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SCOPE OF CELL SHEET ENGINEERING IN PERIODONTICS – A NARRATIVE REVIEW

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ABSTRACT

Tissue engineering including stem cell therapy is an emerging technique used for the regeneration of lost tissues in Medicine and Dentistry. Tissue engineering aims to engineer tissues by selecting optimal concentration of cells which acts as biological mediators and selectively enhance the chances of increase in cell matrix in periodontal defects. It helps in regeneration but has drawbacks such as isolating the correct type of cells and preparing a conducive environment for their growth. To overcome the limitations of present-day regeneration techniques, cell sheet engineering is gaining popularity. Cell sheet engineering offer several advantages over conventional stem cell engineering such as avoiding host inflammatory response, creation of extracellular matrix with intact cell-to-cell junctions, and escaping enzymatic degradation as these are thermo-responsive. Cell sheets appear to have a promising role in periodontics due to their ability to regenerate a variety of tissues such as gingiva, enamel, dental pulp, periodontal ligaments cells, cementum, alveolar bone, peri-implant tissue, and sections of the temporomandibular joint. In this review, tissue reorganization by using cell sheet engineering will be discussed along with sources of cell sheets, steps in preparation, and types of cell sheets both used in-vivo/in-vitro studies.

KEYWORDS: Periodontics, Regeneration, Stem Cells.

INTRODUCTION

Periodontology is a field of dentistry that deals with tooth-supporting structures including gingiva, periodontal ligament, cementum, and alveolar bone. Periodontitis is the most common plaque-induced infection affecting these tissues that can destroy the tissues of the periodontium and affects individuals older than 30.[1] As the severity increases, bone tissue destruction occurs in the form of defects that may be crater-like and surround the roots of the teeth. These infra-bony defects pose a therapeutic challenge. Scaling and root planing which are considered a gold standard for the treatment of periodontitis are inadequate to manage them.[2]

As we progress to the 21st century, new advances in the treatment of periodontal defects like guided tissue regeneration help in gaining clinical attachment and decrease probing depth with minimal drawbacks[3], growth factors along with platelet-rich fibrin[4], navigated computer-aided surgery, gene therapy[5], tissue engineering and cell sheet engineering are the newest techniques used in the treatment of periodontal regeneration. Among these Tissue engineering and cell sheet engineering appears to be more promising in regeneration of lost periodontal structures. Advancements in technology have made tissue engineering possible which was first proposed, in the 1980s, by R. Langer and J.P. Vacanti, and used skills in engineering to fulfill the need for the replacement of lost cells and tissues and to improve function.[6] Tissue engineering uses a scaffold, and pluripotent stem cells[7] to allow the growth of new cells and tissue to replace that lost due to pathology. However, scaffolds have various limitations in their properties. Ansuja.P Mathew has described the limitations of advancements in tissue engineering,[8] such as tissue reconstruction using stems cells using single cells have been done in animals, but this technique did not result in the complete regeneration of the lost tissues. Therefore, cell sheets were introduced which can be used as a single layer or in 2D or 3D form without the loss of cell junction and extracellular matrix which is considered to be main drawback of tissue engineering. Cell sheet engineering was developed to advent the issue of early scaffold dissolution. Takezawa et al was the first to introduce cell sheet engineering and his technique used temperature responsive dishes.[9] Another eminent scientist T.

Okano[10] introduced the various steps involved in cell sheet engineering like sources of cell sheets, its manipulation and harvesting of these cell sheets which showed promising results in periodontal regeneration. Let's discuss in detail about the various steps involved in the cell sheet engineering technology.

Sources for preparation of cell sheet

The complex anatomy of the tooth and its supporting structure provides diverse stems cells from various layers (found during and after tooth development) such as stem cells from the apical papilla, exfoliated deciduous teeth, periodontal ligament, alveolar bone, and extracted third molar region.[11,12]

The various stem cells derived from the stomatognathic region are periodontal ligament stem cells and dental follicle stem cells (DFCs), periodontal stem cells (PDLCs), bone marrow mesenchyme stem cells (BMMSCs), jaw BMMSCs (JBMMSCs) and Dental pulp stem cells (DPSCs). Some other examples of stem cells are osteoblastic cells, apical tooth germ cells (APTGs), human umbilical vein endothelial cells (HUVEC), stem cells from the apical papilla (SCAP), urine-derived stem cells (USCs), and stem cells from human exfoliated deciduous teeth (SHEDs). Cells that have been utilized for cell sheet preparations include induced pluripotent stem cells with mesenchymal cells from periodontal ligament, somatic and embryonic stem cells.[13]

Types of cell sheets manufactured[14]

1. Monolayered cell sheets
2. Multilayered cell sheets
3. Cell sheet fragment
4. Cell sheet pellets
5. Co-culturing and micropatterning
6. Scaffold-based cell sheet tissue.

