BIOMATERIALS AS POROUS SCAFFOLDS FOR TISSUE ENGINEERING APPLICATIONS: A REVIEW

Fatema Tuj Zohora
Bangladesh University of Textiles, Tejgaon, Bangladesh
Abu Yousuf Mohammad Anwarul Azim
Lecturer, Department of Textile Engineering, Primeasia University, Banani, Bangladesh

Abstract

In tissue engineering, biomaterials play a critical role, act as a 3D template, provide mechanical support, and give artificial extracellular matrix environment (ECM) for neo-tissue formation. Only one type of biomaterials is not enough for both hard and soft tissue engineering. That's why all types of biomaterials i.e. metals, ceramics, & polymers have their own importance in making tissue engineering scaffolds. This article presents a brief overview on all types of biomaterials (metals, ceramics, natural and synthetic polymers, composite materials, & hydrogels) that are being developed for soft or hard tissue engineering with their properties, limitations, current developments and future challenges in tissue engineering applications. This article also represents brief discussion on tissue engineering triad i.e. scaffolds, signal, & cells.

Keywords: Biomaterials; Tissue Engineering; Scaffold; Signal & Cells

Introduction

In the human body, degeneration of tissues or tissue defects may occur due to diseases, trauma, accidents, aging or congenital defects, in which case treatments are necessary to facilitate the tissues repair, regeneration or replacement (Fergal, 2011). Typical treatments focus on organ or tissue transplantation as an autograft, allograft, and sometimes isograft & xenograft. Harvesting autografts (obtained from the host), depend on blood supply & need to avoid visceral injuries (Mekala, 2012), where allografts (from one individual to another in the same species) & xenografts (obtained from another species) own the problems associated with infection or disease transmission and immune rejection of the implants (Flanagan, 2006). Another problem with these treatments is dearth of organ donor.

Today tissue engineering has emerged as a rapidly expanding approach to overcome the drawbacks with these classical treatments by regenerating damaged tissues, instead of replacing them (Fergal, 2011 & Mekala, 2012). This approach lead to the development of biomaterials to prepare porous 3D scaffolds as biological substitutes to restore, maintain, or improve defective tissues (B.P. Chan, 2008 & Karp, 2007).Various materials including metals, natural and synthetic polymers, ceramics and even their composites have been developed as tissue engineering scaffolds. While bioceramics & polymers are suitable for bone tissue engineering as native bone consists of a naturally occuring polymer and biological apatite, but ceramics are brittle and polymers do not show enough mechanical properties which limited their applications in load-bearing areas. In contrast, metals are suitable for load-bearing applications because of their high mechanical strength. But metals are bio-inert, generally lack biodegradability and are not suitable for soft tissue engineering. On the other hand, hydrogels synthesized by ionic or covalent cross-linking can encapsulate proteins or bioactive molecules and release them by a mechanism manipulated by swelling of the hydrogels (Berger, 2004). So selection of appropriate scaffolding materials is necessary for successful tissue engineering.

Tissue engineering: An overview

In 1988, the National Science Foundation Workshop first coined the term 'Tissue Engineering' to mean "the application of principles and methods of engineering and life sciences toward the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues & the development of biological substitutes to restore, maintain, or improve tissue function" (Skalak, 1989). Since then, as a new discipline and potential medical treatment, tissue engineering has made rapid advances that holds promises of (1) eliminating re-operations by using biological substitutes which are biodegradable, (2)solve problems of immune rejection of implants, infections or diseases transmission associated with allografts & xenografts , and shortage of organ donation, (3) initiating the natural regeneration process with biological substitutes to repair or replace lost or damaged tissues i.e. to provide long term solutions, (4) offering potential treatments for currently untreatable medical conditions (Zippel, 2010 & Min Wang 2006). Tissue engineering strategies involve combining living cells with a natural/synthetic support or scaffold to develop a biological substitute or 3D living construct which is structurally, mechanically and functionally equal to or better than the tissue that is to be replaced (Zippel N, 2010 & K.M. Kim, 2005). To develop such tissue engineering construct requires careful selection of three major components: (1) scaffolds, (2) signaling factors, & (3) cells (Brochhausen, ISBN:978-953-

307-079-7); generally referred to as tissue engineering triad (B.P. Chan, 2004). Porous 3D scaffolds are generally seeded with cells and occasionally with signaling molecules or subjected to biophysical stimuli in the form of a bioreactor (I.Martin, 2004). These cell-seeded scaffolds are either undergo a pre-implantation differentiation culture in vitro, to synthesize tissues and then transplanted or are directly implanted into the injured site, using the body's own systems, where tissue regeneration is induced in vivo (Fergal, 2011). The general tissue engineering approaches with porous scaffolds are shown in below by figure 1.

