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Scaffold Free Tissue Engineering in Regenerative Medicine: A Review

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Abstract: ***With the rapid progress in the understanding of stem cell biology during the 1990s, the clinical use of stem cells seems to be promising. Recently, novel therapeutics using tissue engineering and stem cell technology, termed regenerative medicine, have been investigated. Cell sheet technology enables novel approaches to tissue engineering without the use of biodegradable scaffolds. Cell sheet technology consists of a temperature responsive culture dish, which enables reversible cell adhesion to and detachment from the dish surface by controllable hydrophobicity of the surface. This allows for noninvasive harvest of cultured cells as an intact monolayer cell sheet including deposited extracellular matrices. The monolayer cell sheet can be transplanted to host tissues without using biodegradable scaffolds and sutures. This paper highlights the various role of cell sheet engineering in periodontics and other tissues.***

Keywords: tissue engineering, scaffolds, stem cells.

1. Introduction

Recently, regenerative medicine is widely regarded as an effective method of achieving radical treatments that overcome the limits of treatments, and has been attracting world wide attention. To establish this visionary treatment method, fundamental studies using embryonic stem cells and induced pluripotent stem cells have been carried out actively, and tissue engineering therapies are also becoming increasingly important for aim at clinical application. Tissue engineering is a novel and highly exiting field of research. With tissue engineering techniques it may be possible to repair damaged tissues or even create replacement organs[1]. It can help in the regeneration of enamel and dentin to restore the lost tooth structure in future. Tissue engineering is the field of functional restoration of tissue structure and physiology for impaired or damaged tissues because of cancer, disease and trauma[2]. This holds the promise of solution to a number of compelling clinical problems in dentistry that have not been adequately addressed through the use of permanent replacement devices. The key elements of tissue engineering are stem cells, morphogens and scaffolds of extra cellular matrix.

2. Definitions

2.1 According to **Langer** and **Vacanti,** tissue engineering is "an inter disciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain, or improve tissue function".

2.2 According to **MacArthur** and **Oreffo** tissue engineering defined as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use"[3].

3. Principles of Tissue Engineering [4]

Representation of three different tissue engineering approaches: conductive, inductive, and cell transplantation.

3.1 Conductive Approach

This approach makes the use of a barrier membrane to exclude connective tissue cells that will interfere with the regenerative process, while enabling the desired host cells to populate the regeneration site. An example of this is the dental implants and guided tissue regeneration membranes. Today implants are considered as standard treatment opinion in conjunction with prosthetic rehabilitation, for replacing single and multiple teeth. GTR membranes are used to regenerate the periodontal tooth supporting structures and use a material barrier to create a protected compartment for selective wound healing [5].

3.2 Inductive Approach

This approach uses a biodegradable polymer scaffold as a vehicle to deliver growth factors and genes to the host site. The growth factors or genes can be released at a controlled rate based on the breakdown of the polymer. Inductive approach uses biodegradable scaffold to deliver growth factor/genes at a controlled rate based on breakdown of polymer. One limitation of inductive approach is that the inductive factors for a particular tissue may not known.

3.3 Cell transplantation

This strategy uses a similar vehicle for delivery in order to transplant cells and partial tissues to the host site. The cell transplantation strategy truly reflects multidisciplinary nature of tissue engineering, requires clinician, bioengineer and cell biologist.

a) **Clinician**: - biopsy of small sample of tissue containing cell of interest.
b) **Cell biologist:** - multiply cells and maintain their function.

c) **Bioengineer:** - manufacturer of tissue, bioreactor and the material on to which the cells will be placed for transplantation. Lastly clinician transplants the engineered tissue- polymer scaffold degrades and is remodeled by host and transplanted cells resulting in complete natural tissue.

d) **Tissue engineering triad [**6]: Tissue engineering is the employment of biologic therapeutic strategies aimed at replacement, repair, maintenance, and or enhancement of tissue function. Tissue engineering is generally considered to consist of three key elements (Fig: 1)

- stem cells/progenitor cells
- scaffolds or extra cellular matrix
- Signaling molecules.

