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Review Article

Dentistry Section

Cell Sheet Engineering in Periodontology: A Review

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ABSTRACT

Cell sheet engineering has developed its own place for the regeneration of tissues. It focuses on phenomena occurring at the cellular level, whereas regenerative medicine and tissue engineering aim to create or stimulate new tissue for the treatment of disease. In medicine and dentistry, tissue engineering, cell-based therapies, and regenerative medicine have been progressing rapidly for regeneration procedures. It has emerged as a promising strategy for scaffold-free cell-based regenerative medicine therapy by means of transplantable single or multi-layered cell-dense sheets to achieve tissue repair and regeneration. Cell sheet engineering could be one of the procedures for regeneration of periodontal tissues, which can yield better results. In this review, the authors will highlight the principal techniques of cell sheet engineering and its application in tissue regeneration in periodontal therapy.

Keywords: Bone regeneration, Osseointegration, Regeneration, Tissue engineering

INTRODUCTION

Periodontitis is mostly attributed to tooth loss in adults, because it leads to the loss of connective tissue and bone support. In addition to the infectious microbes in the biofilm, environmental and genetic factors, particularly tobacco smoking, have a role in the development of these disorders [1].

Under extreme circumstances, the bone tissue around the tooth roots is destroyed, leaving crater-like abnormalities [2]. Additionally, the untreated periodontal pockets can impede on an individual's aesthetics, quality of life, and serve as a catalyst for further devastation and consequently have an adverse effect on the health system and incur a high financial cost. Scaling, root-planing, and surgical cleaning are examples of traditional procedures used to get rid of bacteria and contaminated tissue [3], whereby individuals tend to present with a relapse of illness without maintenance therapy. Numerous regenerative treatments, including enamel matrix derivative and Guided Tissue Regeneration (GTR), have been implemented in clinical practice to address this issue.

The complete restoration of all components of the periodontium including cementum, alveolar bone, Periodontal Ligament (PDL) and gingival connective tissue, to their function and original architecture is the main objective of periodontal treatment [3]. It is challenging to regenerate the entire periodontal tissue structure. Several technologies, including bone barrier membranes, graft materials and protein products, have been produced and utilised to repair periodontal abnormalities therapeutically. In order to overcome the limitations of traditional techniques, cytotherapeutic treatments have recently been developed. Several therapeutic trials have already utilised the in-vitro grown autologous cells obtained from different kinds of tissues. These cytotherapeutic therapies for periodontitis have been proven to be secure and efficient [4].

The tissue engineering theory was initially proposed in the 1980s by Langer R and Vacanti JP [4]. Successful uses of tissue engineering therapies employing biodegradable scaffolds include cartilage, bladder [5,6], and blood vessels [7,8]. To build the scaffold approach, however, advancement is required to strike a critical equilibrium between the rate of scaffold disintegration and the rate of tissue creation. To avoid the restrictions imposed by tissue reconstruction, the development of 'cell sheet engineering' was proposed by Okano T et al., (1993) [9]. This is a freshly developed technique for tissue regeneration. This method is better than the traditional method since it separates cultivated cells without the need of an enzymatic method. Recently, an innovative method to build tissues devoid of a particular scaffold is cell sheet engineering using temperature sensitive dishes. The resulting cell sheets still include the original extracellular matrix and cell-cell contact tissue regeneration technique. Cell sheet engineering is commercially available under the name of UpCell® (CellSeed Inc., Tokyo, Japan) [8,9].

Cell sheet engineering, a method of fabricating the tissue in absence of scaffolds, has emerged as a significant innovation in the field of regenerative medicine. The extracellular matrix, nexin, ion channels, growth factor receptor and other significant cell surface proteins are all present in the cell sheet as a whole layer. The area has advanced quickly in the last two decades in terms of examining manufacturing methods and several uses in biological research and regenerative medicine [10]. Cell sheet engineering technique is used in clinical practices for heart [10], cornea [11], oesophagus [12,13], middle chamber of ear [14], knee cartilage [15], and lung [16]. Recently, it has been also used in periodontal regenerative therapy [17].

