells in periosteum are fibroblastic in appearance. A prominent subpopulation of these cells has been identified as mesenchymal stem cells (MSCs) and progenitor cells.[8-10] However, the cell population of periosteum is diverse, potentially containing fibroblasts, fibroblast progenitor cells, osteoblasts, osteoblast progenitor cells, multipotent stem cells, pericytes.[6,11,12] It has been found that the periosteal stem cells and progenitor cells can differentiate into osteoblasts, fibroblasts, chondroblasts, adipocytes, and skeletal myocytes.[13]

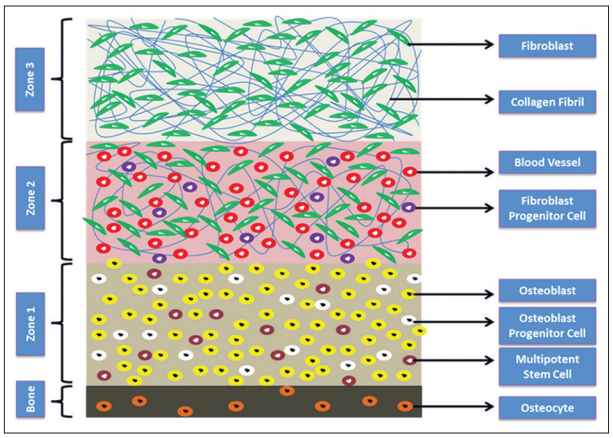

Figure 1: Schematic presentation of three discrete zones of periosteum, (Adapted from reference no. 4: Chang H, Knothe Tate ML. Concise review: The periosteum: Tapping into a reservoir of clinically useful progenitor cells. Stem Cells Transl Med 2012;1:480-91)

A unique property of these periosteal stem cells and progenitor cells is that in all age groups, they retain their ability of differentiation into a variety of cell lineages. However, it has been found that their capacity to differentiate in the direction of chondrogenic and adipogenic lineages reduces with age.[8,14,15] Pericytes have also been recognized as a distinct cell population in periosteum. They may have a function of re-vascularization and promotion of bone formation, but their role in periosteal bone formation is deemed minimal at present.[16]

Physiologic functions of periosteum in normalcy

In a fully developed state of the alveolar processes, periosteum serves several important functions: (1) It forms a variably rigid attachment for gingiva to bone because of its density and numerous fibrous insertions into bone. It also provides attachment linkage of the alveolar mucosa essentially to cortical bone of alveolar processes and jaws. These do not appear to be as tenacious as seen in case of attached gingiva to subjacent bone. The Sharpey's fiber insertions here appear to be frailer, to be fewer with greater spacing over the cortical surface and have shallower penetration into bony cortex, (2) it carries principle vascular supply and lymphatic drainage for alveolar mucosa and gingiva. Additionally, it contributes to the vascularization of the adjacent bone, joining with branches of interseptal vessels to provide liberal blood supply to cancellous bone. Many of blood vessels coursing from periosteum to cortical bone are of minute caliber. They arise from an extensive and delicate vascular plexus located in the matrix layer of the periosteum and enter bone's haversian canals directly joining with an elaborate microvascular arcade within cortical bone. Fluids also diffuse from periosteum's extravascular connective tissue, as a transudate into bone, principally into and through a canalicular maze. In this manner, bone matrix is hydrated, mineral is added to and withdrawn homeostatically within bone, and osteocytes receive nutrients and release wastes. When osseous septa are thin and largely or completely composed of compact bone, the periosteal blood supply may constitute the principle one to the septum, (3) it contains prominent neural fibers, generally myelinated that liberally arborate into gingiva, mucosa, bone marrow and periosteum itself, (4) periosteum responds, generally in an appositional manner by osteogenesis and fibrogenesis through its multipotent stem cells in the inner cambium layer, to exigencies derived from overlying gingiva and mucosa. Thus, the inflammatory response in marginal gingiva is usually contained and restricted from progress through attached gingiva to the alveolar bone by the capacity of periosteum to repair in the face of irritation.

