Regenerative Techniques in Periodontology

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ABSTRACT:

Once upon a time the regeneration and revival of lost oral tissues e.g. periodontium, bone and teeth etc. seemed to be a dream. Current era in dentistry brings with it the key to many doors which have the powers to transform fantasies to reality. Regenerative dentistry is the direction every eye is looking towards these days. This review article aims to give the reader an insight into the most happening progressions pertaining to regenerative periodontology in the most recent times with some directions for future.

KEYWORDS: Regeneration, Periodontium, Bone.


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INTRODUCTION

Regenerative therapy is no doubt a reality! In this era of Hi-tech dentistry it occupies one of the greatly explored aspects of both clinical and laboratory dental medicine. The last few decades have shown an interesting paradigm shift towards procedures directed into conservation, rejuvenation and/or replacement of missing/lost or endangered oro-dental tissues. It has been quite an intriguing, thought provoking and interesting journey to have moved progressively based upon evidence from xenografts, allografts, alloplasts and autografts alone, to the era of growth factors (GF), proteins and stem cells.

We can broadly categorize the use of ongoing regenerative techniques into three main specialties of dentistry namely periodontics, endodontics/conservative dentistry and oral and maxillofacial surgery. Detailed insight into all specialties would be out of scope for this review however the following discussion will focus on the most practically happening periodontal regenerative techniques at the current time concentrating on the biological mediators of regenerative therapy and stem cells and provide some directives towards future of regeneration in periodontology.

All efforts these days are directed towards either the possibility of re-growing the entire periodontium related to an individual tooth or group of teeth or repairing each individual constituent (periodontal ligaments, cementum, bone or gingiva) as close as possible to the lost natural counterpart. Both require the utility of biomaterials, scaffolds, stem cells, and/or growth factors. Biomaterials and scaffolds have been talked about in many publications however the utilization of the latter two techniques are scarce in literature.

The following discussion therefore will be divided into two main parts:

- Advances in Growth factors, signaling molecules and cytokine therapies.
- Advances in Stem cell techniques.

GROWTH FACTORS, SIGNALING MOLECULES, CYTOKINES AND GENE THERAPY

1. Autologous Platelet Products

These products are biological factors extracted from and utilized in the same patients. The main aim behind these techniques is that platelets being a natural source of growth factors are utilized as tools for promotion of regenerative process. These techniques/products could be used solely or in combination with scaffolds like collagen membranes, bone substitutes etc. and include the following:

1.1. Platelet Rich Plasma (PRP)

This is an autologous product derived from patient’s own blood and contains high concentration of platelets suspended in a small amount of plasma. In contrast to Normal platelet count in blood which ranges from 150,000 to 300,000/ µl, PRP by definition contains 1000,000 platelets/µl in 5 ml
volume of plasma. The main idea behind a solution with concentrated platelets is that alpha granules in the platelets contain growth factors which are released by platelet activation initiated by thrombin, calcium chloride or collagen. The released growth factors include platelet derived growth factor (PDGF), transforming growth factor β1 (TGF-β1), insulin-like growth (IGF), vascular endothelial growth factor (VEGF), fibroblast growth factor and epidermal growth factor (EGF), which promote tissue healing and repair of damaged periodontal tissues, modulate inflammatory process and promote angiogenesis.

The formation of PRP includes collection of patient’s blood in a vacutainer/ collection tube containing anticoagulant. Blood is mixed with anticoagulant and the vacutainer tube is then subjected to two step centrifugation process. In the first centrifugation RBC’s are separated from plasma and in the second centrifugation the platelets and leukocytes are separated from a cellular plasma also known as platelet poor plasma. The advantage PRP holds is the ready provision of growth factors (GF) to the site of defect. However as the release is immediate and in high quantities, there is a concern that the GF can be lost without much effect. Therefore a combination of bone substitutes and PRP is more advantageous in sustaining and retaining GF levels to the site of interest.

1.2. Plasma Rich in Growth Factors (PRGF)

This technique has been projected as one step ahead of PRP in respect to duration and sustenance of release of GF. Also the concentration of White Blood Cells (WBCs) is nonexistent in PRGF as compared to PRP. The main difference in the preparation includes onetime centrifugation for PRGF and addition of calcium chloride for activation leading to formation of a gel. The advantage of this gelatinous substance is that it increases the duration of growth factor release. PRGF can be used in a variety of ways i.e. as supernatant, liquid coating of dental implant surfaces, as scaffold gel or as elastic hemostatic fibrin.

