Tissue Engineering- Bridging science and medicine

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I]Abstract

During the past two decades tissue engineering and regenerative medicine have invested in the regeneration and reconstruction of pathologically altered tissues such as cartilage, bone, skin, heart valves, nerves and tendons, etc., the scaffolds combined with other bioactive materials like genes and cells are able to guide the development of functionally engineered tissues. Many of the scaffolding materials used are bioresorbable inorganic materials, natural as well as synthetic biomaterials and this is because of their biological, structural as well as mechanical properties. A diversity of biomaterials to be used as 3d scaffolds for tissue engineering and other emergent technologies for tissue specific considerations are discussed in depth in this chapter. The latest technologies related to 3D behavior and multicellular interactions are also outlined.

II] Introduction

Tissue engineering and regenerative medicine also known as TERM for short is an approach that brings to the table advanced approaches for damaged tissue regeneration and healing. Over the past few decades this emerging field has seen many advances and there has been a multitude of research including biomaterial design and processing, surface characterization or scaffolding and functionalization for improved cell material interactions and imaging. The various approaches include:

1) Direct implantation of new cells into the defects of the cells isolated from the patients.
2) Bioactive materials and growth factors delivery targeting tissue specificity
3) Cell free scaffolding biomaterials
4) Cell laden scaffolding structures that mimic the natural extracellular matrix

The cell laden scaffolds are the most commonly used for tissue engineering which involve 3D porous and hydrogel scaffolds on which cells grow and organize to form an extracellular matrix(ECM) used for regeneration purposes. The scaffolds provide the physicochemical and mechanical maintenance for in vitro ECM formation being slowly degraded resorbed and metabolized on in vivo plantation. The porosity or the pore size as well as the interconnectivity between the structures of the scaffold have a direct influence over the functionality of the cells. High porosity in the scaffold means greater infiltration of the cells and extracellular matrix colonization which again is directly influenced by pore size. Open and interconnected pores contributes to the growth, migration and proliferation of cells to an extent during extracellular matrix production. Thus, maintaining an optimal pore size becomes crucial during the process of TERM. As a result, the vascularization as well as

formation of the new tissue maybe faster. On the contrary microporosity is also required for cell adhesion, spreading and creating an initial mechanical strength between the scaffold and the tissue. Other parameters that are to be kept in mind include biocompatibility, safety, cost-efficient materials and devices as well as methodologies.

A broad variety of natural as well as synthetic scaffold materials have been applied for scaffold processing. Natural based polymers show biological properties better fit to microenvironment of the tissue meaning that they promote cellular response, biocompatibility as well as degradability. Most recent advancements show that materials made from decellularized matrix are being explored in TERM. This approach has been observed to preserve native tissue composition not only in terms of structural proteins such as collagen but also preserves the growth factors and cytokines of the native tissue which can promote cell viability as well as tissue repair and remodeling.

On the other hand, the lack of mechanical strength in natural polymers can be compensated by using synthetic polymers or combining the natural polymers with inorganic and ceramic materials to produce a scaffold with superior strength and bioresorbability. Thus, depending on the TERM strategy, optimal biomaterials and processing technologies are considered for the scaffold. Some of the strategies for scaffold processing include-

1) Solvent casting with particulate leaching
2) Freeze-drying
3) Gas foaming
4) Fiber bonding
5) Phase separation
6) Electrospinning
7) 3D printing technologies

Some of the scaffold biomaterials are discussed in the next section.

III]Scaffold materials- biomaterials

Current strategies in TERM involve usage of a wide variety of biomaterials. They are classified as-

1) Natural polymers
2) Synthetic polymers (poly-glycolic acid, poly-lactic acid, etc.)
3) Inorganic biomaterials (include metals like titanium and its alloys)
4) Ceramics (alumina, zirconia, calcium phosphate cements)

Natural polymers have an advantage that they are readily recognized by the body, their similarity with the extracellular matrix and heir susceptibility to specific enzymes. On the contrary the inorganic biomaterials are best used for their biocompatibility, bioresorbable and osteoconductive properties. Few of the biomaterials are described.

A) Natural and synthetic polymers

Natural polymers are isolated from biological organisms like algae, plant, animal, microorganisms, which are similar to the biological macromolecules like proteins and carbohydrates that are easily recognized by the environment. These materials are similar to the extracellular matrix and hence are called as biopolymers and they prevent inflammation, toxicity as well as immunological reactions that are mostly seen during use of synthetic polymers. Therefore, biopolymers are effective in designing therapeutic systems to be used as bioactive compounds and drug delivery system or even to bioengineer functional tissues. Structural proteins such as elastin and fibrin are used as sutures for scaffolds and as drug delivery systems.