In this article, the authors explore the possible applications for the technology of cell sheets in periodontal regenerative medicine and describe new developments in cell sheet engineering.

METHODS OF CELL SHEET HARVESTING

Harvesting whole cells from the culture surface is one of the most crucial processes in the creation of scaffold-free tissue. It is important to design platforms that allow for cell adhesion and dissociation without consuming the extracellular matrix, which holds the layers of cells together. All signaling proteins and substances that are essential for progressing cellular processes and biological processes should also be maintained on the platform [18].

Various studies described various methods for cell sheet harvesting. One of the techniques is "cell scraping method". Many studies explained the therapeutic applications in osteogenesis in-vitro [19], acceleration of bone regeneration in-vitro [20], treatment of bone defects, in periodontal tissue regeneration [21], and in promoting alveolar bone regeneration, in hastening the development of peri-implant bone and enhancing osseointegration [22]. Another method is "thermosensitive method" for promoting anti-inflammation in periodontitis. However, there are additional instances where chemical and physical techniques like trypsinisation and magnetic stimulation are used [23].

The Temperature-responsive

Two types of Temperature-responsive are using synthetic polymers PIPAAm and methyl cellulose, employing temperature-responsive culture dishes, approach for regenerative treatments [24]. As it's an alternate cell sheet with lower critical solution temperature of 32°C, the temperature-responsive polymer poly (N-isopropylacrylamide) (PIPAAm) may undergo a clear change from hydrophobic to hydrophilic. Thus, PIPAAm was covalently immobilised at a nanometer-scale thickness on common Tissue Culture Polystyrene Surfaces (TCPS), and as a result, cell adhesion and separation may be managed by small temperature changes [25]. In comparison to standard TCPS at 37°C, there were no variations in cell adhesion, dissemination, or proliferation on these surfaces. Then, by lowering the incubation temperature and changing the coated PIPAAm from hydrophobic to hydrophilic, cultivated cells may be removed in the form of a cell sheet.

The temperature-responsive culture dishes were used to harvest a variety of cell sheets from various sources, and the technique is now used in research. For tissue healing, overlapping cell sheets may also be extracted and multilayered cell sheets can be created [24,25].

Non-temperature Responsive

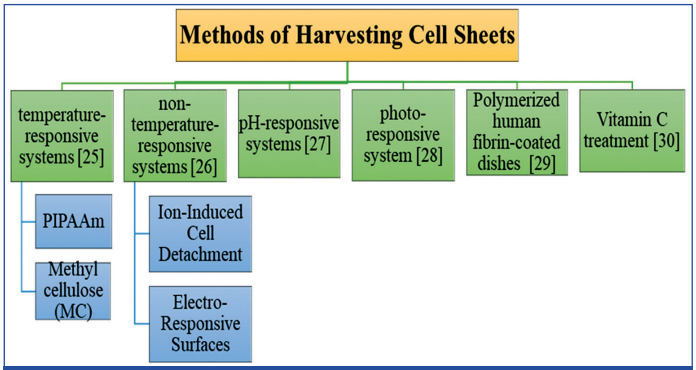
The ion-induced cell detachment technique was created as a straightforward isothermal system to remove cells when needed. The cell-culture surface can be grafted using this approach without the need for vapour-phase polymerisation equipment or infrastructure. Recovering cell sheets using an electro-responsive surface is a frequent method as well. In this system, the signal that initiates cell separation is, in theory, electrical stimulation [26].

Other Methods

The pH of the human body varies. pH-responsive devices have been widely used in drug delivery systems to regulate the distribution of medications to the target location. Cancer drug delivery systems are well-known instances of this system [27].

A fabrication technique that triggers wettability using light is necessary for a photo-responsive system. Due to modifications in a variety of characteristics, such as magnetism, fluorescence, and wettability, light can illuminate and irreversibly alter the conformation of photoresponsive materials. The ability of light to affect cell adhesion is one of these qualities. The most popular photo-responsive materials are metal oxides, particularly zinc oxide (ZnO) and titanium dioxide (TiO_2) [26].