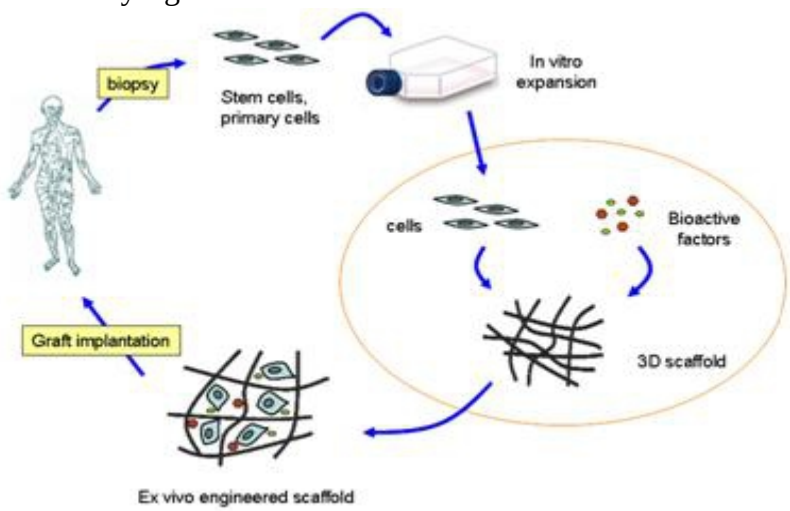

Figure 1: Tissue engineering (nuhs.edu.sg)

Scaffold: Tissue engineering scaffolds mimic the 3D environment of the natural extracellular matrix (ECM), provide short term mechanical support of the transplant, & provide an increased surface area for cells adhesion, proliferation, migration, and differentiation, eventually leading to neo-tissue formation (Zippel, 2010). As blood vessels grow into the new tissue, the scaffold degrades and replaced by the new tissue (Zippel, 2010).

Key factors concerning scaffolds for use in tissue engineering

Biocompatibility: In case of tissue engineering, the biocompatibility of a scaffold or an artificial ECM refers to the ability to perform as a 3D substrate that will possess the right surface chemistry (with the faciliation of molecular and mechanical signaling system) to promote cell adhesion, proliferation, and migration in vitro (Wang HM, Chen CH et al. PLoS ONE 8(11):10.1371). And after implantation, the scaffold must not provoke any undesirable immune reaction that may reduce healing or cause rejection by the body (Fergal, 2011).

Biodegradability: It is one of the crucial factors for scaffolds because by gradual degradation it helps to make space for growing tissues to deposit their own matrix (J.E. Babensee, 1998) and hence, avoids the necessity of second surgery to remove the implant. That is an ideal scaffold should be able to degrade with time in vivo, but at a rate almost proportional to the rate of the tissue formation. The degradation products of the biodegradable materials should be non-toxic to other tissues in vivo. There are two distinct modes of degradation. The first type of biodegradation is bulk erosion, erosion happens throughout the device and it occurs when the rate at which water penetrates, exceeds the rate of conversion of the polymer into water soluble materials (J.C. Middleton, 2000 & J.Kohn, 1996).The second is, surface erosion that occurs when the rate at which water penetrates the device is very slower than the rate of conversion of the polymer into water soluble materials (J.C. Middleton, 2000 & J.Kohn, 1996).Surface erosion happens layer by layer while maintaining its bulk integrity. It is often referred to as bioerosion rather than biodegradation (M.E. Gomes. 2004).

Structural requirements: An ideal scaffold should have void volume for vascularization, neo tissue formation & remodeling, necessary to facilitate host tissue integration upon implantation (B.P. Chan, 2008). Biomaterials should be processed to provide a highly porous structure with interconnected porosity for transporting oxygen, nutrients and waste metabolites in and out of the scaffold without significantly compromising the mechanical stability of the scaffold (B.P. Chan, 2008). A scaffold with too small pore size may enact the cells to penetrate the scaffold initially and subsequently to migrate through these pores to the other regions of the scaffolds where the too large pore size may inhibit the effective neo-tissue regeneration by disabling the cells to bridge pores during cell proliferation (Edwards, 2004). Generally smooth surfaces exhibit less cell adhesion than rough surfaces. Young's modulus as well as Yield, compressive, & flexural strength is greatly related to the pore volume and size i.e. mechanical properties generally reduces with increasing porosity.

Mechanical Properties: An ideal scaffold should have enough mechanical strength to retain shape, to support growing tissues, & withstand in vivo forces (Edwards, 2004). During implantation it must possess enough strength to allow surgical handling.

Manufacturing technology: During developing tissue engineering scaffolds one should concern about the biomaterials and fabrication techniques. Another important consideration is to produce a large quantity of scaffolds at a relatively low or reasonable cost i.e. to turn them suitable for commercialization (Edwards, 2004).

Cells: According to the source, cells are generally classified as follows:

Autologous cells: Obtained from the same individual to whom they will be re-implanted (Cherian, 2011). Transplantation of autologous cells minimizes the immune complications like pathogen transmission and immune rejection which makes them an ideal source for use in tissue engineering (Tran, 2003). However, in some cases, this type of cells might be unavailable like genetic diseases(Cherian, 2011). Also very ill patient, severely burnt patient and even an elderly person may lack affordable quantities of cells to establish useful cell lines (Cherian, 2011). Further, Autologous solutions may not be very quick (Cherian, 2011) & are not cost effective (Knight, 2004).

Allogenic cells: Cells are isolated from the body of a human donor i.e. from the same species but are immunologically inidentical (Kim et al, 2005). Autologous cells offer some advantages like uniformity, standardization of procedure and are cost effective (Kim et al, 2005).