Synthetic polymers have excellent processing characteristics. Hydrolytically degradable polymers are chosen over enzymatically degrading ones as to cater to the patient needs. This is also because hydrolytically degradable polymers have minimal site as well as patient to patient variation. The downside of using synthetic polymers is that they become toxic when combined with certain polymers. The solution is to create hybrids using natural polymers to increase the hydrophilicity of the cells, biodegradability and cell attachment. Few examples of synthetic polymers are poly-ethylene-glycol (PEG), poly-lactic-acid (PLA).

B) Inorganic biomaterials

These types of biomaterials have been established for orthopedic load-bearing coatings, bone grafting, cements and dental restoration . Ceramic biomaterials are called as bio ceramic and they are considered for their osteoconductive as well as biocompatible properties.

Inorganic biomaterials are classified as- bioinert, bioactive and bioresorbable. Bioinert biomaterials are those which have no interaction with adjacent tissue after implantation and are typically used as support implants. Bioactive biomaterials have a direct interaction with the living tissue and are used for filling small bone defects and similar injuries. Bioresorbable materials are gradually absorbed in vivo and are replaced by bone over time.

A number of studies are being carried out in order to enhance the bioactive inorganic materials by the process of doping that is addition of ionic elements, that are slowly released during bone resorption and hence boost the biocompatibility and mechanical strength of implants.

C) Hybrids of organic-inorganic biomaterials

These biomaterials are made as a result of combining organic as well as inorganic biomaterials as the name goes. The aim is to attain good compatibility between phases and maintain the porous structure and mechanical strength of the scaffold. Further nanostructured hybrids have been preferred as the nanoparticles provide a larger surface area thus contributing to upgraded mechanical properties.

Examples include PLG, PLA, PEG, bio-ceramics, bioactive glasses, carbon nanotubes, etc.

IV] SCAFFOLDING STRATEGIES FOR REGENERATION AND TISSUE ENGINEERING:

1. 3D Porous Scaffolds- The requirements needed to be fulfilled by a scaffold are that they should be able to help the cell to proliferate, detachment and attachment. The scaffold material should be able to provide mechanical support. This enables the cell to be stimulated for cell and biomaterial attachment and growth [1].
2. Natural 3D Porous Scaffolds- Marine sources are used to extract various bioactive products and byproducts. This helps in low-cost production and increases in biodegradability index. The fabrication of 3D scaffolds by using the skin of Prionace glauca (Shark) in combination with CaP from the teeth of two different shark species through freeze-drying technique *[2]*. The development of scaffolds using a natural biopolymer containing silk fibroin and β-TCP which incorporates strontium, zinc, etc. The collagen matrix is able to support the cell attachment and proliferation of the osteoblast like-cells. These scaffolds revealed high interconnected microporosity of 500μm. These scaffolds showed globule like structures with apatite crystals and porous spherulite like structures with the ceramic part into the silk when immersed in a biofluid *[3].*

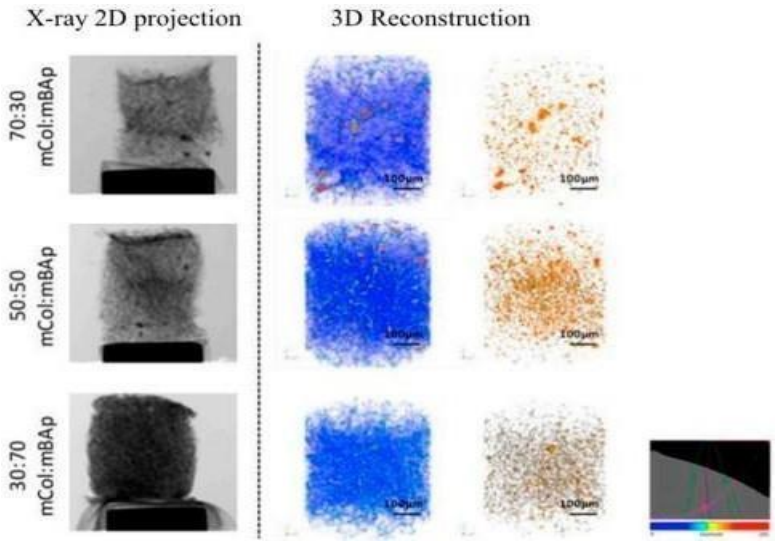


Figure 1 Scaffolds Of marine collagen. [3]

3) 3D Printed Scaffolds- Recently the 3D printed technology involving tissue engineering is termed as TERM. This allows in providing high freedom for the cell and biomolecule positioning in various designs and geometries *[4]*. The usage of collagen increased hydrophilicity from 87.8° to 76.7° and improved mechanical properties *[5]*. Silk fibroin with β-TCP usage gave significant responses of cell proliferation and differentiation with varying dopants in the scaffolds. Polycaprolactone gave the highest osteogenic values.

