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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[1]. 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[2]. 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[3,4]

- 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 (pectinic acid), galactan, curdlan, gellan, levan, emulsan, dextran, pullulan, gluten, elastin, fibroin, hyarulonic 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[5]. Properties, advantages and disadvantages of Natural biomaterials are described in Table 1.

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

Scaffold	Properties	Advantages	Disadvantages
(1) Chitosan	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.	Biologically renewable , Biodegradable, Biocompatible, Non-antigenic, Non-toxic, Biofunctional, Bioadhesive materials.	Inducing rapid bone regeneration at initial stages. Bone formation after implanting these matrices occurs over a long period.
(2) Fibrin	Fibrin a complex network formed by polymerization of fibrinogen in the presence of the enzyme thrombin.	Induce improved cellular interaction, High biocompatibility	Rapid degradation in vivo. Difficult to maintain structural integrity.
(3) Silk fibroin	Fibroin is a fibrous protein constituting the core of silk, while sericin is a glue-like protein surrounding fibroin.	Biocompatibility, Slow degradability , Excellent mechanical properties	Spider silk production very less
(4) Gelatin	Derived from collagen, Insoluble in water	Biodegradability and biocompatibility in physiological environment, Low antigenicity	Poor mechanical properties, Brittle
(5) Collagen	Component of natural extra cellular matrix(ECM) Component of natural ECM, Role in natural Wound healing	Biocompatible, Good cell recognition	Poor mechanical properties Poor mechanical properties
(6) Hyaluronic acid	Originates from seaweed, Structurally similar to natural glycosaminoglycan.	Biocompatible, Easily functionalized, Good cell recognition	Poor mechanical properties
(7) Alginate		Biocompatible, Simple gelation methods	

(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, polyanhydride, poly (propylene fumarate), polycyanoacrylate, polycaprolactone, polydioxanone, and polyurethanes etc. and nonbiodegradeable polymers include polyvinyl alcohol, polyhydroxyethymethacrylate, 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[6]. Properties, advantages and disadvantages of Synthetic biomaterials are described in Table 2.

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

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

(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 [$9CaO.Ca(OH)_2 .3P_2O_5$],, and biodegradable or resorbable (noninert) like calcium phosphates, aluminium calcium phosphates, coralline, tricalcium phosphates ($3CaO.P_2O_5$), zinc calcium phosphorus oxides, zinc sulphate calcium phosphates, ferric calcium phosphorus oxides, and calcium aluminates etc[6]. 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

Scaffold	Properties	Advantages	Disadvantages
(1) Calcium phosphates (Hydroxyapatite, Tricalcium Phosphate)	Found naturally as a component of mineral phase of bone Compositional similarity to mineral phase of bone.	Excellent Biocompatibility Good osteoconductivity, Adequate mechanical strength	Slowly degradable , Brittle, Non resorbable, Poor mechanical properties
(2) Bioactive glasses and glass ceramics (bioglass, phosphate glasses)	Show the capability to bond to both bone and soft tissue and to stimulate bone growth	Good biocompatibility , Good osteoconductivity, Tailorable resorption, Good angiogenetic , Regulation of gene expression in osteoblasts, Adequate mechanical strength	Slowly degradable (crystalline structures), Brittle(amorphous structure)

(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[6]. 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

Scaffold	Properties	Advantages	Disadvantages
(1)Polymer-Ceramic	Natural or synthetic polymers combined with ceramics often combined for bone tissue engineering applications	Ability to tailor mechanical, degradation and biological properties	Fabrication techniques can be complex
(2)Polymer –Polymer	Combinations of (1) synthetic– synthetic, (2) synthetic –natural and (3) natural–natural polymers possible	Good biocompatibility , Good osteoconductivity Tailorable degradation rate, Improved mechanical properties	Compromise between 'best' qualities of individual components with overall scaffold properties

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 μm[2,6].

Gas foaming method

Sieved effervescent salt particles (Ammonium bicarbonate) in the form of polymer gel paste was cast in a mold 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[1,2,6].

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 thickness[2,6].

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 CO_2 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 PLGA[1,2,6,7].

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 infiltration[2].

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

of biomedical sensors, laser materials or for delayed drug delivery. The disadvantages are high cost of raw materials, large shrinkage during processing, residual fine pores, residual hydroxyl, residual carbon, health hazard of organic solution long processing time[8].

Melt moulding technique

Scaffolds are prepared by melting polymers/ceramics in the presence of porogens (such as sodium chloride, sugar crystals), once the mixture is cooled, porosity is achieved by dissolving the porogens in water; finally, the porous scaffolds are usually lyophilized. The advantage of this technique is independent control of porosity and pore size, macro shape control and disadvantages is high temperature required for nonamorphous polymers and residual porogens. Thompson et al in 1995 they used the compression moulding principle where a teflon mould was used with PLGA and gelatin micro spheres[9].

