Scaffold: Tissue Engineering and Regenerative Medicine

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ABSTRACT

Scaffolds are the central components, which are used to deliver the cells, drug and gene into the body. Polymeric scaffolds may be prepared as typical 3-D porous matrix, nanofibrous matrix, thermo sensitive sol-gel transition hydrogel or porous microsphere, which provide suitable substrate for cell attachment, cell proliferation, differentiated function, and cell migration. Scaffold matrices have specific advantage over other novel drug delivery systems by achieving high drug loading. This study has been conducted to illustrate the various fabrication techniques of scaffold like Particulate leaching, freeze-drying, Supercritical fluid technology, thermally induced phase separation, Rapid prototyping, powder compaction, sol-gel, melt moulding etc. These techniques allow the preparation of porous structures with regular porosity. The main conclusion of this study is Scaffold provides adequate signals (e.g., through the use of adhesion peptides and growth factors) to the cells, to induce and maintain them in their desired differentiation stage and for their survival and growth and their successful utilisation in various fields like bone formation, joint pain inflammation, tumor, periodontal regeneration, In-vivo generation of dental pulp, diabetes, osteochondrogenesis, wound dressing, inhibit bacterial growth, heart disease, repair of nasal and auricular malformation, cartilage development, regulated non-viral gene delivery, as artificial corneas, as heart valve, antiepileptic effect, tendon repair, ligament replacement, plasmid delivery, etc.

KEY WORDS: Scaffolds, Cell proliferation, Freeze-drying, Bone formation, Osteochondrogenesis, Ligament replacement

INTRODUCTION

Tissue Regeneration is also known as tissue engineering and regenerative medicine. Regenerative medicine combines the physical nature of a product with living cells. It is an emerging revolutionary approach in modern medicine as it delivers living tissue, stimulating the body's own natural healing process by activating the body's inherent ability to repair and regenerate. Innovative regenerative medicine therapies are now available that aim to heal or reconstruct diseased tissue and support the regeneration of diseased or injured organs. The best example of tissue engineering/ regeneration device is scaffold. Scaffold is the central components, which are used to deliver cells, drug and gene into the body. The Tissue regeneration cycle is shown in Fig. 1. Definition of the scaffold is categorised into two main category-(a) cell delivery scaffold and (b) drug delivery scaffold. When cells are often implanted or seeded into an artificial structure, capable of supporting three-dimensional tissue formation, these structures typically called cell delivery scaffold and when Drugs are loaded into 3D artificial porous structure capable of high drug loading efficiency and sustained release of drug for longer duration. These structures, typically called drug delivery scaffolds. Different forms of polymeric scaffolds for cell/drug delivery are available: (A) a typical 3-D porous matrix, (B) a nanofibrous matrix, (C) a thermo sensitive sol-gel transition hydro gel, and (D) porous microsphere. In these, a typical 3-D porous matrix and nanofibrous matrix are the implantable form and a thermo sensitive sol-gel transition hydro gel, porous microsphere are the injectable form. Scaffold for tissue engineering (cell delivery) have mechanical properties that are sufficient to shield cells from tensile forces without inhibiting biomechanical cues, have possess acceptable biocompatibility, be bioadsorbed at pre determined time period, support cell adhesion and proliferation, facilitating cell-cell contact and cell migration. Tissue regeneration is mainly dependent on cells, scaffolds, bioreactors and signals (Fig. 2).

Importance Of Scaffold Matrices In Cell Delivery

- Scaffolds provide growth of cells either seeded within the porous structure of the scaffold or migrating from surrounding tissue.
- Scaffold matrices can be used to achieve cell delivery with high loading and efficiency to specific sites.
- Scaffold must provide a suitable substrate for cell attachment, cell proliferation, differentiated function, and cell migration.
- To permit the transport of biological signalling factors, nutrients and wastes to allow for cell survival.
- Possess relatively easy process ability and malleability into desired shapes.
- Minimal stimulate the immune or inflammatory responses in vivo
- Highly porous with a large surface/volume ratio which provides high cell attachment

Biomaterials For Scaffold Fabrication

A number of different categories of biomaterials are commonly used as scaffold for cell delivery (tissue engineering) (Fig. 3) like-

(A) Natural polymers

Natural polymers include alginate, proteins, collagens, gelatin, fibrins, albumin, elsinan, pectin (pectin acid), galactan, curdlan, gellan, levan, emulsion, dextran, pullulan, gluten, elastin, fibroin, hyaluronic acid, cellulose, starch, chitosan (chitin), scleroglucan, heparin, silk, chondroitin 6-sulfate, polyhydroxyalkanoates, etc. They can be used as biomaterials for cell/drug/gene delivery purposes. Advantage of natural polymers is their biocompatibility, commercial availability, easy processing and they can more closely mimic the natural extracellular matrix of tissues. But the limitations are short supply, expensive, batch-to-batch variation, and are susceptible to cross-contamination.