Despite its routine performance of these essential functions, periosteum is a "sleeping giant" only mildly stirred on a generative level by functional stimuli and gingival inflammatory processes. When surgical injury assails periosteum, it quickly springs to action, both appositionally and resorptively. In repair of periodontal wounds, the periosteum has a marked capacity for the following: (1) Multipotent stem cells in periosteum differentiate into osteogenic and fibrogenic cell lineages, (2) secretion of protein-polysaccharide complexes of the matrices of connective tissue, bone and blood vessels, (3) endothelial proliferation and the formation of new blood and lymphatic vessels, (4) after both partial-thickness and full-thickness flap surgery periosteum responds by providing literally a "river of regenerative tissues" moving centripetally into the wound, (5) periosteum's vasculature responds rheologically and by dilatation and permeability to provide hydration and nutritive materials to adjacent tissues and itself in the healing process. This is especially apparent in the sustenance of free gingival autografts and partial-thickness pedicle grafts that are largely or completely dependent on periosteum of the recipient site during the 1st week after grafting. Periosteal vessels serve as progenitors for new blood vessels required to link to those of the graft. A comparable lymphatic anastomoses occur, assisting substantially in removing exudate and cellular and other debris from the graft, (6) periosteum is a "springboard for nerve regeneration" into overlying gingiva, mucosa, or graft and into subjacent bone.

It has been noted that the periosteum, in particular, the inner, relatively quiescent cambium layer is stimulated to osteogenic, fibrogenic, vasculo-proliferative, and neuro-trophic activities by surgical trauma.[17] Since, periosteum offers a rich source of stem cells and progenitor cells, it has high vasculo-proliferative and neuro-trophic activities, and therefore, the regenerative potential of periosteum is enormous and should be harnessed for periodontal regeneration.

Harnessing the regenerative potential of periosteum

Remarkably, Duhamel can be regarded as the first researcher to study the osteogenic capacity of periosteum. He observed that agitation of the periosteum results in the formation of new bone.[18] More than a century later, Ollier found that the periosteal tissue when transplanted was able to form new

bone *de-novo*.[19] One of the oldest experimental reports to demonstrate periosteum's osteogenic capability was by Urist and Mclean. They transplanted periosteum to the anterior chamber of the eye in rat and reported that periosteum formed bone.[20] Melcher reported the formation of new bone in parietal bone defects in rats and was laid down by periosteum that was neither previously elevated nor disturbed.[21] Since then a number of experiments were attempted to "tap" the periosteum for tissue reconstruction with some attention in the field of dentistry also.

The necessity of a graft, which has its own vascular supply, and can be acquired in adequate amounts from adjacent to the defect site and has a capacity for providing periodontal regeneration, is yet to be fulfilled. In this context, the periosteum can prove to be a very close option. In addition, recently the periosteal cells have been found to produce vascular endothelial growth factor that helps in angiogenesis and wound healing [Figure 2].[22] The application of periosteum in periodontology is not so new and started with the researches in which the periosteum harvested from the palate was used as a barrier membrane to successfully treat furcation and intrabony defects.[23-25] Following this many techniques were introduced and attempted to take the advantage of periosteum's regenerative potential for achieving periodontal regeneration.

Gaggl *et al.* proposed a periosteum eversion technique or perioplasty to cover the denuded roots. Patients with severe gingival recessions were successfully treated with this technique. The technique involves the reflection, eversion and coronal repositioning of periosteum from a full-thickness flap that is then placed over the denuded roots. The basis of tissue regeneration here is same as the conventional connective tissue graft.[26] A case series describes the successful treatment of severe gingival recessions with this technique.[27] Both the case series concluded that the periosteum eversion technique is suitable for the treatment of gingival recessions with a clear improvement in aesthetics.[26,27] Further studies are required to assess the effectiveness of the technique.