1.3. Platelet Rich Fibrin (PRF)

This is a fibrin membrane consisting of a collection of immune and platelet concentrate. The PRF membrane has three fold benefits in that it provides a platform for development of angiogenesis, wound coverage and immune support at the site of interest. The technique for PRF generation is much easier as compared to the previously mentioned methods. It has the advantage of exclusion of addition of any sort of activator or anticoagulant and therefore is less technique sensitive. There is a handsome constituency of leukocytes in PRF which release cytokines, and therefore PRF acts as a double edge sword by regulating immune process on one hand and promoting bone and tissue regeneration on the other. The biggest advantage PRF has is that the duration of release of growth factors is enhanced up to several days as compared to PRP and PRGF. In addition to this the PRF membrane has better mechanical properties than PRGF membrane.

2. Enamel Matrix Derivatives

These proteins are available in the market by the name Emdogain since the past nineteen years. It is mainly used for periodontal regeneration. The main constituents of Emdogain include enamel matrix derivatives (mainly amelogenins isolated from developing porcine teeth), water and propylene glycol alginate (carrier). Its use and effectiveness is backed by considerable evidence however it faces some religious issues in countries which do not use porcine products for health care purposes. Moreover in a fairly recent Cochrane review it was summarized that, although there are less post-operative complications, post one year application Emdogain has neither been able to save more compromised teeth as compared to the other regenerative methods nor has been able to demonstrate patient’s perceived esthetic improvement one year post procedure. The techniques of using Emdogain is simple. After open flap debridement and root surface conditioning, the gel is applied directly to the denuded root and flap is repositioned.

3. Recombinant / Synthetic Products Produced by Virtue of Gene Therapy

As opposed to their autologous counterparts there are a few commercially available products manufactured using recombinant (genetic) technologies. One of the uses of gene therapy is to introduce vectors into the target cells which are then programmed to yield the required protein or growth factor. These provide a source of one growth factor/protein in high potency to fulfill specific regenerative needs.

3.1. Recombinant Human Platelet Derived Growth Factor (rhPDGF)

PGDF is a potent cytokine with the ability to mediate wound healing and regeneration. This is also released by platelets as mentioned above, however the concentration available varies depending upon the platelet product and its generation technique used. Commercially a genetically engineered Food and drug Administration (FDA) approved product available in the market is GEM-21S (growth-factor enhanced matrix) which contains 0.5 ml (at a concentration of 0.3mg/ml i.e. 0.15mg) of PDGF along with beta tri calcium phosphate (β-TCP) 0.5 cc and is indicated for use in intra bony defects, furcation defects and gingival recession associated with periodontal defects.
3.2. Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2)

This is one of the most happening and researched upon product these days in my humble opinion. It belongs to the transforming growth factor β (TGF β) family. It regulates the differentiation of stem cells located in the bone tissues. The main reason for its popularity is its osteoinductive nature. Recombinant human BMP-2 (rhBMP-2) is available in the market by the name of Infuse an FDA approved product from Medtronic. The Infuse kit is available in various sizes from 0.7 cc to 8.0 cc and consists of sterile water vial/vials, absorbable collagen sponge, and vial/vials of lyophilized rhBMP-2. The sterile saline is mixed with lyophilized rhBMP-2 and the absorbable collagen sponge is dipped in the resulting solution. The BMP dipped collagen sponge is then placed in the defect. This product has provided with more than satisfactory results to be considered a promising solution for bone regeneration.

3.3. Recombinant Human Fibroblast Growth Factor-2 (rhFGF-2)

Recombinant human Fibroblast growth factor (rhFGF-2) has been reported to exert potent angiogenic and mitogenic effects on mesenchymal cells. To date very few randomized control trials have been carried out utilizing rhFGF-2. A phase II trial previously conducted did not reveal any significant findings. However a recently published phase III study provides some more promising results, however post marketing stage of this product in the near future will enlighten us more about rhFGF-2’s efficacy and capability in periodontal regeneration.

3.4. Recombinant Human Growth Differentiation Factor-5 (rhGDF-5) or BMP-14, rhBMP-7 and rhBMP-6

These are other potential products undergoing animal trials and would be commercially available in the near future for oral and maxillofacial purposes.

ADVANCES IN STEM CELL TECHNIQUES

In the quest for approaches to restore oral structures to their normal function and form, and possibly to engineer the entire tooth and periodontium, novel technologies have come up in the form of stem cell tissue engineering techniques.

1. Stem Cells Types and Utility

These are unspecialized and immature cells with self-renewal programmability potential and the ability to differentiate into a variety of cell lines. Due to their unique traits, stem cells have gained popularity in tissue engineering to regenerate or replace missing or damaged tissues and/or organs.

