TISSUE RE-ENGINEERING TECHNIQUES: ADVANCES AND APPLICATIONS
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Abstract
Tissue re-engineering is an emerging field which has tremendously developed in the last decade. It is an innovative technology which uses cells, scaffolds, signals and bioreactors for cell differentiation. Tissue re-engineering follows the principles of cell transplantation, materials science and engineering toward the development of biologic substitutes that can restore and maintain normal function. It is used to re-engineer either a part of an organ or an entire organ. It can be done by in-vivo or in-vitro methods. It is widely used in various tissue re-engineering applications such as skin, orthopedic, cardiac tissue, vascular grafts and also whole organ re-engineering. This field has still some challenges to overcome and is working its way out.

Keywords: Tissue re-engineering, components, applications.

Introduction
Tissue re-engineering, as the name suggests, is the regeneration or the renewal of damaged tissue which uses a multidisciplinary approach and includes the principals and fundamentals of life science and engineering to restore, replace or maintain a normal function or a part or whole organ.12. Tissue re-engineering is used interchangeably with the term ‘Regenerative Medicine’.

Tissue re-engineering strategies require interaction and integration with tissue and cell through incorporation of appropriate physical and cellular signals.1. Thus, the tissue re-engineering triad includes cells (which grow into the desired tissue), signalling molecules (which tell the cells what to do) and scaffolds (which support the cells and signalling molecules). All these components together in a conducive environment, provided by a bioreactor, produce the desired tissue (as shown in figure 1). The cells proliferate and differentiate as the scaffold degrades. Tissue re-engineering can be in-vitro (when bioreactor is used and cells are grown outside the patient’s body) or in-vivo (when the cell-seeded scaffold is placed in the patient’s body which itself acts like a bioreactor)2
**Tissue Re-Engineering Components:**

The tissue re-engineering triad consists of main components called cells, scaffold and signalling molecules placed in a bioreactor as shown in Figure 1.

![](image)

**Figure-1: Components of Tissue re-engineering.**

**CELLS:** Tissue re-engineering starts with a few cells which grow into a tissue and then further into an organ. So, it is very important to choose this component wisely.

Stem cell- A stem cell is characterised by its ability to self-renew and differentiate along multiple lineage pathways. Stem cells can be embryonic stem cells or adult stem cells such as bone-marrow derived mesenchymal stem cells (differentiate into bone, cartilage, muscle, tendon), hemopoietic mesenchymal stem cell (from blood cells), umbilical cord derived stem cells (undergo multiple lineage cell differentiation). Induced pluripotent stem (iPS) cells- These are similar to human ES cells in their morphology, expression of genes, and the epigenetic status of pluripotent cell specific genes. Somatic cells or terminally differentiated cells can be transformed into iPS cells and made to behave like ESC by genetic reprogramming.

**SCAFFOLD:** A scaffold in tissue re-engineering is a template which provides a 3D structure for the cells to grow and differentiate. The best scaffold for an engineered tissue should be the extracellular matrix (ECM) of the target tissue in its native state. If this is not possible then biodegradable polymers are used for scaffolding. Various scaffolding approaches are used. Pre-made porous scaffolds include material from natural sources, such as ECM, inorganic ceramics such as calcium phosphates and organic polymers such as proteins, polysaccharides, lipids and polynucleotides. Porogens such as carbon dioxide and water can be used to form porous scaffolds. Decellularized extracellular matrix from allogenic or xenogenic tissue can be used after removing the cellular antigens which cause
immunogenicity after implantation. This method gets closest to the nature as possible due to its acellular component. Cells encapsulation using a hydrogel is used for injection type scaffolding approach unlike the previous techniques which are used for implantation approach. These hydrophilic, cross-linking polymers contain about 60-90% water. Natural biomaterials (sodium alginate, chitosan, agarose) as well as synthetic polymers (poly ethylene glycol, polyvinyl alcohol) can be used. The polymer can be injected which undergoes in-situ gelation or polymerisation. This causes the formation of scaffold with minimum invasion.

Types of biomaterials used can be classified as natural materials, ceramics (usually used for bone tissue re-engineering) and synthetic polymers as shown in Table No 1. These biomaterials will interact with the host system so that it can produce its desired function of replacing or treating any organ of the body.