Properties, advantages and disadvantages of Natural biomaterials are described in Table 1.
Advantages and disadvantages of Bioceramics are described in Table 3.

Calcium phosphorus oxides, zinc sulphate calcium phosphate or resorbable (noninert) like calcium phosphates, aluminium calcium phosphates, coralline, tricalcium phosphates (3CaO.P2O5) are examples of bioceramics. They have good mechanical properties, Biocompatible, Biodegradable, Non-toxic, Biofunctional, Bioadhesive materials. Inducing rapid bone regeneration at initial stages. Bone formation occurs over a long period.

(b) Bioactive or surface active (semi-inert) like calcium phosphates, aluminium calcium phosphates, coralline, tricalcium phosphates (3CaO.P2O5) are also used. They have good mechanical properties, Biocompatible, Biodegradable, Non-toxic, Biofunctional, Bioadhesive materials. They cannot be completely replaced after implanting these matrices occurs over a long period.

Advantages of Synthetic biomaterials are described in Table 2.

Synthetic polymers are largely divided into two categories: biodegradable and Nonbiodegradeable. Biodegradable polymers are polyglycolide, polylactide and its copolymer poly (lactide-co-glycolide), polyphosphazene, polyanhydrides, polyorthoesters, polyhydroyethyimethacrylate, and poly (N-isopropylacrylamide). Advantage of this scaffold is easily controlled physicochemical properties and quality, no immunogenicity, processed with various techniques and consistently supplied in large quantities. Polyvinyl alcohol, poliglyco, polyglycolide, polylacticco-glycolide, polyphosphazene, polyanhydride, poly (propylene fumarate), polyorthoesters, polyhydroyethyimethacrylate, and poly (N-isopropylacrylamide). Poor mechanical properties, Brittle, Poor mechanical properties, Poor mechanical properties.

(B) Synthetic polymers

Synthetic polymers are largely divided into two categories: biodegradable and Nonbiodegradeable. Biodegradable polymers are polyglycolide, polylactide and its copolymer poly (lactide-co-glycolide), polyphosphazene, polyanhydrides, polyorthoesters, polyhydroyethyimethacrylate, and poly (N-isopropylacrylamide). Advantage of this scaffold is easily controlled physicochemical properties and quality, no immunogenicity, processed with various techniques and consistently supplied in large quantities. Polyvinyl alcohol, poliglyco, polyglycolide, polylacticco-glycolide, polyphosphazene, polyanhydride, poly (propylene fumarate), polyorthoesters, polyhydroyethyimethacrylate, and poly (N-isopropylacrylamide). Poor mechanical properties, Brittle, Poor mechanical properties, Poor mechanical properties.

Table 1. Properties, advantages and disadvantages of Natural biomaterials used as scaffolds for cell delivery

<table>
<thead>
<tr>
<th>Scaffold</th>
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<th>Advantages</th>
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<tbody>
<tr>
<td>(1) Chitosan</td>
<td>Chitosan, the fully / partially deacetylated form of chitin. Its wide variety of application ranging from skin, cartilage, bone and vascular grafts to substrates mammalian cell culture. Fibrin a complex network formed by polymerization of fibrinogen in the presence of the enzyme thrombin.</td>
<td>Biologically renewable, Biodegradable, Biocompatible, Non-antigenic, Non-toxic, Biofunctional, Bioadhesive materials.</td>
<td>Inducing rapid bone regeneration at initial stages. Bone formation occurs over a long period.</td>
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<td>(2) Fibrin</td>
<td>Fibroin is a fibrous protein constituting the core of silk, while sercin is a glue-like protein surrounding fibrin.</td>
<td>Induce improved cellular interaction, High biocompatibility</td>
<td>Rapid degradation in vivo. Difficult to maintain structural integrity.</td>
</tr>
<tr>
<td>(3) Silk fibroin</td>
<td>Derived from collagen, Insoluble in water</td>
<td>Biocompatibility, Slow degradability, Excellent mechanical properties</td>
<td>Spider silk production very less</td>
</tr>
<tr>
<td>(4) Gelatin</td>
<td>Component of natural extra cellular matrix(ECM)</td>
<td>Biodegradability and biocompatibility in physiological environment, Low antigenicity</td>
<td>Poor mechanical properties, Brittle</td>
</tr>
<tr>
<td>(5) Collagen</td>
<td>Component of natural ECM, Role in natural Wound healing</td>
<td>Biocompatible, Good cell recognition</td>
<td>Poor mechanical properties</td>
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<td>(6) Hyaluronic acid</td>
<td>Origins from seaweed, Structurally similar to natural glycosaminoglycan.</td>
<td>Biocompatible, Easily functionalized, Good cell recognition</td>
<td>Poor mechanical properties</td>
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<tr>
<td>(7) Alginate</td>
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</table>