Xenogenic cells: Cells are obtained from donors of different species (Cherian, 2011 & Kevin, 2014). For instance, animal cells like bovine, equine, and porcine tissues have been used extensively for cardiovascular implants (Kevin, 2014).

Isogenic or Syngenic cells: Isolated from clones, twins who are genetically identical (Cherian, 2011).

Stem cells: Stem cells are undifferentiated cells (Cherian, 2011), have the ability to self-renewal, & cell potency. Stem cells, according to their plasticity or developmental versatility, can be classified into three groups: (Cherian, 2011 & www.aaas.org)

1. **Totipotent stem cells:** Totipotent stem cells are early embryonic cells of 1-3 days from oocyte fertilization. It has the potential to form all cell types in a body, also extra embryonic or placental cells. An entire functional organism is possible by this type of cell.
2. **Pluripotent stem cells:** The totipotent stem cells are continues to divide & specialized further into pluripotent stem cells, approximately 4days after fertilization & up to 14^{th} days. Pluripotent stem cells can differentiate into any of the three germ layers: endoderm, mesoderm, and ectoderm but like totipotent stem cells can not give rise to an entire organ.
3. **Multipotent stem cells:** Embryonic cells, specialized further from the pluripotent stem cell division. Multipotent stem cells can differentiate into multiple but limited cell types.

Stem cells are broadly divided into two groups:

1. **Adult stem cells/Somatic stem cells:** Adult stem cells are multipotent stem cells (Pandit N, 2011),found in specific niches or tissue compartments. They are available in various sources like trabecular, bone, muscle, blood, bone marrow, liver, skin, cornea, and

retina of the eye, dental pulp, GI tract, & pancreas (Kim et al, 2005). At least twenty major categories of adult stem cells have been identified in mammals (Kim et al, 2005). Recently some evidence proves that adult stem cells have greater plasticity than was thought before.

2. **Embryonic stem cells:** They are totipotent cells (Pandit N, 2011) and isolated from the inner cell mass of blastocysts (Kim et al, 2005). But its use has been limited to the tissue engineering research field due to ethical concerns regarding use of human embryonic stem cells and the potential tumorogenicity & immunological incompatibilities (Pandit N, 2011).

Signaling molecules: Signaling molecules provide stimuli for cell adhesion, growth, differentiation, vascularization, & other functions. There are several kinds of signaling molecules used such as growth factors, cytokines, hormones, small molecules like neurotransmitters (Aneta, 2010 & Jin Gao, A neuroinductive biomaterial based on dopamine. 103(45):16681-86), proteins, morphogenes, iRNA & e.t.c. Among these signaling molecules, multifunctional proteins like growth factors and cytokines have studied more for tissue engineering applications (Aneta, 2010).

Table 1: Functions of various growth factors.

Growth Factor	Abbreviation	Functions
Platelet-derived growth factor	PDGF-AA PDGF-AB PDGF-BB	Proliferation and chemoattractant agent for smooth muscle cells; ECM synthesis and deposition (Ruth R, 2003).
Epidermal growth factor	EGF	Proliferation of mesenchymal, epithelial, & fibroblast cells (Ruth R, 2003).
Transforming growth factor-α	TGF-α	Migration and proliferation of keratino-cytes; ECM synthesis and deposition (Ruth R, 2003).
Transforming growth factor-β	TGF-β	Stimulates recruitment & proliferation of mesenchymal cells, their differentiation into osteoblasts and /or chondrocytes, & ECM production (Bose, 2012).
Fibroblasts growth factor	aFGF/FGF-1 bFGF/FGF-2	Mesenchymal condensation, Osteogenic differentiation (Brochhausen, ISBN:978-953-307-079-7).
Vascular endothelial growth factor	VEGF	Vascularization, ingrowth of osteoblasts & chondroblasts (Brochhausen, ISBN:978-953-307-079-7).
Bone morphogenetic protein	BMP-2 BMP-7	Critical in embryonic skeletal development, bone formation, maturation, & repair. They differentiate and migrates bone forming cells (Bose, 2012).
Insulin-like growth factor	IGF	Regulates several key cellular process, including proliferation, movement, and inhibition of apoptosis (Bose, 2012).
Hepatocyte growth factor	HGF	Participate in the bone remodeling process(Pandit N, 2011).

Bioreactor: In tissue engineering, a bioreactor, used for 3D cell culture is a device that provides the pre-defined chemical, biochemical, physical, and mechanical environments for the seeded scaffolds so that cells

can proliferate & differentiate to form neo-tissues in vitro (Hua, 2007). However enough oxygen and nutrient supply to the growing thick tissue with removal of waste metabolites, specifically from the center of the construct, has made the 3D dense tissue formation a real challenge because most bioreactors can not deal with these requirements (Hua, 2007 & Leor J, 2005). For instance, human heart muscle is about 1cm thick, but in a bioreactor, tissue growth typically stops when it is about 100 micrometer. Moreover, cells at the innermost position are too far from the supply of fresh growth medium (Leor J, 2005). To overcome the diffusional problems, researchers have developed hollow fiber membrane bioreactors so that the membrane network can replicate the blood capillary system (Hua, 2007).