Combination of techniques

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[10].

APPLICATION OF SCAFFOLD: MATRICS/ 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[11] (Fig. 5). Tissue engineering application of Polymer(s)/carrier/scaffold structure with Active bio molecule and Encapsulated/seeded cell type are described in (table 5) with diagrammatically results (fig. 6).

Table 5. Tissue engineering application of Polymer(s)/carrier/scaffold structure with Active bio molecule and Encapsulated/seeded cell type

TYPE OF APPLICATION	POLYMER(S)/ CARRIER/ SCAFFOLD STRUCTURE	ACTIVE BIOMOLECULE	ENCAPSULATED/ SEEDED CELL TYPE	RESULTS
Bone formation[12]	Chitosan, Chitosan-PLLA composite	Platelet derived growth factor-BB	Rat calvarial osteoblast	Scaffolds promoted bone healing and regeneration for 10 days in Rat
Bone formation (osteogenisis)[13]	PLGA scaffold	Dexamethasone, Ascorbate-2-phosphate	Human marrow stromal cell	In vivo bone formation into athymic mice
Osteochondral tissue engg.[14]	Silk fabroin microsphere impregnated alginate scaffold	Growth factor: bone morphogenetic protein 2,insulin like growth factor 1	Human bone marrow derived mesenchymal stem cell	Exhibited osteogenic and chondrogenic differentiation within 5 weeks.
Dental pulplike tissue engg.[15]	Collagen scaffold	Dentin matrix protein 1	Human dental pulp stem cell	Newly derived pulp tissue was seen in the six weeks in mice.
Heart valve[16]	Acellular aortic valve scaffold	----------	Myofibroblast cell, Endothelial cell	The valve leaflets were completely reconstructed at the end of the 10th week in vivo.
Periodontal Regeneration[17]	Glycidyl methacrylated dextran/gelatin scaffold	Bone morphogenetic proteins	Human periodontal ligament cell	Periodontal regeneration after 4 weeks implantation
In diabetics[18]	PLGA scaffold	----------	Islet like cell from human embryonic stem cell	Continuous release insulin for 7 weeks in mice.
Ligament replacement[19]	Polylactide-co-glycolide	----------	Anterior cruciate ligament	Growth of ligament tissue with in 1 week
Chondrogenic differentiation[20]	PEG AND PCL Scaffold	----------	Rabbit chondrocytes	Growth of new cells within 3 weeks in rabbit

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 minimally 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.

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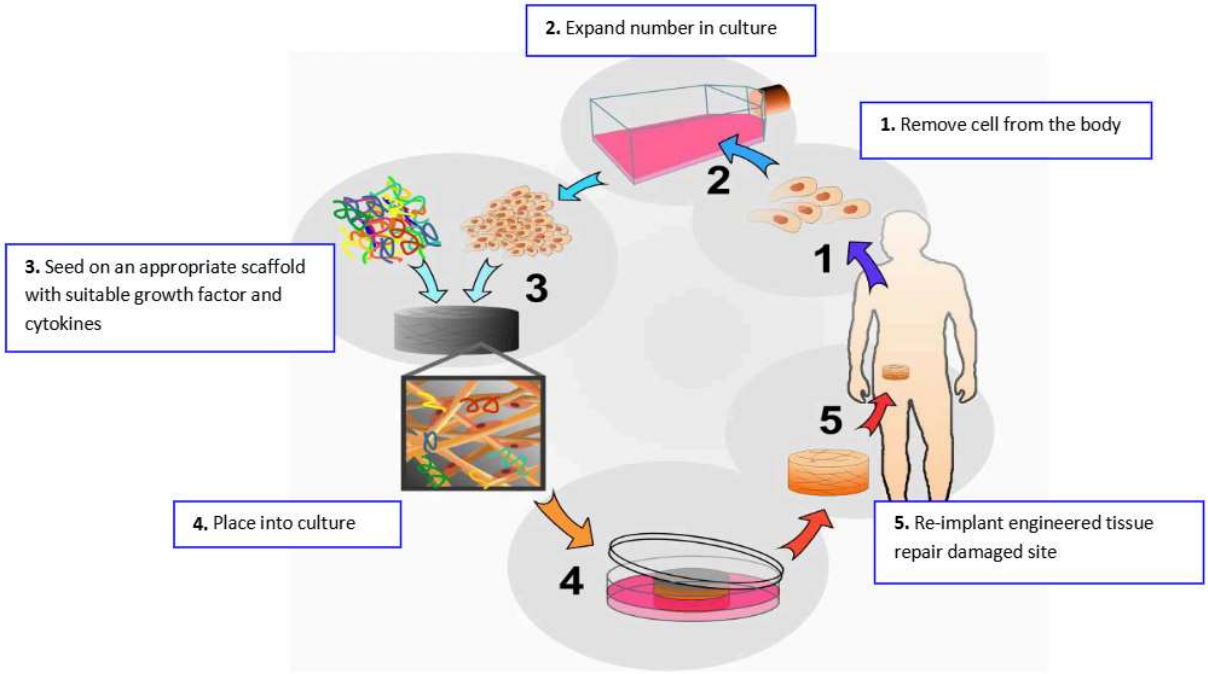