(B) Synthetic polymers

Synthetic polymers are largely divided into two categories: biodegradable and Nonbiodegradeable. Biodegradable polymers are polyglycolide, polylactide and its copolymer poly (lactide-co-glycolide), polyphosphazene, polyanhydrides, polyorthoesters, polyhydroyethyimethacrylate, and poly (N-isopropylacrylamide). Advantage of this scaffold is easily controlled physicochemical properties and quality, no immunogenicity, processed with various techniques and consistently supplied in large quantities. Polyvinyl alcohol, poliglyco, polyglycolide, polylacticco-glycolide, polyphosphazene, polyanhydride, poly (propylene fumarate), polyorthoesters, polyhydroyethyimethacrylate, and poly (N-isopropylacrylamide). Poor mechanical properties, Brittle, Poor mechanical properties, Poor mechanical properties.

Table 2. Properties, advantages and disadvantages of Synthetic biomaterials used as scaffolds for cell delivery

<table>
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<tr>
<td>(1) Bulk Biodegrad- able polymers like (poly lactic acid , poly glycolic acid, poly lacticco-glycolic acid, poly propylenefuumurate)</td>
<td>Mechanical and degradation properties, Can be tuned by varying polymer segments</td>
<td>Excellent Biocompatibility, Good Biosorabable, Excellent biodegradation rate.</td>
<td>Produce local acidic condition from degradation products. Poor cell adhesion, Poor compression strength</td>
</tr>
<tr>
<td>Poly(ethylene glycol)</td>
<td>Used as an injectable gel, Mechanical and degradation properties can be tuned by varying polymer segments</td>
<td>Biocompatible, Hydrophilic</td>
<td>Poor cell adhesion</td>
</tr>
<tr>
<td>2) Surface bioero-dible polymers (polyorthoesters, polyanhydrides, polyphosphazene)</td>
<td>Commonly used in advanced drug delivery due to its surface erosion properties</td>
<td>Excellent biocompatibility, Retention of mechanical integrity possible</td>
<td>They cannot be completely replaced by new bone tissue</td>
</tr>
</tbody>
</table>

(C) Bioceramics

Melting of inorganic raw materials to create an amorphous or crystalline solid body, which is known as bioceramics, and these Porous final products are mainly used for scaffolds. Bio ceramics classified as (a) nonresorbable (relatively inert) like Alumina, zirconia, silicon nitride, (b) bioactive or surface active (semi-inert) like glass ceramics, such as dense hydroxyapatites [9CaO.Ca (OH)2 .3P2O5], and biodegradable or resorbable (noninert) like calcium phosphates, aluminium calcium phosphates, coralline, tricalcium phosphates (3CaO.P2O5), zinc calcium phosphorus oxides, zinc sulphate calcium phosphates, ferric calcium phosphorus oxides, and calcium aluminates etc. Properties, advantages and disadvantages of Bioceramics are described in Table 3.
Table 3. Properties, advantages and disadvantages of Bioceramics used as scaffolds for cell delivery