Biomaterials

Metals: Porous metallic scaffolds are considered as the most suitable implants for hard tissue engineering in load bearing areas as metals have superior fatigue resistance (Garrett, 2006) and high compressive strength, required for load bearing applications such as femur, vertebra, skull, & mandible reconstruction, replacement of hip and knee bone e.t.c. However, metallic scaffolds have some limitations: (1) lacking of biological recognition on the material surface or bioactivity (XIAO, 2012), considered as the main disadvantages of metallic scaffolds, (2) Biomolecules can not be integrated into the metallic scaffolds (Bose S, 2012), (3) Metallic scaffolds are generally not biodegradable (Bose S, 2012),(4) The another concern with metallic scaffolds is the possible release of toxic metallic ions (Bose S, 2012) or particles through corrosion or wear, (5) The control of architecture of porous metallic scaffold is another problem. For some metals that are too weak, it is difficult to create a controlled porous structure while some materials are too stiff to be arranged into certain architectures (Kelly, 2009). An essential requirement of already established biocompatible metal for use in tissue engineering scaffold is the surface modification which reduces some of the limitations of metallic scaffolds (XIAO, 2012 & Kelly, 2009).

Non –biodegradable metals

Ti and Ti alloys: Porous Ti and Ti alloys are considered as the most promising biomaterials for bone tissue engineering scaffolds due to their new bone tissue in-growth capability and lower modulus of elasticity similar to that of natural bone (Li Y, 2009). Ti alloys specifically Ti-6Al-4V is widely used in various orthopedic applications because it possesses good biocompatibility & better mechanical properties than conventional stainless steel, Co-based alloys, & even more than pure titanium (Kelly, 2009 & Li Y, 2009). But Ti-6Al-4V contains an element, vanadium which, when isolated has possessed cytotoxic outcomes that has led the researchers to develop new

β- Ti alloys with nontoxic elements like Nb, Zr, & Ta (Kelly, 2009). Porous Ti-6Ta-4Sn alloy scaffold fabricated with a Space Holder Sintering method was studied by Liu et al as a great feasibility in an orthopedic implant because of its excellent biomechanical properties (Liu Y, 2009). Though Ti is found to be well tolerated and bio-inert it does not bond directly to bone unless the application of bioactive materials that induce a specific biological activity i.e. form a biologically active bone-like apatite layer on the material surface in vivo (Garrett, 2006). Some growth factors like TGF-β, BMP-2, often applied via Ti scaffold to accomplish craniofacial reconstruction or augmentation of bone, enhanced regeneration of bone e.t.c. (Kelly, 2009 & Jansen, 2005). Ti and Ti alloys are non-ferromagnetic, so titanium implanted patient may safely be examined with magnetic resonance imaging (Kelly, 2009).

Nitinol: Nitinol (metal alloy of Ni & Ti with roughly equiatomic parts of Ni and Ti) exhibit a mixture of unique properties where two closely related properties are thermal shape memory effect and superelasticity, & other properties include enhanced biocompatibility, and high damping properties (Garrett, 2006 & Greiner, 2005). These properties made it one of the most promising metallic biomaterials. Shape memory effect allows the researchers to create such a scaffold that can change its shape after implantation, initiated at the temperature of the human body (Kelly, 2009).

Tantalum(Ta): Porous Ta possesses some unique mechanical and physical properties. Its unique mechanical properties are mainly because of its high porosity (>80%) with fully interconnected pores which allows rapid bone in-growth (Kelly, 2009). It has a low modulus of elasticity close to that of natural bone like subchondral and cancellous bone which leads to better load transfer and ultimately less stress shielding phenomenon (Kelly, 2009). Like Ti, Ta is a non-ferromagnetic, do not cause harm to the patient with Ta implant undergoing MRI units. Ta is biologically inert so it is restricted to bond directly to the bone. This obstacle can be overcome by thermal processing of porous Ta in an alkaline environment which forms an extensive hydroxyapatite layer on its surface (Christos, 2012).

Biodegradable metals

Magnesium(Mg): Mg and its alloys have been gaining particular interest as promising biomaterials for bone tissue scaffold due to their superior mechanical properties, fast corrosion (F. Witte, 2007 & Renáta, 2013) in physiological solution, & non-toxic corrosion by-products. Mg and its alloys are very light weight metals having density 1.74-2 g/cm^3 closer to that of natural bone (1.8-2 g/cm^3)(M.P. Staiger, Biomaterials 27:1728-1734). Mg has greater fracture toughness and modulus of elasticity ranging from 41-45 GPa is closer to the bone which is necessary to avoid stress

shielding effect (A.H. Yusop, 2012). Addition of alloying elements and also some thermo-mechanical manufacturing process may improve the mechanical properties of Mg alloys (A.H. Yusop, 2012). However there is a concern about the use of pure Mg which corrodes very rapidly in the physiological environment. So Mg implant may lose its mechanical and structural integrity before completely healing the tissue (Renáta, 2013). In addition, this rapid corrosion reaction produces H_2 at a higher rate that is difficult for the host tissue to deal with. To control the corrosion rate some measures have taken like provided with ceramic coating, titanium coating or the use of Mg alloys (A.H. Yusop, 2012).