**Table no 1. Sources of materials used in tissue re-engineering.**

<table>
<thead>
<tr>
<th>Natural</th>
<th>Ceramic</th>
<th>Synthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>Poly saccharides</td>
<td>Poly nucleotides</td>
</tr>
<tr>
<td>collagen</td>
<td>cellulose</td>
<td>DNA</td>
</tr>
<tr>
<td>fibrinogen</td>
<td>dextran</td>
<td>RNA</td>
</tr>
<tr>
<td>elastin</td>
<td>chitin</td>
<td>amylose</td>
</tr>
</tbody>
</table>

(PLA-polylactic acid, PGA-polyglycolic acid, PLGA- poly(lactic-co-glycolic acid)

SIGNALLING MOLECULES: They affect the development and functioning of the cells and direct the cells to grow into the desired phenotype. Various growth factors such as transforming growth factor (TGF-alpha and beta), fibroblast growth factor (FGF) family-alpha and beta (Heparin binding growth factor α, adipose growth factor, bone-derived growth factor), platelet-derived growth factor and insulin-like growth factor are used. Growth factor delivery (at times along with a drug) is an important part of tissue re-engineering. Here, the growth factor can be incorporated directly into the scaffold (diffusion release of growth factor as the scaffold degrades) or it can be fabricated into nanoparticles, microparticles (controlled or programmed release is possible)

BIOREACTORS: A tissue re-engineering bioreactor can be defined as a device that uses mechanical means to influence biological processes. It provides nutrients, mechanical stimulus and regulatory signals to the cells to grow differentiate and proliferate. The advances in bioreactors include dynamic conditions such as stirred bioreactor, rotating bioreactor and flow perfusion bioreactor where a constant movement of nutrients and gases take place so that
even a larger scaffold is sufficiently nourished. The culture mode used in these newer types is microcarriers and aggregates. Microcarriers are small spherical shapes of 100-300μm diameter. Floating aggregates devoid of scaffolds or external addition of ECM matrices is possible in stirred bioreactors. Controlled environment regarding pH, temperature can be automated to improve efficiency, reproducibility along with reliability.

**Applications of Tissue Re-Engineering:**

1. **Skeletal muscle re-engineering:**

Muscular defects can be due to trauma, injuries, and accidents or due to the congenital defects. A skeletal muscle has the ability to self repair but does not maintain its integrity as before. Hence their regeneration should be such that their integrity is maintained.

Skeletal muscle regeneration can be in-vitro or in-vivo (also called myoblast transfer therapy). The characteristics of ideal engineered skeletal muscle are that they should be appropriately large and thick and the myofibres should be packed and differentiated\(^8\).

In in-vitro re-engineering, satellite cells of primary myoblasts are isolated from muscle biopsy and cultured outside the patient’s body. Other cell types differentiating into myoblast cell lines are mesenchymal stem cells, hematopoietic stem cells, and embryonic stem cells. The cells along with signalling molecules are placed in a scaffold. For in-vitro type of scaffolds, acellularised skeletal muscle tissue is used\(^9\). Myoblasts are then seeded on the acellular scaffold and cultured. Another scaffold for in-vitro technique is fibrin. It allows diffusion of nutrients and growth factors such as fibroblast growth factor-2 and vascular endothelial growth factor. Before implantation, the muscle which is engineered can be subjected to pre-conditioning for demanding host environments. When implanted, the engineered muscle gets incorporated into the body and starts functioning as per its design.

In in-vivo re-engineering, the initial steps are the same as that of in-vitro re-engineering. Cells, growth factors and a scaffold are placed in the patient’s body which acts like a bioreactor. For in-vivo techniques, hydrogels (natural as well as synthetic) are also used as scaffolds and are superior to the porous scaffolds. They allow uniform cell entrapment and high cell density due to gel compaction. A new class of polymeric temperature responsive hydrogels that polymerize in presence of temperature changing has also been studied. Combination of tri-block polymer Pluronic®F127 to fibrinogen, polymerizing at 37°C\(^10\). A newer technique includes micromoulding of hydrogel or cell. Polydimethylsiloxane (PDMS) moulds are microfabricated by photolithography or photopatterning which are
porous in nature. The cell/hydrogel mixture injected into PDMS moulds. This gives a controlled pore size which increases the nutrient and oxygen transport thereby improving the cell survival.\textsuperscript{8}

The novel in-vivo approach include a gene therapy for the treatment of muscular dystrophies \textsuperscript{11}. Skeletal muscle comprises 40\% of total body fat and is accessible for gene transduction. Intra-muscular injected plasmid DNA-vectors are largely taken up by only skeletal myocytes because they do not transduce to other tissues. The transduced muscle precursor cells deliver soluble hormones and growth factors for longer period of time. Muscle-based delivery system using bioartificial muscle for recombinant-human growth factor showed advantages such as increased survival rate and fusion efficiency.