<table>
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<tr>
<td>(1) Calcium phosphates</td>
<td>Found naturally as a component of mineral phase of bone Compositional similarity to mineral phase of bone.</td>
<td>Excellent Biocompatibility Good osteoconductivity, Adequate mechanical strength</td>
<td>Slowly degradable, Brittle, Non resorbable, Poor mechanical properties</td>
</tr>
<tr>
<td>(2) Bioactive glasses and glass ceramics</td>
<td>Show the capability to bond to both bone and soft tissue and to stimulate bone growth</td>
<td>Good biocompatibility, Good osteoconductivity, Tailorable resorption, Good angiogenic, Regulation of gene expression in osteoblasts, Adequate mechanical strength</td>
<td>Slowly degradable (crystalline structures), Brittle (amorphous structure)</td>
</tr>
</tbody>
</table>

(D) Composites
Due to some of the problems associated with using scaffolds synthesised from a single phase biomaterial (poor mechanical properties and biocompatibility of natural and synthetic polymers respectively, and poor degradability of bio ceramics), a number of researchers have developed composite scaffolds comprising two or more phases to combine the advantageous properties of each phase. When the Combinations of (1) synthetic– synthetic, (2) synthetic –natural and (3) natural–natural polymers combination then it have Ability to tailor mechanical, degradation and biological properties. But Compromise between ‘best’ qualities of individual polymers with overall scaffold properties”. Properties, advantages and disadvantages of Composites are described in Table 4.

Table 4. Properties, advantages and disadvantages of Composites used as scaffolds for cell delivery

<table>
<thead>
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<tr>
<td>(1)Polymer-Ceramic</td>
<td>Natural or synthetic polymers combined with ceramics often combined for bone tissue engineering applications</td>
<td>Ability to tailor mechanical, degradation and biological properties</td>
<td>Fabrication techniques can be complex</td>
</tr>
<tr>
<td>(2)Polymer – Polymer</td>
<td>Combinations of (1) synthetic– synthetic, (2) synthetic –natural and (3) natural– natural polymers possible</td>
<td>Good biocompatibility, Good osteoconductivity, Tailorable degradation rate, Improved mechanical properties</td>
<td>Compromise between ‘best’ qualities of individual components with overall scaffold properties</td>
</tr>
</tbody>
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SCAFFOLD FABRICATION TECHNIQUE
A variety of techniques have been used for processing biodegradable polymers into 3-D porous scaffolds. The conventional methods include fiber bonding, melt molding, solvent casting/particulate leaching, gas foaming/particulate leaching, phase separation, and high-pressure processing, Electrospinning and rapid prototyping etc (Fig. 4). Some of the important techniques are described below-

Emulsification/freeze-drying method
Scaffolds are generally prepared by dissolving/suspending polymers/ceramics in water or in an organic solvent followed by emulsification with a water phase. After pouring this mixture into a mould, solvents are removed by freeze-drying and porous structures areobtained.

This method allows a faster preparation but pore size is relatively small and porosity is often irregular. Using this technique, hang et al. have prepared PLGA scaffolds with porosity of up to 95% and pore sizes of up to 200 μm²⁶.

Gas foaming method
Sieved effervescent salt particles (Ammonium bicarbonate) in the form of polymer gel paste was cast in a mould and subsequently immersed in hot water. The evolution of ammonia and carbon dioxide gas, along with the leaching out of ammonium bicarbonate particulates from the solidifying polymer matrix, resulted in the formation of pores (100 to 200 μm) with high inter-connectivity. Nam et al. Synthesised poly (lactic acid) [PLA] scaffolds using ammonium bicarbonate which acted as both a gas foaming agent and as a solid salt porogen.²⁶

Particulate leaching method
Particulate leaching methods categorised into two categories: (A) solvent casting–particulate leaching and (B) Melt moulding–particulate leaching. In Solvent casting–particulate leaching, a polymer dissolved in a solvent is mixed with salt particles in a mould; the solvent is then evaporated to give a polymer monolith embedded with the salt particles, these are then removed by washing the scaffold with water, resulting in the formation of a porous scaffold. In Melt moulding–particulate leaching, where the polymer is cast into a mould with the embedded solid porogen. The polymer is set by applying heat and pressure, and again the porogen is leached away by washing the resulting product with water to yield a porous polymer scaffold. This approach allows the preparation of porous structures with regular porosity, but with a limited thickness²⁶,⁷.

Supercritical fluid technology
The dry polymer is dissolved in super critical carbon dioxide to forms single phase polymer /gas solution. The pressure is then reduced to create thermodynamic instability of the dissolved CO₂ and results in nucleation and growth of gas cells to generate pores within the polymer matrix. The porous structure is quite uniform and higher mechanical strength and useful for incorporation of heat sensitive bio molecules. Mooney et al. utilized this technique to fabricate highly porous scaffold of PLGA¹²,³⁶,⁷.