Iron(Fe): Fe exhibit high mechanical strength & corrodes very slowly which makes them suitable for higher load carrying implants (P. Quadbeck, 2007). It has been tested in vivo as biodegradable metal with no toxicity during 18 months of study (A.H. Yusop, 2012). The corrosion rates and mechanical properties could be increased by various alloying elements (Renáta, 2013).Chou DT et al have assessed the 3D printed Fe-30Mn (wt.%) scaffold as a bone scaffold material where 3D printed parts were maintained an open porosity of 36.3% and they found that 3D printed Fe-Mn corrodes significantly more rapidly than pure iron. The tensile mechanical properties were similar to that of natural bone and the scaffolds also possessed good cell infiltration & in vitro cytocompatibility. This preliminary study has made the Fe-Mn alloy a promising biomaterial for craniofacial biomaterial applications (Chou DT, 2013).

Ceramics: Ceramics are not generally used for soft tissue engineering. Ceramic scaffold possesses many aspects like being bioactive, biocompatible, biodegradable, mechanically stiff (Young's modulus) (Qizhi, 2012), less elastic and brittle (Qizhi, 2012). They also exhibit shaping difficulties. Bioceramics can be classified into the following three groups: (Shikinami, 1999)

1. Bioinert groups. E.g. alumina and zirconia.
2. Surface bioactive groups. E.g. high temperature sintered hydroxyapatite(s-HA), bioglass.
3. Bioresorbable groups. E.g. low temperature sintered hydroxyapatite(u-HA), α-Tricalcium Phosphate (α-TCP), β-TCP, octacalcium phosphate (OCP), tetracalcium phosphate (TTCP).

For bone tissue engineering, various calcium phosphates (CaPs) specially HA, β-TCP, & biphasic calcium phosphate, BCP (mixture of HA & β-TCP) have long been studied as porous scaffold materials (Burg, 2000 & Hench, 1993). As natural bone consists of large amounts of HA ($Ca_{10}(PO_4)_6(OH)_2$), so it might seem ideal to use HA, β-TCP as they closely mimic the chemical and crystalline nature of the mineral phase of the native bone (Q Chen, 2008 & Jarcho, 1981) and hence, will be biocompatible. HA is known

to its bioactivity, biocompatibility, non-toxicity, non-inflammatory, osteoconductivity, & biodegradability. In comparison with β-TCP, HA degrades slowly (Mohamed, 2011) (the degradation rate is generally ordered as following: OCP> α-TCP > β-TCP > u-HA>> s-HA (Yang, 2001)) & after implantation undergoes little conversion to a bone like material (Martin,1993). However, for the same porosity β-TCP scaffolds often exhibit lower mechanical strength than HA scaffolds, making them difficult for use in load bearing applications (Mohamed, 2011) . The degradation rate and other properties can be manipulated by varying HA to β-TCP ratios in BCP (Mohamed, 2011). In recent years, researchers have shown that dopant addition in the scaffolds of CaPs can control the biocompatibility, densification behavior, dissolution rates, & mechanical strength (Bose, 2011 & Aneta, 2010).

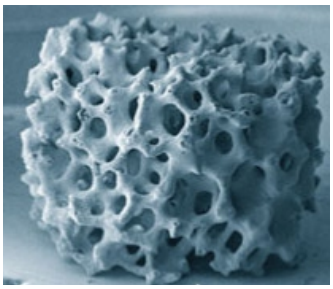

Figure 2: Calcium Phosphate based scaffold (Randall, 2004)

Bioactive glasses have already shown their excellence as promising biomaterials for tissue engineering due to their ability to support bone cell growth, bonding to both hard and soft tissues (Min Wang, 2006), capability to repair defect sites & controllable degradation rate in vivo. Glass compositions as well as the microstructure of the scaffolds are important factors that determine the degradation rate & conversion to an HA-like material, mechanical properties and response to cells. Bioglasses, doped with various elements such as Cu, Zn & Sr, promotes the healthy bone growth (Hoppe, 2011). Recently researchers have shown that bioglass can enhance angiogenesis (formation of blood vessels), critical to tissue engineered constructs (M.E. Gomes , 2004 & Mohamed, 2011) and soft tissue wound healing (Mohamed, 2011).And this capacity of bioglasses has provided an alternative approach to the use of expensive growth factors for stimulating neovascularization of engineered tissues (Mohamed, 2011).

Silicate bioactive glasses: The composition of 45S5 glass is 45% SiO_2, 6% P_2O_5, 24.5% CaO, 24.5% Na_2O & the low SiO_2 content (<55% SiO_2), high content of network modifiers like Na_2O & CaO, high CaO/P_2O_5 ratio contributes to the bioactivity of 45S5 glass (Mohamed, 2011). 45S5 glass has long been established as highly bioactive, biocompatible (Wilson, 1981), & biodegradable. It is considered as class A bioactive materials