The cell sheet could be easily separated intact from the polymerised fibrin layer because the intrinsic protease had broken down the fibrin by the time it was added to the cell [28]. Wei F et al., cultivated PDL stem cells that had received varying doses of vitamin C. At concentrations of vitamin C >20 g/mL, the cells produce cell sheet structures because vitamin C can boost cell matrix formation [29]. Various methods of harvesting cell sheets have been highlighted in [Table/Fig-1] [25-29].



[Table/Fig-1]: Methods of harvesting cell sheets [25-30].

CELL SHEET ENGINEERING IN USE

Periodontal Ligament (PDL) Regeneration

The PDL is a soft connective tissue inside of the alveolar socket and the tooth roots. The cementum of teeth is joined to the gingiva as well as alveolar bone by collagen bands, the majority of which are type I collagen. Fibroblasts, the major cells of the PDL, generate, maintain, and repair alveolar bone and cementum [30,31].

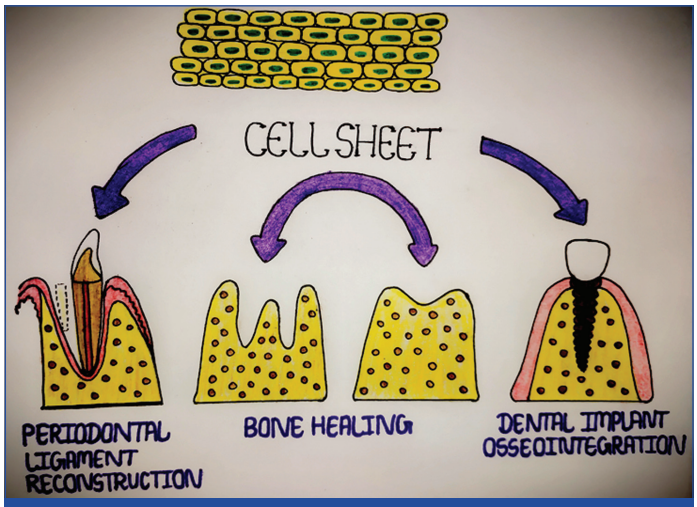
The supporting and enclosing tissue of the tooth is destroyed by periodontitis, one of the most prevalent diseases affecting teeth [21]. Cell sheets that express genes with anti-apoptotic, angiogenic, antibacterial and anti-inflammatory capabilities may be used to treat periodontitis.

Experimental periodontitis and periodontal abnormalities in beagle dogs were created and treated with Human Beta Defensin-3-Periodontal Ligament Cell (HBD-3-PDLC), which secretes an antimicrobial peptide [21]. This approach of treating periodontitis in dogs prevented root exposure, gingival recession, and tooth loosening.

Hepatocyte Growth Factor-Dental Pulp Stem Cell (HGF-DPSC) sheets were implanted in miniature pigs with artificially generated periodontitis lesions. Significant periodontal tissue regeneration, including alveolar bone and PDLs, was seen in lesions transplanted with HGF-modified DPSC sheets [30]. As a consequence, scientists came to the conclusion that HGF-DPSC sheets promoted tissue regeneration and would work well as a periodontitis therapy.

Tsumanuma Y et al., employing three different mesenchymal tissue-derived cell types (peridontal ligament, alveolar periosteum, and bone marrow), cell sheet transplantation was accomplished in a canine severe defect (one-wall intrabony defect) model [32]. Three-layered cell sheets that were autologously transplanted from each cell source were applied to the denuded root surface. These sheets were supported by woven polyglycolic acid. Beta-Tricalcium Phosphate (β-TCP) and collagen were used to fill one-wall intrabony defects. Eight weeks after transplantation, periodontal regeneration with freshly produced cementum, PDL fibres that were well aligned, and alveolar bone regeneration were seen in all the groups. The PDL sheet group had the greatest degree of bone and periodontal regeneration.

Genetically, modified cell sheets were transplanted into animal models of periodontitis, and these animals showed therapeutic responses. There are no clinical trials available for this strategy right now. Applications of cell sheet engineering in periodontal treatment have been explained in [Table/Fig-2].



[Table/Fig-2]: Applications of cell sheet engineering in periodontal treatment.