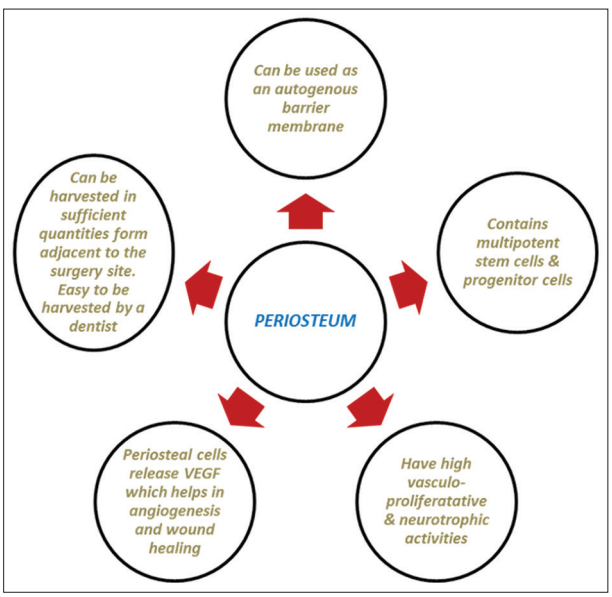

Figure 2: Why periosteum should be harnessed for periodontal regeneration??

Steiner *et al.* introduced an inverted periosteal graft (IPG) technique. In the IPG, the normal anatomy of the periosteum is reversed or inverted i.e. the cambium layer (containing osteoblasts, osteoblast progenitor cells and multipotent stem cells) cover the fibrous layer (containing fibroblasts, fibroblast progenitor cells) which is placed immediately adjacent to the root surface. The cells having capability to produce cementum and periodontal ligament (i.e., fibroblasts, fibroblast progenitor cells) are the immediate cells put forward to the root surface, while osteoblasts, osteoblast progenitor cells and multipotent stem cells lie immediately outside the fibroblasts and fibroblast progenitor cells and produce the osseous counterpart. Thus, IPG seats the appropriate cells in the correct site for the periodontal regeneration.

There are two different techniques of IPG. One technique is to reflect a partial-thickness flap, leaving the periosteum on bone from which it is reflected, inverted, coronally advanced and placed over the periodontal defect. The second technique involves the reflection of full-thickness flap, lifting the periosteum off the flap, inverting and coronally advancing it followed by placing over the defects. It is clinician's decision to opt any of the two techniques because both the techniques achieve the same objective.[28]

Gamal and Mailhot illustrated a marginal periosteal pedicle (MPP) graft to be used as a biologic barrier membrane for the treatment of deep angular 2- and 3- wall infrabony defects. They reported a significant improvement in clinical and radiographic parameters of deep infrabony defects. MPP graft consists of a facial partial-thickness and a lingual full-thickness flap followed by the creation of a facial marginal periosteal strip adjacent to the defect. Periosteum is then separated laterally on the facial aspect, keeping it attached to its base to be used as a pedicled biologic barrier membrane.[29,30] One study reported significant improvements in clinical and radiographic parameters for localized two-wall intrabony defects employing MPP graft with alloplasts.[31] Further research is needed to evaluate the effectiveness of MPP grafts as autogenous barrier membranes.