(Hench, 2002) that are both osteogenetic and osteoconductive while class B bioactive materials (like HA) possess only osteoconductivity (Q Chen, 2008). In contact with the body fluid, 45S5 glass forms HCA (carbonate substituted hydroxyapatite which is similar to the mineral constituent of the bone) layer on its surface that significantly promotes osteoblast activity. As a porous 3D scaffold material 45S5 glass has some limitations like processing difficulties into a scaffold, low mechanical strength, slow degradation rate & conversion to an HA-like material (Mohamed, 2011). But it has recently been discovered that during scaffolding 45S5 glass can be heated to high temperatures (>950°c), to make a mechanically strong crystallize phase (bioactive glasses are amorphous while glass ceramics possess crystallize structure) that can convert to a biodegradable, amorphous calcium phosphate at the body temperature & in a biological environment (Chen QZ, 2006). This process enables the mechanical competence and biodegradability to be incorporated in a single scaffold, making it promising as tissue engineering scaffold (Q Chen, 2008). Compared to 45S5 glass, 13-93 bioactive silicate glass has slower degradation rate (& conversion to an HA-like material) and has better processing characteristics by viscous flow sintering (Mohamed, 2011).

Borate bioactive glass: Researchers have shown that borate or borosilicate bioactive glasses promote cell proliferation and differentiation in vitro, as well as tissue infiltration in vivo (Mohamed, 2011).Compared to 45S5 or 13-93 glass, borate bioactive glasses degrade faster and more completely convert to an HA-like material because of their lower chemical stability (Huang, 2006). The degradation rate can be controlled by manipulating the glass composition (Huang, 2006 & Yao A, 2007). However, there is a concern about the toxicity of boron released into the solution as borate ions $(BO_3)^{3-}$ (Mohamed, 2011).

Phosphate Bioactive glass: (Mohamed, 2011) Phosphate bioactive glasses are based on P_2O_5-glass forming networks where CaO & Na_2O acts as network modifiers. These glass show a chemical affinity towards bone because of their constituent ions are present in the organic mineral phase of the bone. The degradability can be controlled by modifying their composition, & this flexibility has made it potential resorbable biomaterials for tissue engineering.

Natural Polymers: Natural polymer based scaffolds show excellent bioactivity, biodegradability, but poor mechanical properties which reveal their successful use in soft tissue engineering & not for load bearing applications. Moreover, there is immunological concern associated with naturally derived polymers. Scaffolding natural polymeric materials with homogeneous & reproducible structures are other problems which limited their wide applications (Fergal, 2011).

Collagen: Collagen is considered as an ideal choice for tissue engineering scaffolds because it is the major fibrous protein in the extracellular matrix (ECM), & provides strength and structural integrity to connective tissues including skin, bone, tendons, cartilage, blood vessels, & ligaments (Amoabediny, 2011). Twenty seven types of collagen have been identified (Amoabediny, 2011) where 80-90% of the collagen in the body consists of type *1, 2, & 3* (Chunlin, 2004). In tissue engineering, collagen scaffolds are used in various forms such as porous sponges, thin sheets or gels (Dhandayuthapani, 2011& Amoabediny, 2011). Collagen scaffolds possess the excellent biocompatibility, high porosity & permeability, hydrophilicity, biodegradability, but poor mechanical strength for bone tissue engineering in minimally weight bearing applications. However, the degradation rate, compressive and tensile strength can be enhanced by physical and chemical cross-linking methods (Cuy J, 2004).

Chitosan: Chitosan, derivative of chitin, is a linear polysaccharide polymer composing copolymers of β (1-4)-glucosamine & N-acetyl-D-glucosamine. Chitin exists in the exoskeleton of crustaceans (such as crabs, shrimps), cuticles of insects & cell walls of bacilli (Amoabediny, 2011). Chitosan has gained special attention from researchers owing to its biocompatibility, low toxicity, biodegradability, controllable mechanical and structural properties, & capability of being processed in many forms, sizes and shapes. But pure chitosan as a polymeric tissue engineering scaffold is limited because of their weak mechanical properties (Mohammad, 2012 & Amoabediny, ISBN:978-953-307-609-6) and inconsistent behaviour with seeded cells (Madihally, 1999). However, chitosan can be physically & chemically modified (Mohammad, 2012), and produce materials with wide range of properties.

Alginate: Alginate is a linear polysaccharide isolated from brown sea algae. It holds to a family of linear block polyanionic copolymers consisting of (1-4)-linked β-D-mannuronic acid (M fragments) & (1-4)-linked α-L-guluronic acid (G fragments) residues (BouhadirKH, 2001 & Sutherland, 1991). Alginate can form stable and well characterized hydrogels in the addition of certain divalent cations (e.g. Ca^{2+}, Sr^{2+}, Ba^{2+} (Wang, 1993 & Honghe. 1997), except Mg^{2+} (BouhadirKH, 2001)) at low concentrations. To prevent immune responses after implantation alginate must undergo extensive purification (Willerth S.,2007). Alginate possesses biocompatibility, hydrophilicity, non-cytotoxicity, biodegradability, & also it is thermally stable and relatively economical. But some drawbacks have limited their applications in tissue engineering such as weak mechanical properties, poor cell adhesion (for highly hydrophilic nature) and uncontrollable degradation (BouhadirKH, 2001). These limitations can be improved by mixing with other materials like hydroxyapatite and also with

natural polymers, chitosan and agarose (Amoabediny, ISBN:978-953-307-609-6 & Sutherland, 1991).