Fig 1. Tissue Regeneration Cycle

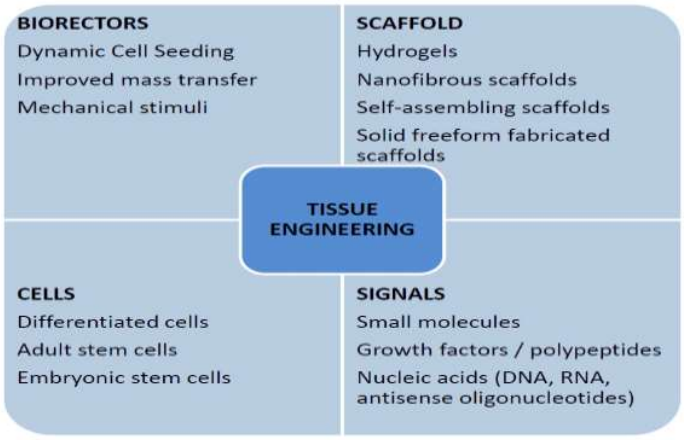


Fig 2. Tissue Engineering Factors (Bioreactors, Scaffolds, Cells and Signals)

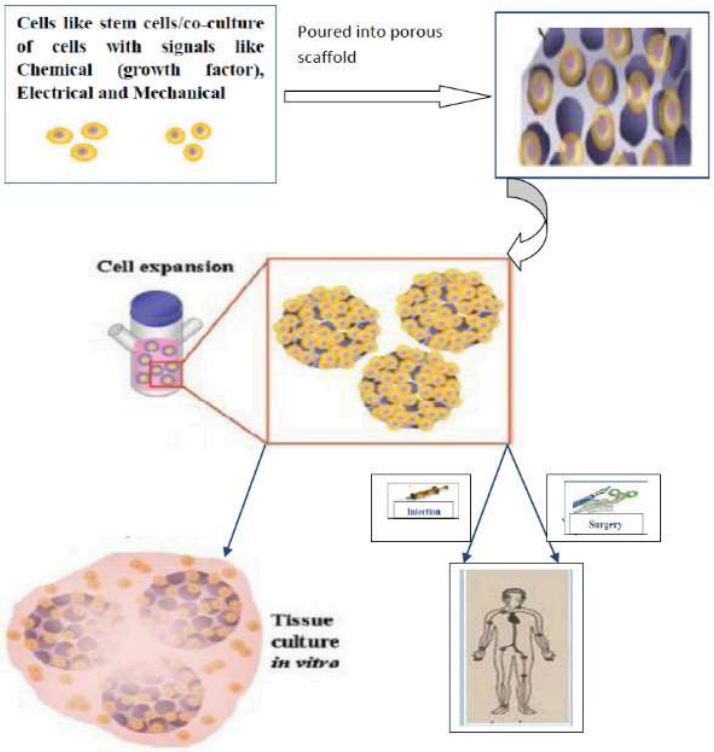
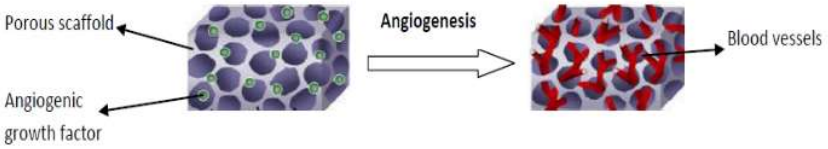
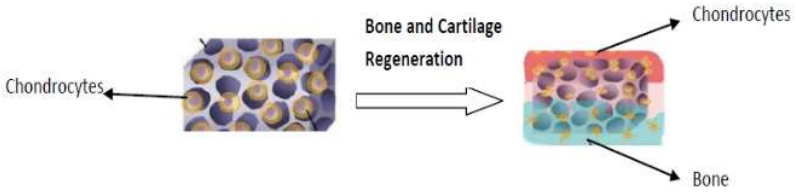


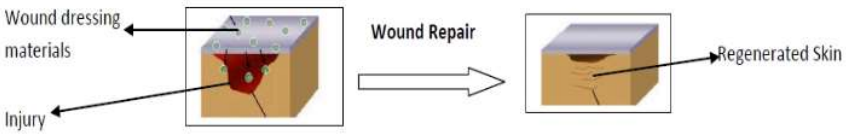
Fig 5. Matrics/ scaffold for Cell delivery used in different Tissue Eng. Application



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



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



(c) Growth factor Releasing film as Wound dressing Materials

Fig 6. Multifunctional scaffold for regeneration of various tissues