Steps in preparation of cell sheet engineering

Various steps involved in Cell sheet tissue engineering are.[15]

1. Cell sheet preparation
2. Cell sheet harvesting/manipulation
3. Cell sheet transplantation

Cell sheet preparation

Main technique involved in preparation of cell sheets is the isolation of seed cells from the source and transfer them to culture dishes invitro. They are grown on poly N-isopropylacrylamide (PIPAAm) and tissue culture polystyrene (TCPS) dishes. These cells grow at a temperature 37°C. The conflux of the cells is achieved with the formation cell sheets maintaining the cell junctions and extracellular matrix. There are always accelerators like proteins and decelerators which disrupt the bond between cells like enzymes play a major role in preparation.[16]

Importance of PIPAAm polymer

poly N-isopropylacrylamide (PIPAAm) a thermo-responsive polymer, shows remarkable changes of temperature. PIPAAm is considered as one of the most intelligent polymers because of its properties like reversible temperature- responsive soluble/insoluble character as it changes in aqueous solution below and above a lower critical solution temperature (LCST) of 32^0. The thermo- responsive polymer shows full-hydrated and extended conformation at low temperature. Over 32 ^{0}C, however, it extensively dehydrates and changes to compact chain conformations. Efficient recovery of cells from culture substrates is an essential process for their passage and characterization. Typically, such a process requires enzymatic treatment of adhered cells using trypsin, collagenase, etc. These proteolytic enzymes inflict damage on cell membranes by hydrolyzing various membrane-associated protein molecules, resulting in the impairment of cell function. Generally, in order to subculture cells, cultured cells must demonstrate resistance against enzymatic treatment for detachment from culture dishes prior to selection and subculturing. From this point of view, a technique for subculture which does not require enzymes has long been desired.[17]

Cell sheet harvesting /manipulation

Critical part in cell sheet devising is harvesting of sheets without the loss of cell junctions and extracellular matrix by which capability of cells is retained in generating a viable tissue. In order to avoid physical damage and chemical disruption, scientists have come up with the clever use of temperature-controlled cell sheets. Various other methods have also been used for harvesting the cell sheets. Among the harvesting methods thermos-responsive culture dishes are more efficient. At 37°C, these facets are water-insoluble, like any other tissue culture dishes available in the market, but change to water-soluble below 32°C. When the temperature decreases these cells instantly separate from the surface without any need for enzymes.

The thermo-responsive is the most accepted technique. Many modifications exist such as polymer coating, irradiation, grafting, micropatterning.[18]

Other methods of harvesting cell sheets that gained popularity include:

1. Electro-responsive systems
2. Photo-responsive systems
3. pH-responsive systems
4. Magnetic systems
5. Mechanical harvest methods.

Progressing technology favors the novel methods of harvesting such as, ion attachment, reactive oxygen species, and reduces fabrication time of thermo-responsive culture dishes.[19] According to Zhou et al.[20] mechanical harvesting or brushing of the sheets from the base of the culture dishes, is reliable, economical and more convenient in manipulation of sheets. Usage of manipulators like plunger device helps in layering and stacking of the sheets to form 3D and multilayered cell sheets.[21]

Cell sheet transplantation

The deciding step is to transfer the harvested sheets to the desired areas for regeneration, sites are mostly categorized into three aspects in which first category is reconstructing a slash defect or tear in sensitive areas like cornea in which single layer can be used.[22] The second category defects includes areas where impaired function is also noted, like stacking a layer of cell sheets infused with hormone and cytokines with a slow release to certain organ cells.[23] The third category which is considered as future of cell sheet engineering is where not only a part of torn or lost tissue is regenerated but regeneration of the entire organ is done using multilayered exactness of cell sheets along with its blood supply.[24]

Purview in field of Periodontology

Periodontium which undergoes continuous injury due to various microorganisms, trauma from occlusion and deleterious habits. This results in periodontal tissue destruction. Therefore, there is a need for reconstruction of tissues to avoid loss of function.

Gingival mesenchymal cells[25] have high potential for soft tissue regeneration, enhancing wound healing and also helps in replacement of tissue lost due to gingivitis, desquamation, recession and trauma.[26]

Periodontal ligament fibers which are the pillars of periodontium are most effected to trauma and their regeneration have become a challenge. Reconstruction of periodontal ligament fibers using autologous pdl cells are used as seed and defects were treated.[27]

Cementum, a mineralized tissue covering the tooth, can also be regenerated using cell sheets, not only on natural tooth but also covering implants to increase their stability.[28]

Main reason from loss of tooth is loss of alveolar bone, reconstruction of bone has been cell sheets challenging due to various factors. Periodontal ligament cells or bone derived mesenchymal cells are used in cell sheet engineering in resetting of intra bony defects.[29]

Studies conducted by Gao et al demonstrated cell sheet to be effective in regenerating osseous tissues around implants. In the study by Washio et al. successful formation of cementum and periodontal ligament on titanium mesh surface was achieved. These studies suggest a future for cell sheet complex in implant dentistry. Cell sheet can also be used in combination of various bone grafting material such as tricalcium phosphate, hydroxyapatite, bioactive glass and platelet rich fibrin.[30]

Advantages of cell sheet engineering

1. Cell sheets harvested from thermo-responsive substrates bond to tissues of host without the need for sutures.
2. Cell sheet engineering avoids the use of scaffold materials which elicit strong inflammatory host responses due to release of byproducts from degraded scaffold materials.
3. Conventional culture dishes use enzymes which are harmful to cell junctions, can be avoided using thermos-responsive culture dishes.
4. Cell sheet engineering offers better control over cell seeding, with the tissue constructs having varying densities for usage of differing tissues.

Drawbacks of cell sheet engineering

1. Cell sheets are extremely delicate because of which their damage during manipulation and transportation are unavoidable.
2. Numerous layers like multilayered are required for regeneration of lost tissue as thickness of cell sheet is very thin.
3. The high-cost factor in this procedure is a major impeding in making this treatment procedure a reality.

CONCLUSION

To conclude, Cell sheet engineering has been used for regeneration of various tissues in the medical field and in dentistry. Many studies including animal studies, and clinical trials have demonstrated periodontal regeneration of lost tissue with minimum side effects. Advancements in cell sheet engineering should be made available and as it is beneficial in achieving adequate reconstruction and regeneration of the lost tissues of the periodontium.

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