Agarose: Agarose is a polysaccharide extracted from red algae and seaweed. It is a linear polymer composed of D-galactose and 3,6-anhydro-L-galactopyranose linked by α-(1-3) & β-(1-4) glycosidic bonds. Agarose is purified from agar (which has two principle components, agarose and agaropectin), by removing agar's one component, agaropectin. The properties of agarose i.e. good mechanical strength and capacity to retain chondrocytes phenotype make it suitable for the scaffolds of cartilage tissue engineering. Agarose hydrogels are popular owing to their biocompatibility, native tissue like viscoelastic mechanical properties and ease of casting into complex shapes and sizes (Bose corporation, 2012).

Fibrin: Fibrin is a fibrous, non-globular protein, & is a critical blood component involved in the hemeostasis (Ahmed, 2008). Fibrin hydrogel is formed by combining commercially available fibrinogen (fibrinogen is a soluble, plasma glycoprotein, consisting of six chains-two α, two β, & two γ chains, linking by disulfide bonds (Wnek GE, 2002)) and thrombin (Wnek GE, 2002 & Ahmed, 2008). One advantage of fibrin is that it can be autologously sourced (Linnes, 2007). Fibrin scaffolds are biodegradable, biocompatible, non-toxic, easy processable with various size and shape, & fibrin is relatively cost effective than synthetic polymers or collagen gels (Ahmed, 2008). The function of fibrin scaffold can be improved by incorporating bioactive peptides and growth factors through heparin-binding delivery system (Ahmed, 2008). The mechanical properties of fibrin scaffold can be controlled by varying concentrations of components, according to the needs of surrounding or encapsulated cells. Biodegradation can be managed (Wozniak, 2003) with the help of fibrinolysis inhibitors (Cholewiaski, 2009) or fiber cross-linkers.

Fibronectin: Fibronectin, exists outside cells and on the cell surface, in blood plasma & other body fluids (Amoabediny, ISBN:978-953-307-609-6), is a high molecular weight glycoprotein of the ECM, that can bind other ECM components like collagen, fibrinogen, & glycosaminoglycans (Amoabediny, ISBN:978-953-307-609-6). Fibronectin is considered as a key component of the ECM and it involves in both the structural integrity and functional properties of the living tissues. It contributes to cell adhesion, proliferation, migration, & differentiation (Stoffels, 2013).

Synthetic polymers: Numerous synthetic polymers have been tried to produce scaffolds because of their several advantages as a scaffold material and also due to their availability. Synthetic polymers can be biodegradable and non-biodegradable. Synthetic polymeric materials can be fabricated with a tailored architecture and properties (e.g. porosity, degradability, & mechanical properties), according to their applications. That

means they can be produced under controlled conditions with large uniform quantities and long shelf life (Dhandayuthapani, 2011). However there is a risk of rejection of these polymeric scaffolds owing to their reduced bioactivity. During the degradation process, formation of acidic products & consequently lowering the local pH is a common problem with synthetic polymers which can result in diminishing mechanical strength of material and cell-tissue necrosis.

Poly (α-hydroxy esters):

1. Polylactic acid (PLA): PLA is the biodegradable, thermoplastic aliphatic polyester. The presence of pendant methyl group on the alpha carbon of PLA differentiates it physically, mechanically or chemically from the structurally very similar PGA (Yang S, 2001). Due to this structure which causes chirality; & so several distinct L, D, & DL isomers are possible (Yang S, 2001). PLLA is resulting from polymerization of L, L-lactide. PLLA has a semicrystalline structure (around 45%) with glass transition temperature ranging from 60-65°C,melting temperature from 170-180°C and tensile modulus between 2.7-16 GPa. The melting temperature and heat deflection temperature of PLLA can be increased by physically blending the polymer with PDLA (Poly-D-lactide) in which they form a highly regular stereocomplex with increased crystallinity, where (Yang S, 2001) PDLLA with a more or less random distribution of the stereo sequences is an amorphous material with a glass transition temperature between 50-60°C, depending on molecular weight. PDLLA degrades faster than PLLA if all other conditions remain same (Yang S,2001). Y.M.Lin et al studied the feasibility of growing lung cells on PDLLA scaffolds (scaffolds in two forms: polymer discs and 3D foams) to engineer pulmonary tissue for human implantation & their study has been demonstrated that PDLLA is nontoxic to pneumocytes and it actively supports their growth (Lin YM, 2006)

2.Poly glycolic acid (PGA): PGA is the simplest, linear aliphatic polyester prepared by polycondensation or ring-opening polymerization, starting from glycolic acid. PGA exhibits an elevated degree of crystallinity (generally 45-75%) with glass transition temperature ranging from about 25-65°C, & melting temperature between 185-225°C (Yang S, 2001). PGA has a good thermal stability, as an absorbable material (Shalaby, 1994). Because of its highly crystalline nature, PGA is not soluble in water. High molecular weight form of PGA is not soluble in almost all common organic solvents (exceptions are highly fluorinated organic solvents like hexafluoroisopropanol(HFIP) & hexafluoroacetone sesquihydrate) while low molecular weight oligomers differ in their physical properties and show more solubility.