Tissue engineering is a new outlook for tissue regeneration by utilizing appropriate cells, bioactive signaling molecules and a scaffold (which is made up of a bio-degradable material either natural or synthetic.[32] Stem cells are being employed clinically for the regeneration of lost or missing tissues re-establishing natural form and function in many disciplines of medicine and dentistry. MSCs are simply cultured, multipotent, immune-privileged cells, making them perfect candidate option for tissue engineering.[33,34] A huge body of literature describes the use of MSCs in regeneration of tissues. Many sources of MSCs have been identified, i.e., bone marrow, adipose tissue, umbilical tissue, muscle, periosteum etc. Bone marrow is the most widely used source of MSCs. But, the morbidity at the donor site and the complexity in obtaining sufficient amounts of tissue to extract MSCs still remains barriers for their clinical application.[4]

Periosteum has been tapped and recognized as a reservoir of clinically useful stem cells and progenitor cells. As periosteal

cells possess robust potential to differentiate into osteoblasts, fibroblasts, chondroblasts, adipocytes and skeletal myocytes,[13] periosteum can offer a unique cell source for periodontal tissue engineering. In addition, periosteum can be harvested from many convenient sites to extract MSCs, such as sites adjacent to surgery. Regarding the donor site, it is simpler in the dental set-up to harvest periosteum as compared to more widely used bone marrow-derived MSC (bMSCs), because dentists often access the mandibular periosteum during routine oral and periodontal surgeries. Many researches have reported periosteum-derived stem cells (PDCs) to be as good as, if not superior to bMSCs regarding healing and regeneration of bone.[35-37]

The use of PDCs for periodontal tissue regeneration is still in its infancy. Studies using PDCs with different scaffolds have shown considerable bone formation in a variety of bone defects.[35,38] One study compared the PDCs with the bMSCs (maxillary tuberosity) and found that both the cell types depicted similar phenotypes and generated bone upon ectopic transplantation *in-vivo*.[39] The collection quantity of PDCs required for tissue regeneration is yet to be determined. Still the PDCs are under continuous research.

With the advancements in tissue engineering, Mizuno *et al*. illustrated a technique to regenerate periodontal defects using autologous membranous cultured periosteum (CP) or human CP (HCP) sheets. They reported considerable regeneration of the bone defects. Regarding the technique, a prior surgery is required before actual regenerative surgery to harvest a piece of periosteum (usually from the posterior mandibular body). The periosteum specimen is then placed directly onto a culture dish in a pre-defined culture medium: 10% foetal bovine serum, 25 mg ascorbic acid, antibiotics (penicillin [100 IU/ml], and streptomycin [100 mg/ml]), and an antifungal agent (amphotericin-B [250 ng/ml]) and is incubated at 37°C; 10% CO_2. Culture medium has to be changed every 2-3 days. The periosteum samples are incubated until the cells form a sheet-like structure (4 weeks approximately).[40] Several reports have showed the efficacy of HCP sheets in the treatment of periodontal intrabony defects. All the cases showed significant improvements in clinical and radiographic parameters of the defects.[32,41-43]

One of the distinctive features of this technique is that the cell scaffold is autologous which eliminates the risk of host-immune responses to more commonly used exogenous scaffolds (polyglycolic acid or polylactic acid) often leading to graft rejection. Periosteal cells, during the formation of CP produce extracellular matrix that acts as a natural scaffold. Another advantage is the presence of stem cells and progenitor cells in CP. So, having a membranous structure with robust regenerative potential owing to the presence of stem cells and progenitor cells serves two purposes in regenerative therapy, enhancing the therapeutic efficiency. Moreover, dispersion cell cultures from periosteum require exogenous growth factors to attain a robust regenerative capacity. These exogenous factors are not needed with CP to uphold the regenerative potential of cells. Further research (including histologic evaluations) is required to precisely understand the role of CP in periodontal regeneration.[32]

Conclusion

To summarize, the periosteum has desired stem cells and progenitor cells, high vasculo-proliferative, and neuro-trophic activities, therefore, the regenerative potential of periosteum is enormous. Various surgical and/or tissue engineering approaches have been proposed to exploit this capability. PDCs are under continuous research using different types of scaffolds, in an attempt to regenerate periodontium. In addition, the designing of CP has become a dynamic research field in the past 6-7 years. Further research is required, using or combining a variety of techniques to ensure development in clinical usage of periosteum, and documentation of protocols is the key to success.

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