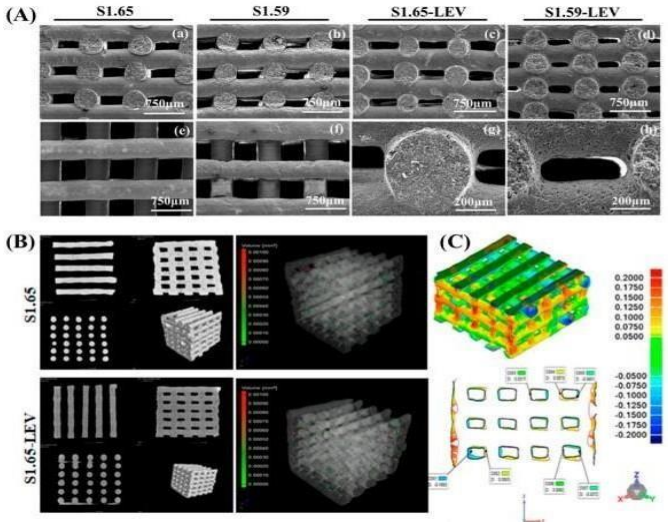
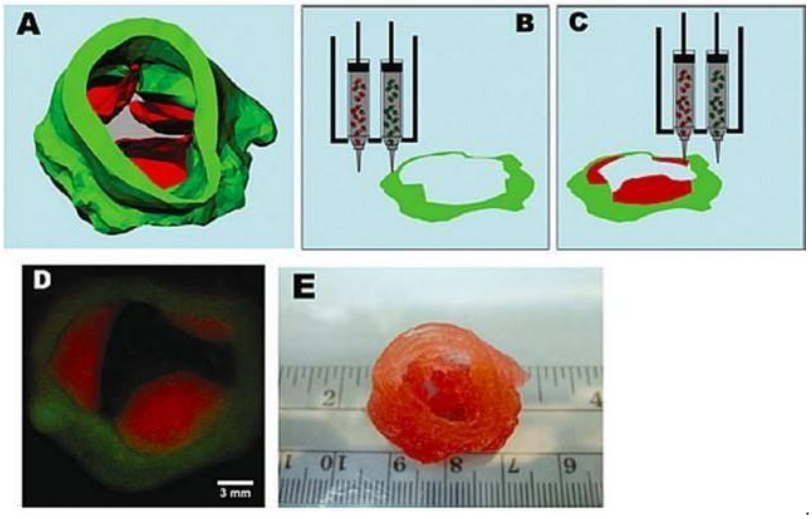


Figure 2 (A) Electron micrographs, (B) 2Dand 3D images, (C) 3D and 2D with color coding, [6]

3. Injectable Hydrogel- They are mainly fillers and of soft and hard tissues which promotes good physical integration into a defective site without requiring any possible surgeries or removal of the tissue. These hydrogels have high water content which make them easy to manipulate for the induction inside e the cells and other growth factors. They are injected at the site or injury or wound in way that the solution turns to gel transition is in optimal crosslinking parameters [7]. The chemically induced hydrogels form a covalent bond between the polymeric chains promoted by agents like genipin and enzymes [8] [9]. The physical methods for crosslinking involve thermal gelation which is easy to process and does not limit the injection depth [10]. The crosslinking mechanisms can be harder to control inn case of natural polymers. This is due to property of natural polymers change the pH of the solution which can affect the gelation time, temperature, and its biocompatibility within the gel matrix [11].

   A way this issue was addressed was as in case of chitosan to produce thermosensitive injectable which are pH dependent hydrogels, was investigated in combination with starch. It showed that the addition of starch to the chitosan gel solution did not change the transition temperature and allowed heating induced hydrogelation for applications in minimal invasive injectable systems [12].