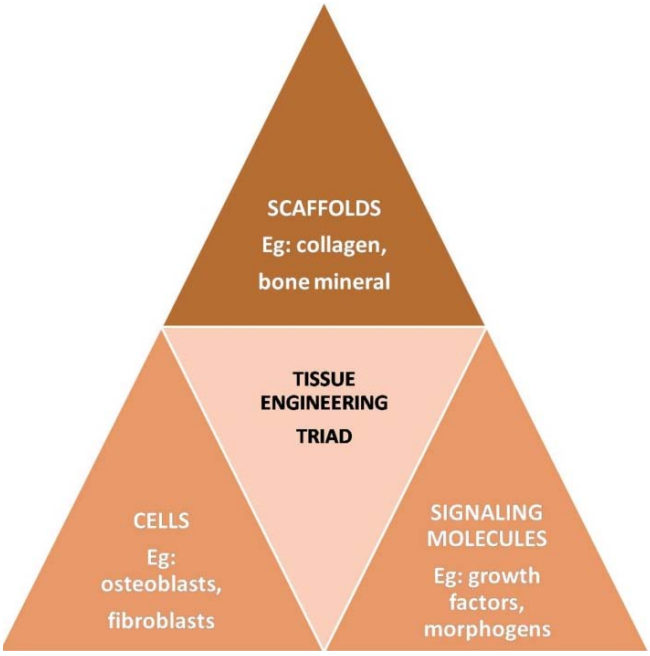

Figure 1: Tissue engineering triad

4.1 Major approaches to tissue engineering [7]

a) Ex-vivo approach: In this technique the target tissue is created in a laboratory by culturing cells in biodegradable scaffolds in the presence of specific trophic factors before their transplantation into the body.

b) In – vivo approach: This technique involves induction of intrinsic healing activity at the site of tissue defect using these three elements (cells, scaffolds, signalling molecules).

4.2 Scaffold free tissue engineering

Recently, novel therapeutics using tissue engineering and stem cell technology, termed regenerative medicine, have been investigated. In experimental transplantation into small animals, such as rats and mice, the injection of single cell suspensions sometimes works. However, single cell suspension injection does not seem to be suitable for large tissue reconstruction, since only a few percent of the injected cells are integrated into host tissues. It is apparent that we currently lack successful methods of tissue reconstruction [7].

Cell sheet engineering technique has been developed in order to avoid the limitations of tissue reconstruction using biodegradable scaffolds or single cell suspension injection. The concept of cell sheet engineering is tissue reconstruction, not from single cells, but from cell sheets. This is the reason why 'cell sheet engineering' is used to overcome these problems and in order to give better solutions for tissue repair and regeneration filed among others. Conventionally, cells are harvested using proteolytic enzymes such as trypsin and dispase. These enzymes degrade cell adhesion molecules and the deposited Extracellular matrix (ECM) to detach cultured cells. But at the same time, cell-cell junction proteins, as well as receptor proteins expressed on the cell membrane, are often damaged. Harvest of cultured cell sheets, therefore, is only achieved with exceptional cell types whose cell-cell junctions are less susceptible to such enzymes.

In this regard, new techniques to detach the cultured cells without using enzymatic approaches were expected to develop. Okano et al developed a new method to control cell-surface adhesion, utilizing changes in cell culture temperature and a surface grafted temperature-responsive polymer named Poly–N- Isoprophylacylamide(PIPAAm). This thermo-responsive polymer surface technology allows for the detachment of cells from their polymer surface without compromising cell function. This is possible because the polymer itself experiences a change in its attraction to water molecules as temperature changes over a certain range. Poly–N- isoprophylacylamide(PIPAAm) is the most notable polymer utilized for this type of cell harvesting. At temperatures lower than 32^0C, PIPAAm is fully hydrated, at temperatures higher than 32^0C, PIPAAm is extensively dehydrated [8]. Okano[9] et al used these cellular characteristics of PIPAAm to develop temperature responsive culture dishes by grafting PIPAAm onto tissue culture-grade polystyrene dishes by irradiation with an electron beam. This dish allowed intact cells to be harvested with just low temperature treatment.

4.3 Temperature-responsive culture dishes [10]

A temperature-responsive polymer, Poly-N-isopropylacrylamide (PIPAAm), is covalently grafted onto a culture dish surfaces and is be controlled by varying temperatures. Its reversible temperature responsive characteristics allows the cells to preserve their functionality. Temperature –responsive culture dishes are prepared several ways, but the most common is by the addition of a monomer, N-isoproplyacrylamide(IPAAm), to a culture dish and applying electron beam irradiation. This process covalently immobilizes PIPAAm to the culture surface. PIPAAM surfaces are typically 20-30nm thick. A PIPAAm thickness greater than 30nm thickness inhibits cell adhesion and response from temperature variations, therefore the polymer thickness is usually maintained at 20nm.

A grafted polymer layer of ~20 nm thickness allows control of temperature- responsive cell adhesion/detachment. PIPAAm coated dishes are temperature responsive culture dishes where the surface becomes either hydrophilic or hydrophobic in a reversible manner, depending on the temperature. This characteristic has been exploited to detach

an intact cell sheet from the culture dishes.