One of the challenges faced is vascularisation. The demand for oxygen and nutrient has to be fulfilled or else it will result in nutrient limitation or cell death. Integrating of the cells with the nervous and vascular system of the host has to take place for the working and survival of the engineered muscle. Pre-vascularisation can be done to help vascularisation and perfusion of blood. Arteriovenous (AV) loop can be used for this purpose.

2. Cardiac tissue re-engineering:

Cardiac tissue re-engineering is most challenging because cardiac muscles involve voluntary as well as involuntary muscles. Stroke, myocardial infarction, hypoxia are some of the reasons which damage heart and may require tissue re-engineering to repair and regenerate the cardiac tissue.\textsuperscript{12}

Various approaches can be used for cardiac regeneration. Cell sources are bone-marrow derived stem cell\textsuperscript{12}, adipose-derived stem cell\textsuperscript{3}. A viscous cell suspension can be injected at the injured part. These cells once inside the body form ECM around them, but the survival rate is less. Another approach is by using a cardiac patch, wherein, the cells are combined with a scaffold and grown in-vitro in a bioreactor. This cardiac patch is then placed at the damaged part. Natural and synthetic biomaterials can be used in this approach. Scaffold free approaches have recently emerged, where cells can self-assemble into sheets or circular structures\textsuperscript{13}. Injectable hydrogels are used to form scaffolds. Cells along with growth factors, genes or other therapeutics can be injected along with the hydrogel. These proteins or other molecules get entrapped into the scaffold and show better cell survival rate.\textsuperscript{12,14} These injectable hydrogels are easier to handle, have ability to reach very deep tissue defects, have excellent defect margin and complete defect filling leading to neovascularisation from healthy tissue\textsuperscript{15}.

The newer advances include tubular scaffolds which are used for in-vitro culturing of small calibre arteries. Biodegradable polymers such as PGA, PLA and copolymers such as PLGA can be used. Arterial prosthesis using
tubular PGA scaffolds and PLGA films were used but they did not show the physical strength required for vascular conduit. Hence pulsatile intra-luminal pressure and flow is used to mimic in-vitro conditions. The other advancements include developing vascular media by self-assembly. Vascular grafting becomes difficult for small calibre vessels and synthetic possess the risk of thrombogenicity. Hence in such cases, tissue engineered blood vessels (TEBV) can be used. Mesenchymal cells produce and assemble extracellular matrix by self assembly. These cells grow into a tissue sheet which is rolled into a tubular construct and forms TEBV. A tissue engineered vascular media (TEVM) can be developed from vascular smooth muscle cells (VSMC) by self-assembly. They can be used as vascular substitutes and also as a model for physiological, pathological, pharmacological and toxicity studies.

Fibrous scaffolds can also be made by nanofibrous electrospinning instead of decellularization technique. Electrospinning includes an electrically charged polymer solution which is solidified after passing through an orifice forming nanofibres. The ‘bottom-up’ approach used for newer techniques help in controlling various parameters such as fibre alignment, fibre diameter and scaffold porosity. These parameters cannot be controlled in the decellularized techniques which are fabricated with the ‘top-up’ method.

3. Connective tissue re-engineering:

Bone- Bone tissue re-engineering can be in-vitro or in-vivo. Cell sources are adult stem cells, adipose-derived stem cells. iPS cells can be differentiated into osteoblasts, or iPS-derived mesenchymal stem cells which can then be differentiated into osteoblasts. Other types of cells such as cells originated from periosterum have the desired properties of proliferation, osteogenicity, mineral deposition. Growth factors for osteogenesis, ECM formation, and angiogenesis are incorporated into the scaffold. The scaffolds which are used have to be osteoinductive, osteoconductive. Natural materials such as collagen, hydroxyapatite can be used. Synthetic materials such as PLA, PGA and PLGA have superior mechanical properties. Newer fabrication technique such as laser microstereolithography (L-MSTL) which selectively cures photopolymer on a moving platform layer by layer. Such constructs allow better diffusion of biological molecules and regeneration of cells. Recent studies confirm that addition of ceramic nanoparticle into polymer matrix improves mechanical properties of matrix. Growth factors help in cell growth, proliferation and differentiation. Extracellular matrix has growth factor regulatory function which has inspired the growth factor during the bone regeneration process which helps in the speedy recovery of bone healing. Bone morphogenic protein (BMP) -2, -4, -6, -7, -9 are known to be the most osteoinductive growth factors. FGF carry out transmission of signals via tyrosine kinases. The most abundant ligand FGF-2 enhances formation of
Other growth factors include insulin-like growth factor (IGF-1 and IGF-2) for collagen synthesis, osteogenesis as well as chondrogenesis and platelet-derived growth factor for proliferation of chemotaxis, collagen activity.