Alveolar Bone Regeneration

All age groups have extensive alveolar bone loss, which continues to pose a serious threat to periodontal health. The pattern of bone loss

most frequently observed in periodontitis is horizontal alveolar bone loss. The least predictable kind of periodontal defect, horizontal alveolar bone loss has only recently been treated in periodontal clinics using a few regeneration techniques [33,34].

Commonly used components in regenerative therapy strategies for horizontal bone loss include graft materials, GTR membranes, growth and differentiation agents, and cells [35].

Hepatocyte Growth Factor (HGF) is a growth factor for angiogenic tissue, and the HGF gene has been introduced into Dental Pulp Stem Cell (DPSCs) [36]. In miniature pigs, HGF-DPSC sheets were implanted into periodontitis lesions that had been induced artificially. In terms of bone regeneration, the HGF-DPSC sheet group outperformed the HGF-DPSC injection group. The stimulation of tissue regeneration by HGF-DPSC sheets may hold promise as a periodontitis treatment [37].

Dental Implant Osseointegration

Osseointegration is a lengthy healing process that entails the strong attachment of alloplastic components to the bone while they are still functionally loaded and clinically asymptomatic [38,39].

For the implants to function normally, osseointegration is necessary. The implant-tissue interface is a very dynamic region of interaction. This complex relationship has an impact on the mechanical environment in addition to problems with biomaterials and biocompatibility.

However, in unfavourable bone diseases such as diabetes, osteoporosis and radiation-damaged bone, osseointegration is reduced [40]. Genetically, modified cell sheets were developed to assist rigid osseointegration following the placement of dental implants [41,42].

In order to construct antimiR-138 MSC Sheet-Implant Complexes (MSICs), Ti implants were wrapped in Bone marrow-derived Mesenchymal Stem Cell (BMSC) sheets that had undergone antimiR-138 alteration. These MSICs were subsequently subcutaneously implanted into immune compromised mice [22]. An increase in the expression of proteins associated to osteogenesis and angiogenesis was found by histological examination and micro-Computed Tomography (micro-CT), leading to significant bone growth and healthy surrounding vascularisation of the implants. On the other hand, MSICs without antimiR-138 transfection only made a little contribution to the growth of new bone, highlighting the significance of gene alteration in such circumstances.

The results of histological investigation and micro-CT showed that the expression of proteins associated to osteogenesis and angiogenesis had increased, leading to significant bone growth and healthy vascularisation around the implants.

Two more trials were successful in coating the implants with BMSC sheets that overexpress Nell-1/LRP5 [40,41]. Wrapping the implants with genetically altered BMSC sheets, the implants were then inserted into the rat tibias. As a result of the in-vivo studies, osseointegration was dramatically accelerated. Following the implantation of Ti implant complexes with hPDLCs sheets into mandibular bone defects, histological analysis of the Ti surface revealed the production of cementum and PDL-like tissue [42]. These results are encouraging for attempts to develop a stable periodontal complex around dental implants in the future.

As a result, the use of genetically engineered cell sheets in conjunction with dental implants has generated creative ideas for addressing challenging clinical issues, such as slow and ineffective osseointegration surrounding the implants. Further research on the efficacy and safety of the implants wrapped with genetically modified cell sheets is highly necessary as this method has not yet been adopted by dental clinics.

Bone Healing

It was shown that BMSC sheets could activate BMSC osteogenic differentiation and enhance bone formation to cure large bone defects in-vivo by showing accelerated bone healing in these defects along with abundant bone creation. Notably, such therapeutic benefits were more pronounced in the group of cell sheets that had been gene-modified with BMP-2, corroborating this theory. In a different study, scientists created BMSC sheets that were administered antimiR-138, and they used in-vitro and in-vivo studies to investigate how osteogenic these sheets were [43]. Therefore, it can be used in bone regeneration and healing following periodontal and implant therapy. There hasn't been an experimental investigation on this topic yet. The advantages and disadvantages of different methods of cell sheet engineering are presented in [Table/Fig-3].