The surface of the dishes is relatively hydrophobic, and therefore suitable for cell culture, when the temperature is 37^0C or higher. When the temperature is reduced to 32^0C or lower, however, the surface of the dish becomes very hydrophilic, and hence confluent sheets of cultured cells can be spontaneously released from the dish surface (Fig.2).

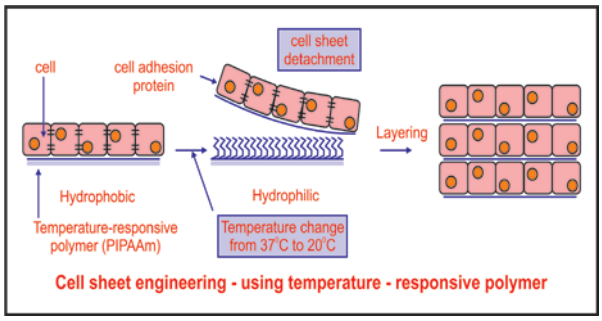

Figure 2: Temperature responsive culture surface

Various cell types adhere, spread, and proliferate on the surface at 37^0C. On reducing the temperature to below 32^0C, cells spontaneously lift up from the surface without the need for trypsin. Highly trypsin susceptible cells, such as hepatocytes and glial cells retain their differentiated native cell functions after this noninvasive cell harvest. Confluent cells are recovered as a single contiguous cell sheet with intact cell-cell junctions and deposited extracellular matrix (ECM). Harvested, viable cell sheets can be transferred to other culture dishes in vitro or to tissue surfaces in vivo. We call this two-dimensional cell sheet manipulation. Since the ECM associated with the basal side of the cell sheets shows adhesion, the harvested cell sheets can be stratified to reconstruct thicker or more complex tissue architectures, such as cardiac muscle, liver lobule, and kidney glomeruli (three-dimensional cell sheet manipulation).

In the following sections, we demonstrate how these cell sheets can be used in regenerative medicine. On the other hand, in cell sheet technology which employs temperature responsive culture surfaces, cells are harvested as a single contiguous cell sheet upon temperature reduction. These Harvested cell sheets have been used for various tissue reconstructions, including ocular surfaces, periodontal ligaments, cardiac patches and bladder augmentation.

4.4 Advantages of cell sheet engineering compared with more conventional approaches [11]

a. Cell sheets harvested from thermo-responsive substrates adhere to the host tissue without the need for sutures.
b. Cell sheet engineering avoids the use of scaffold materials, which can have potential for inflammatory or foreign body reactions or other complications arising from the byproducts of scaffold biodegradation.
c. Thermo-responsive substrates avoid the use of deleterious enzymes that typically are used to remove cell monolayers from conventional culture dishes.
d. Cell sheet engineering offers better control over cell seeding; that is when cells are seeded at a cell concentration similar to that observed in a confluent monolayer, final tissue like constructs have greater cell densities and less ECM, reflecting the varying cell densities of the different native tissues.

4.5 Regeneration of various tissues [10]

1) Ocular surface regeneration
Noninvasive cell sheet harvest and transplantation using temperature-responsive culture surfaces has also been applied to ocular surface regeneration. Ocular trauma, such as alkali burns and severe ocular diseases including Stevens-Johnson Syndrome and ocular pemphigoid, cause corneal opacification and visual loss because of limbal stem cell deficiency. Corneal epithelial stem cells are known to localize in the limbus, the border area between the cornea and conjunctiva.

Limbal stem cells were isolated and expanded on temperatu-reresponsive culture dishes at 37°C. Multilayered corneal epithelial cell sheets are harvested intact simply by reducing the temperature to 20°C without the use of proteases. Cell-cell junctions and the ECM on the basal side of the sheet, critical to sheet integrity and function, remain intact. A viable population of corneal progenitor cells, close in number to that originally seeded, is found in the sheets by colony-forming assay. Harvested sheets are easily manipulated, less fragile, transplantable without any carriers, and readily adhere to corneal stroma so that suturing is not required. In all cases, significant improvement of visual acuity can be observed.

2) Bladder augmentation
Gastrointestinal flaps are generally used as augmentation method of urinary bladder, severe complications such as lithiasis, urinary tract infection, and electrolyte imbalance results from gastrointestinal mucosa in the flap. Conventional bladder augmentation procedure is modified with use of urothelial cell sheet, harvested from temperature responsive culture dishes.