Pre-vascularisation is necessary for optimum oxygen and nutrient supply and for good cell survival. The bone tissue construct formed in the bioreactor is pre-vascularised (using AV loop) and implanted at the site of injury. The local delivery of angiogenic growth factors certainly accelerates vascularisation of an implanted graft. Increased blood vessel ossification can be resulted by application of vascular endothelial growth factor to intramembranous as well as endochondrial bone defects. Stem cells along with progenitor cells can induce vascularisation. This can be done by co-culture system of cells from different lineages.

In-vivo approach involves using patient as a bioreactor. There is no implantation of the bone tissue construct as the construct is grown in the body itself. Hydrogels are of great use here. They mimic the native ECM and help in tissue regeneration. Polymer-ceramic composites can be used for in-vivo approach.

### 4. Skin tissue re-engineering:

Skin injury can occur due to accidents, injuries, trauma, especially thermal trauma. Skin tissue re-engineering can help at such times. The integrity of the re-engineered skin should be maintained.

Earlier, autografts and allografts were used to treat skin burns. But autografts cannot be used if there is extensive skin injury. Allografts have rejection problems. Tissue re-engineering uses cells such as keratinocytes and fibroblasts grown in a scaffold along with growth factors. Melanocytes are used for repopulating the burn scars. The cells interact and organize to form the final structure. The re-engineered graft is then transplanted onto the site of injury where new skin is gradually generated. Scaffolds of natural origin are polypeptides, hydroxyapatite, hyaluronan, alginate. Synthetic materials are polyglycolide, polylactide, polylactidecoglyolide. A newer approach uses polyethylene oxide-based macromer substrate. They can incorporate biologically active ligands. Growth factors are critical for the regeneration of skin. Platelet-derived growth factor activates the mesenchymal cells and stimulates chemotaxis and proliferation. TGF-β is the most potent growth factor in wound healing. β1 and β2 show cutaneous scarring, whereas β3 shows an anti-scarring effect. BMP, FGF and vascular endothelial growth factor are the other growth factors which can be used for improved efficiency and growth of skin substitutes. The recent advancement shows that mechanical stretching fastens the skin regeneration by upregulating the mesenchymal stem cell expression of genes related to vascularisation and cell proliferation. This MSC expression provides homing to the expanded skin and transdifferentiation into epidermal and endothelial cells.
In burns where only the upper epidermal lining is lost and dermal layer is intact, then the epidermis can be grown from the epidermal keratinocytes lining the dermis layer. But if both, the epidermis and dermis is lost, then there is no formation of epithelium by body and tissue re-engineering is required. Pre-made scaffold can be used or else the cells are encouraged to produce their own matrix which is freeze-dried, sterilised and used as scaffold. Epidermal cover, dermal replacement and epidermal/dermal replacement can be provided as per the degree of skin injury. Dermal replacement is used when both the epidermal and dermis layer are to be replaced. Dermal vascularisation has to be provided. An alternative to split-skin graft is known as the epidermal/dermal replacement. This is the most advanced and sophisticated construct.

Vascularisation is still one of the major problems faced by skin tissue re-engineering. Adequate blood supply to the skin graft has to be maintained for a long term survival. Anastomosis between graft vessels and angiogenesis can be done for revascularisation. The other problem is of scarring. The skin graft which is re-engineered is not of the original skin and leads to scar formation at the graft margin after integration. This can be aesthetically problematic. A technique known as the foetal wound repair is characteristic for its scar-free and fibrosis-free regenerative nature this is because the wound healing in foetal tissue takes place by a different mechanism and hence less scarring is observed. Re-engineered skin substitutes do not mimic the functional normal skin perfectly. It does not control the temperature nor does it have sebaceous and sweat glands. These differentiated structures should be incorporated into the skin constructs for the better functioning of skin tissue re-engineering. Also, keratinocytes cannot be delivered as small sheets because they are too fragile. Hence they can be grown on a carrier on a temperature sensitive polymer sheet which collapse as temperature decreases and others release the cell sheet.