The two main sources of stem cells in dentistry are adult stem cells and pluripotent stem cells. The latter includes embryonic stem cells and induced pluripotent stem cells (iPS) Pluripotent variant are the most promising, having the ability to develop into all cell types from all germ layers in contrast to adult stem cells which can only differentiate into limited cell types.

1.1. Adult Stem Cells

There are many variants of these somatic or postnatal stem cells depending upon the specific ability of differentiation and include:

- Mesenchymal stem cells or mesenchymal stromal cells (MSCs)
- Bone marrow derived MSC (BMSCs)
  - From iliac crest
  - From orofacial bones
- Dental tissues derived stem cells
- Epithelial stem cells
- MSC-like cells: These are called MSC-like because they have similar phenotypic characteristics of BMSCs and include:
  - Dental Pulp Stem Cells (DPSC)
  - Stem cells from human exfoliated deciduous teeth (SHED)
- Periodontal ligament stem cells (PDLSCs)
- Dental Follicle Stem Cells (DFSCs)
- Tooth Germ Progenitor Cells (TGPCs)
- Stem cells from apical papilla (SCAP)

Oral Mucosa derived stem cells/ Gingiva derived Mesenchymal Stem Cells (OMSCs/GMSCs)

- Periosteum derived stem/progenitor cells
- Salivary gland derived stem cells
- Adipose tissue derived stem cells (ASCs)

A detailed account of each of the above would require another review as it is not possible to accommodate the details they deserve, in current manuscript. Moreover the above mentioned adult stem cell techniques are in their infancy and it will take considerable amount of time for all to reach the clinical settings. Currently only periodontal...
ligament stem cells (PDLSCs) have been introduced in the clinics and are discussed below.

1.2. Pluripotent Stem Cells

1.2.1. Embryonic Stem Cells (ES Cells)

ES are extracted from undifferentiated inner mass of blastocyst, which is an early stage of embryonic development post fertilization. This is the main reason for major ethical and moral concerns whenever the topic of ES cell extraction from human beings comes up. Therefore ES cell use in regenerative dentistry is still a grey area with unpredictable future.

1.2.2. Induced Pluripotent SC (iPS Cells)

When human or mouse somatic cells undergo nuclear reprogramming by introduction of certain genetic transcription factors, they can be transformed to embryonic state thus unlocking pluripotency. These induced cells then act similarly to ES cells. Ever since their discovery, iPS cells have been the research focus of regenerative medicine and dentistry. To the dentist advantage luckily the oral cavity is a unique and rich source of stem cells. iPS have been successfully generated from SCAP, DPSCs, gingival fibroblasts and Periodontal ligament fibroblasts.

Induced pluripotent stem cells have great potential in regenerative dentistry in the upcoming future however their clinical utility currently is limited to animal models.

CURRENT PRACTICAL SUITABILITY OF STEM CELLS IN CLINICAL DENTISTRY

Apart from the ability of differentiation into specific target tissues, and ease of collection and preparation, in order to ensure patient safety in regenerative dentistry, complete control of cellular fate is a mandatory requirement for all stem cells as well. Based upon this only adult MSCs currently seem to have a convincing clinical potential. Initial case reports and randomized control trials (RCTs) utilizing stem cells for periodontal regeneration are coming up.

The autologous periodontal ligament stem cell technique reported in very recent literature involves prior extraction of third molars from subjects scheduled to receive stem cell treatment for periodontal defects. Post Extraction, production of single cell suspensions is carried out by cell isolation process, which involves separation of Periodontal ligament (PDL) cells from the roots followed by digestion, straining and centrifugation processes. The cells then undergo culture and cell characterization procedures, which are followed by creation of cell sheets. Once the PDLSC sheets are ready for use, freshly prepared Bone particles e.g. Bio-Oss® etc. (in a concentration of 0.25g/sheet) are sprinkled over each sheet to be used. The periodontal ligament stem cell sheets are then rolled up to pack the bone particles and introduce into the defect.

Although the results of Chen and co-worker’s RCT showed no significant difference between the control group and stem cell group for pocket depth fill, the emergence of such clinical trials are affirming and encouraging for us to start preparing for the future of stem cell use in clinics.

We must therefore not hesitate to incorporate the above mentioned techniques in our daily practices as soon as they are available, but after achieving adequate skills through continuing education programs. The utility of regenerative techniques might, not only, serve to provide the patients with comparatively non-invasive treatment plans but also bring more and more promising results as the use becomes widespread. We are therefore looking towards a future that would be the beginning of the end of material based therapies and start of stem cell based regenerative therapies.

CONFLICT OF INTEREST

Declared none.

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Declared none

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