4. 3D printed Hydrogels- These hydrogels are produced with the help of computer assisted technologies. This allows the fabrication of engineered tissues hence providing superior control over the shape and reproducibility. Controlled physical and mechanical properties of different layers and gradients allows complex tissue mimicking architecture. The 3D printing technologies have been proposed by using different hydrogel technologies/systems coupled with conventional TERM strategies *[13]*. E.g.: Li et al. proposed 3D printed hydrogels acting as OC defect fillers by using alginate and hyaluronic acid as photo-polymerized bioinks. 3D printing technologies is actually being applied to tissue engineering involving not only computing and material sciences but also cell loading and development biological sciences. This coordination between different sciences allowed microarchitecture of the organs and tissues *[14]*.



. Figure 3 (A) 3D reconstruction of aortic valves, (B) 3D reconstruction of Sinus smooth muscle cells, (C) Aortic valve leaflet interstitial cells, (D) Fluorescent image of 3D bio printed layers of aortic valve conduit and Macroscopic image of a 3D printed aortic valve conduit [15].

5. Porous Hydrogels- The big problems of hydrogels include maintaining control over the porosity and some mechanical properties, some tissues require a certain amount of scaffold porosity for cell infiltration and secretion for tissue formation. Increased porosity increases the diffusion of nutrients and oxygen in the absence of blood vascularity [16] [17]. The combination of salt-leaching and freeze-drying technologies allowed the increase of macro and micro pores. Salt leaching allowed to improve the hydrogel's structural stability by allowing efficient protein folding. The formation of bioactive polymers/inorganic hybrid hydrogels enabled to increase mechanical stability and porosity of the hydrogels. This strategy helped in the production of hard tissues such as bone and OC complexes which can include the incorporation of certain of growth factor inductions [18].

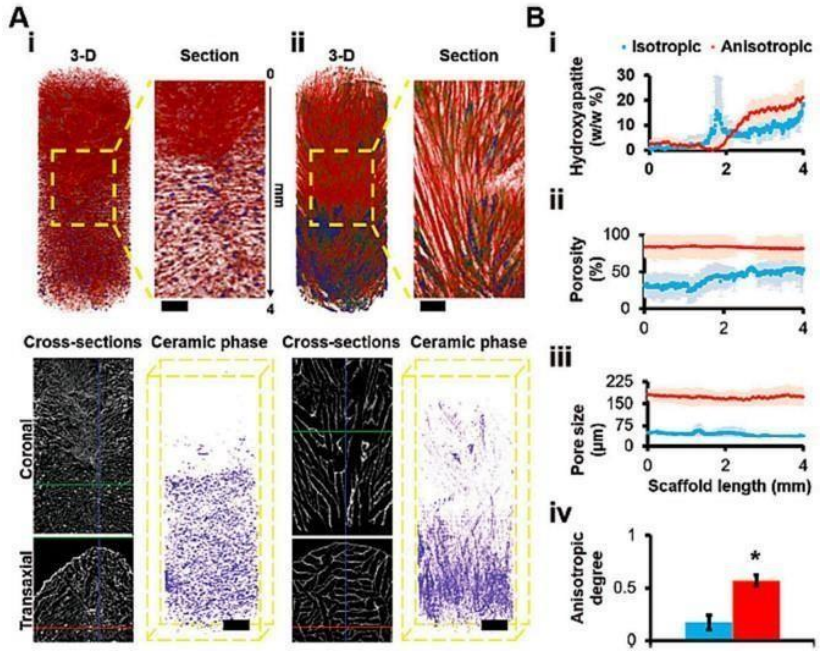


Figure 4 (A) 3D reconstruction of isotropic and anisotropic porous architectures, (B) Quantification profiles [19].

V] FUTURE PROSPECTIVES:

The TERM technology is an upcoming innovative field involving material sciences, biology and medical sciences has helped in providing and alternative for better tissue regeneration and repairing the damaged tissue. The socioeconomic need to make sure that these strategies to be an effective tool for treating a patient and allow them to have a normal life again can make this field extremely important.

All of the above-mentioned tool of scaffold technologies enables to make a new, efficient tissues, the new methods and technologies are still in progress which can help in producing different or more complex types of tissues. The future of tissue engineering and its development will ensure that even in this changing world, the ability to minimize the raw material usage to produce new organs will be vital. This field will in future will be normalized as more and more people become acquired with its knowledge and them comprehending it as technology of giving people new health improvements. The further methods and technologies in this field will ensure that there is accessibility provided and even more options of tissue regeneration in the case of the usage of scaffolds.

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