5. Whole organ re-engineering using decellularized matrices:

Organ transplants are time-consuming as the patient has to wait for the correct match from a donor. The wait can be long and painful. Patients even die while waiting for the transplant. Even if a correct immunological match is received, there is always a risk of rejection and a life-long therapy of immunosuppressant is required. This compromises the quality of life.

Tissue re-engineering can be the alternative solution to overcome the problems of organ transplantation. It uses a decellularized matrix as a scaffold. A cadaveric organ is obtained and treated with sodium dodecyl sulphate (SDS) and Triton X-100 for decellularization. This process forms an acellular matrix with no cells but with the large and small circulatory vessels and extra-cellular matrix. This provides a good scaffold ready for re-population with
6. 3D bioprinting:

3D bioprinting is the recent advancement in the field of tissue re-engineering and uses a technique to print scaffolds and tissues or organs directly. It uses a layer-by-layer approach to print the 3D structures. Desired parameters such as packing density, flowability and shape is optimised for the printing of scaffold. The 3D bioprinter uses a drop of viscous binder to bind the powder (biomaterial) into the desired packing density. The accuracy of printing depends on the powder wettability.

The binder is dried after printing by using a strip heater. Biomaterials such as ceramics, polymeric and composite materials can be used. The resulting printed material is stronger than the body materials. Bioprinting of tissues or organs uses a bioink which acts as a carrier of cells as well as a scaffold. It also provides a favourable environment for cell growth.

Bioinks are hydrogels containing cells. These cells can be clustered together so that printing of these pre-assembled constructs will be easier and will require less time for self-organising after printing. But clogging of the nozzles due to cell-settling and clumping can take place. 3D bioprinting also includes the vascular network printing so as to provide nutrients to the cells. This technique is very useful because of its custom-made approach of designing tissues and organs.

Challenges of tissue Re-engineering:

a) Biocompatibility:

Patient’s own stem cells are used to avoid immunogenic responses, but foreign matter reaction still poses a danger because a foreign matter is either injected or implanted into the body. The biomaterials are tested in-vitro to choose more suitable material for the tissue construct. The recommendations for testing should comply with the document ISO 10993.

b) Regulatory aspects:

Tissue re-engineering products are considered different from medical devices and hence the regulations of medical devices do not apply to these products. Also, tissue re-engineering products cannot be sterilised, though freedom from infectious organisms is a mandatory criteria of selection. Assessment of these products is difficult because its performance depends on the function of donor cells and scaffold degeneration in-vivo.
c) **Vascularisation:**

Inside the body, sufficient supply of nutrients and oxygen is provided. But in laboratory, diffusion process provides supply only to the outer layer of cells, leaving the inner layer depleted from the nutrients. Thus, pre-vascularisation and vascularisation play an important role for the survival rate of cells.

d) **Carcinogenicity:**

This is the major challenge faced by tissue re-engineering. The other challenges can be taken care of to some extent, but this challenge poses a great danger to the patient’s life as there are cells growing inside the body. The growing cells can come out of the scaffold and spread to the other body parts leading to metastasis. This can lead to tumour formation.

**Future:**

Tissue re-engineering is an emerging field and has a lot of potential. It has developed tremendously since the last decade and is still on its way to development. It started with only making few cell layers to tissues, but has now reached to making new organs. Few organs are still in the clinical investigation stage, but they too will come in the market soon. 3D printing which is an innovative technology is trying to print personalized body parts.

**Conclusion:**

Tissue re-engineering incorporates the fields of cell transplantation, material science and engineering. Hence it is a collaborative work involving efforts from every field. With the advancement in medical field, the average life of man has increased and tissue re-engineering helps to improve the quality of that increased life. The field has many hurdles to overcome, but still it is developing at a tremendous speed. It has already helped to save the lives of many people and is yet to save more.

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**LIST OF ABBREVIATIONS:**

ECM- Extra cellular matrix

PLA- Polylactic acid
PGA- Polyglycolic acid
PLGA- Polylacto-glycolic acid
TEBV- Tissue engineered blood vessels
VSMC- Vascular smooth muscle cells
TEVM- Tissue engineered vascular media
TGF- Transforming growth factor
FGF- Fibroblast growth factor
PDMS- Polydimethylsiloxane
MSC- Mesenchymal stem cell

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