Electrospinning
The droplet of polymer solution obtained gets sprouted followed by solvent evaporation leading to the formation of fine fibers that mats in to porous scaffold. Developed a novel PLGA nanofibrous mesh with fiber diameter ranging from 500 to 800 nm and pores that were well inter-connected. The limitation of this method is that they produced 2-D mesh structure with a nanoscale pore size which is not suitable for cell seeding and infiltration⁷.

Sol-gel technique
Scaffolds are prepared by dissolving inorganic metal salts or metal organic compounds in a solvent where a series of hydrolysis and polymerization reactions allow the formation of a colloidal suspension (‘sol’), after casting the ‘sol’ into a mould, a wet ‘gel’ is formed, with further drying and heat treatment, the ‘gel’ is converted into dense ceramic or glass articles. They are used for construction...
The techniques discussed above can also be combined with each other depending on the exact requirements of the scaffold, e.g. phase separation (freeze drying) techniques can be combined with emulsion templating processes. For example, Whang et al. created an emulsion that was quenched using liquid nitrogen, which was then freeze dried to produce porous PLGA polymeric monoliths. 

**APPLICATION OF SCAFFOLD: MATRICES/ SCAFFOLD FOR CELL DELIVERY**

In these, the cells with growth factor are encapsulated or seeded into the scaffold, and administered into the body. Local and sustained delivery of paracrine factors, either by inducing or inhibiting cell proliferation, survival, migration and/or differentiation, may greatly enhance tissue remodelling or organogenesis. Growth factors can be incorporated into the scaffold matrix either by bulk encapsulation, specific or non-specific surface adsorption, and adding microspheres encapsulating them. 

**Combination of techniques**

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**CONCLUSION AND FUTURE STUDIES**

Scaffold provides adequate signals (e.g., through the use of adhesion peptides and growth factors) to the cells, to induce and maintain them in their desired differentiation stage and for their survival and growth. Thus, equal effort should be made in developing strategies on how to incorporate adhesion peptides and growth factors into the scaffolds to influence cell behaviour, and to establish the concentrations and distributions required for successful outcomes. Additionally, the incorporation of drugs (i.e., inflammatory inhibitors and/or antibiotics), into scaffolds may be used to prevent infection after surgery. The field of biomaterials has played a crucial role in the development of tissue engineered products. An alternative to using prefabricated scaffolds is to use a polymer system that is injected directly into the defect site which is polymerised in situ using either heat, thermo responsive polymers, or light (photo responsive polymers). The advantages for the patient with this approach are that injectable delivery systems fill both regularly and irregularly shaped defects, and so "get a custom fit", they represent a minimal invasive procedure therefore avoiding surgery and its potential risks, eliminate the need for donor tissue or a donor site, and waiting time for treatment is reduced. At present, there is a vast amount of research being performed on all aspects of tissue engineering/ regenerative medicine worldwide. As the field progresses, one of the key challenges is to try to mimic the sophistication of the natural ECM more accurately in synthetic substitutes. As more advanced biomaterials and bioreactors are developed, and as research leads to more knowledge of the cell signalling mechanisms required to trigger the chain of tissue development, we will approach our goal of reducing the number of patients waiting for donor tissues.

**REFERENCES**


Fig 1. Tissue Regeneration Cycle

Fig 2. Tissue Engineering Factors (Bioreactors, Scaffolds, Cells and Signals)
Cells like stem cells co-culture of cells with signals like chemical (growth factor), electrical and mechanical.

Poured into porous scaffold

Cell expansion

Tissue culture in vitro

Fig 5. Matrices/scaffold for Cell delivery used in different Tissue Eng. Application

Porous scaffold
Angiogenic growth factor

Angiogenesis → Blood vessels

(a) Scaffolds delivering Angiogenic growth factor to Induce Blood vessel

Chondrocytes

Bone and Cartilage Regeneration

Bone

(b) Scaffold seeded with Chondrocytes for Bone and Cartilage Regeneration

Wound dressing materials

Injury

Wound Repair → Regenerated Skin

(c) Growth factor Releasing film as Wound dressing Materials

Fig 6. Multifunctional scaffold for regeneration of various tissues