3) Cardiac tissue
Recent progress in cell transplantation therapy to repair impaired hearts has encouraged further attempts to bioengineer three dimensional heart tissues from cultured cardiac myocytes. Cardiac tissue engineering has also been pursued using conventional technology with biodegradable polymer scaffolds as a temporary ECM. However, the inflexible and bulky properties of the scaffolds significantly hamper the dynamic pulsation of cardiac myocytes. A new method to fabricate pulsatile cardiac patches by cell sheet engineering, layering several cell sheets three dimensionally has been developed. Cardiac tissue engineering based on this technology may prove useful for heart model fabrication and cardiovascular tissue repair.

4) Periodontal regeneration [12]
Periodontal diseases are very common in the elderly. Cell sheet engineering has emerged as a novel alternative approach for periodontal tissue engineering involving the covalent grafting of a temperature responsive polymer poly N isopropylacrylamide(PIPAAm) surface without the use of scaffolds. Using cell sheet engineering methods, cells can be

harvested as intact sheets along with their deposited ECM. Due to the presence of deposited ECM on the basal sheet surface, cell sheets harvested from temperature responsive culture surfaces can be directly attached to host tissue without the use of any mediators such as fibrin glue or sutures.

Since conventional methods are insufficient to attain complete and reliable clinical regeneration of periodontal tissues, patients suffer from periodontitis, halitosis, and tooth loss. Cell sheet engineering was implemented to solve this problem. Periodontal ligament cells (PDL) cells can be derived from the periodontium of autogenous extracted teeth such as the third molar and premolar for orthodontic purposes and cultured on PIPAAm culture dishes. After two weeks of culture at 37^0C, these dishes can be transferred to another incubator set at 20^0C. After 2h incubation at 20^0C, all PDL cells spontaneously detach from temperature responsive culture dishes and float in the culture medium. Detachments would be monitored under a phase contrast microscope. Then, the periodontal ligament cell sheet can be transplanted into the implant beds before inserting the implants. Following 1-3 months healing period, the histomorphometric methods were used to assess the periodontal regeneration around the cell-grafted implants.

Human periodontal ligament cell sheets harvested from temperature-responsive culture dishes were transplanted into a mesial dehiscence model (where the gum has pulled away from the front of the tooth) in athymic rats (where the cell sheets will not be rejected) to examine whether these cell sheets can regenerate periodontal tissues. In this study, periodontal ligament-like tissues, which include an acellular cementum like layer and fibrils anchoring into this layer, were identified. The fibril anchoring resembles native periodontal ligament fibers. Such regeneration was not observed in nontransplanted controls. These results suggest that this technique could be useful in periodontal tissue regeneration (Fig.3).

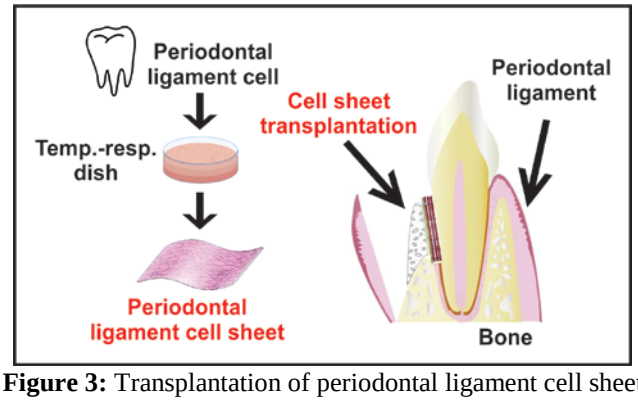

Figure 3: Transplantation of periodontal ligament cell sheet into the area of Periodontal defect

Akizuki [13] et al investigated periodontal healing after the application of periodontal ligament cell sheet in beagle dogs. These results demonstrated that, in the experimental group, periodontal tissue healing with the formation of bone, periodontal ligament and cementum occurred in three out of five defects. However, in the control group, such periodontal tissue formation was not observed except in one defect.

Hasegawa [14] et al studied the characteristics of human periodontal ligament cell sheet retrieved from culture dishes and examined whether these cell sheets can regenerate periodontal tissues. These results suggest that this cell sheet technique can be useful for periodontal tissue regeneration. Flores [15] et al evaluated whether human PDL cell sheet could reconstruct periodontal tissue and found that transplanted PDL cell sheet cultured with osteogenic differentiation medium induced periodontal tissue regeneration containing an obvious cementum layer and sharpeys fibers. All these results suggest that the cell sheet technique could be useful in periodontal tissue regeneration.

4. Conclusion

The cell sheet manipulation techniques described here can be applied to many types of cell and tissue structures, including tubes, bags, and solid masses. It is believed that two- and three-dimensional cell sheet manipulation – cell sheet engineering – should prove useful as a fundamental, generalized technique in next-generation tissue engineering and regenerative medicine.

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