Methods	Advantages	Disadvantages
MC-coating surface [26]	Effective cell detachment Simple method Economical Reusable surface culture	Not available commercially Temperature affect the procedure Methyl cellulose may leads to proliferation of cells
Ion-induced cell detachment [27]	Economical Reusable surface culture Can be used for sensitive cells	Ion depletion buffer exposure can have an impact on cellular signaling and metabolism
Electro-responsive surface [26]	Economical High level of cell patterning precision	May harmful for sensitive cells
Photo-responsive surface [26]	Economical	Different cell types is not yet etected
pH-responsive system [26]	Economical	Limited studies
Polymerised human fibrin-coated dishes [28]	Economical Easily available	Time consuming procedure
Vitamin C treatment [29]	Economical Easily available	Lower concentration of vitamin C did not show results

[Table/Fig-3]: Advantages and disadvantages of different methods of cell sheet engineering.

Current Evidences for Periodontal Regeneration

Specific drawbacks of traditional tissue engineering techniques can be solved using cell sheet approaches. However, certain problems remain. Because a cell sheet is thin, it is frequently overlaid layer-by-layer to produce thicker cell sheets. However, insufficient nutrition or hypoxia may cause necrosis to develop in the centre of multilayered cell sheets. In addition, possible in-vivo ischaemia may reduce cell sheet survival. An earlier investigation showed that pretreatment with hypoxia might boost in-vivo angiogenesis and enhance the survival rate of transplanted MSCs [44]. Cell sheets that have been primed for hypoxia may thus have higher rates of survival and greater therapeutic effectiveness in-vivo. Furthermore, research into cell sheets made from iPSs and ESCs is still in its infancy. Cell sheets generated from ESCs or iPSs are yet to be used in more extensive applications.

Akizuki T et al., in their pilot investigation on Beagle dogs, surgically produced dehiscence defects on the buccal surface of the mesial roots of each dog's bilateral mandibular first molars before covering the flaws with PDL cell sheets and strengthened hyaluronic acid carriers [45]. In three out of five problems, periodontal tissue recovery was seen along with the production of bone, PDLs, and cementum. By repeatedly layering cell sheets, 2D manipulation (temperature-responsive) can be used to create thick biological tissues, a process known as "3D manipulation". Coherent contact between layered cell sheets occurs due to an affinity between the apical receptors and basal ECMs, which makes detachable cell sheets from a temperature-responsive surface, flexible and able to hold ECMs basally. Additionally, due to the creation of gap junctions between the stacked cardiomyocyte sheets, the synchronisation of beating between the sheets happened within an hour [46]. Therefore, we

may build in-vitro 3D tissues that are cell-dense and have cell-cell connections throughout the tissues by manipulating cell sheets in 2D and 3D.

It takes a long time to manually handle cell sheets, and only a small quantity of layered cellar tissues can be generated. The creation of large-scale, clinical-grade tissues is made possible with the integration of automated robotics and the plunger device for cell sheet manipulation. We can accurately align and stack five cell sheets into a myoblast tissue that is 70-80 micronmeter thick utilising an automated system that uses a plunger cell sheet manipulator [47]. Based on this technology, the "Tissue-Factory (T-Factory)" automated cell sheet production system was created to produce clinical-grade myoblast tissues [48].

CONCLUSION(S)

The ability of PDLSCs to repair periodontal tissue has been extensively studied since their discovery. The majority of findings showed that PDLSCs could regenerate periodontal tissue in diverse experimental contexts. The use of PDLSC transplantation in "cell sheet engineering" is one of the most effective ways to regenerate periodontal tissues. A novel idea in dental treatment implants with periodontal tissues could result from experimental studies that are made possible by PDLSC sheets' capacity to produce periodontal tissues. Although such a therapy has not yet been realised, it is anticipated that future advancements in tissue engineering will offer a revolutionary dental treatment alternative. This could be one of the novel techniques in periodontal applications, including PDL, cementum and alveolar bone regeneration, as well as in peri-implant diseases. Moreover, clinical trials should be conducted to obtain more potential applications of cell sheet engineering in periodontology.

Future therapeutic options for treating damaged periodontal tissues will unavoidably be made possible by the development of cell sheet engineering constructions and growth factor delivery methods. Concerns of safety, predictability, level of control, cost, etc., will determine whether or not this technology is implemented in clinical settings.

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