3.Poly (lactic-co-glycolic) acid (PLGA): PLGA is synthesized by random ring-opening co-polymerization of glycolic acid and lactic acid

monomers. It has a glass transition temperature ranging from 45-55°C. PLGA possesses unique properties like good mechanical strength, excellent biocompatibility, low toxicity and immunogenicity, & versatile degradation kinetics (M S Muthu, 2009 & PLGA-PEG, 2003). The properties of PLGA is tailored by vayring the monomer ratios and molecular weight (Polyscitech.com/PLGA/PLGA.php & M S Muthu, 2009). PLGA prepared from L-poly lactide (L-PLA) and poly glycolide(PGA) are crystalline in nature while PLGA from D, L-PLA & PGA are amorphous (M S Muthu, 2009). Generally PLGAs show crystallinity if there is a higher percentage of lactide units in the co-polymer composition. The mechanical strength, degree of crystallinity, melting temperature and glass transition temperature of the polymers are directly influenced by the molecular weight of the polymer. The higher the molecular weight, the greater the mechanical strength found. Also researchers have found that the glass transition temperature of PLGA decrease with the decrease of lactide content in the monomer ratios with decreasing molecular weight (M S Muthu, 2009).

4.Poly (e-caprolactone) (PCL): PCL is relatively inexpensive, highly elastic, and an aliphatic polyester which is established as bioresorbable and biocompatible. Semicrystalline PCL polymer is made by ring-opening polymerization of e-caprolactone (Yang, 2001). Melting temperature of the polymer is around 60°C & a glass transition temperature of -60°C. This polymer is degraded by hydrolysis of its aliphatic ester linkages in physiological environments (Yang, 2001& Amoabediny, ISBN:978-953-307-609-6). The degradation time of PCL homopolymer is very slow which can be accelerated by synthesizing co-polymers. For example, co-polymers of e-caprolactone with D, L-lactide have produced materials with faster degradation rates (Yang, 2001). Not only controllable degradation rates, PCL co-polymers (co-polymerized with other hydroxyacids or polymers like glycolide or lactide) also provide better control over mechanical properties without sacrificing biocompatibility (Qizhi, 2012). PLA, PGA, PCL are rigid and poorly flexible while PCL co-polymers with lactide or glycolide are elastomeric, can provide sustainable elasticity & structural integrity (Christian, 2007) that are thought to be mechanically more advantageous than thermoplastic polymers because elastic stretchability is an important mechanical characteristic of living tissues including collagens of different bone types (Qizhi, 2012).

Poly β-hydroxy butyrate: Poly (hydroxyalkanoate)s (PHAs) are bioderived, biocompatible, & biodegradable thermoplastic polyesters that are produced by various microorganisms (Leng J, 2010). Among various PHA, PHB is extensively studied for bone tissue engineering (Leng J, 2010), which is a linear homopolymer of (R)-β-hydroxybutiric acid that forms crystalline cytoplasmic granules in the wide variety of bacteria strains (Amoabediny,

ISBN:978-953-307-609-6). The properties of this polymer are closer to that of polypropylene, possesses low thermal stability with glass transition temperature in the range of -5 to 20°C & melting temperature between160-180°C. After implantation, it degrades slowly at body temperature and produces a non-toxic metabolite, secreted through the urine (Mosahebi, 2001). The mechanical properties, biocompatibility, & biodegradability of this polymer can be manipulated by blending, & surface modification or composition with other polymers, enzymes, or inorganic materials to enhance the range of clinical applications (Leng J, 2010 & Sérgio, 2006). However, high crystallinity, high fragility, poor processability (Sérgio, 2006) or the time consuming extraction (Leng J, 2010) of these types of bacterial culture polymers has limited their applications.

Poly(glycerol sebacic acid) (PGS): PGS, also called bio-rubber (Amoabediny, ISBN:978-953-307-609-6), can be obtained by polycondensation of glycerol & sebacic acid. It has ester cross-links & hydroxyl groups, directly attached to its backbone (Wang, 2002). Appropriate cross-link density makes PGS tough and elastomeric while hydroxyl groups make it highly hydrophilic (Wang, 2002). PGS is biocompatible both in vitro and in vivo (Wa Christian, 2007 & Wang, 2002). PGS features robust mechanical properties, surface erosion biodegradation. The degradation rate, hydrophilicity, and other properties can be tailored by grafting hydrophobic moieties to the hydroxyl groups (K.N. Jayachandran, 2000 & A.Laschewsky, 2001). However, harsh processing conditions like higher temperatures,& longer reaction times may limit its ability to polymerize directly in a tissue or to incorporate cells or temperature sensitive molecules (Christian, 2007).

Poly (2-hydroxyethyl methacrylate): It is a soft, flexible, highly biocompatible, & water-absorbing plastic widely used to make soft contact lenses. Hydro soluble monomer, HEMA, can be polymerized (under various circumstances) at low temperatures (-20 to +10°c) and can be used to prepare various hydrogels (Amoabediny, ISBN:978-953-307-609-6). Though HEMA is not biodegradable (Mabilleau, 2004) but this property can be achieved through cross-linking with PCL (Rice, 2006) which is hydrolytically and enzymatically degradable polymer. pHEMA is an attractive and potential synthetic polymer for cardiac and other tissue engineering scaffolds because of its elasticity, reasonable mechanical strength, & easy fabrication into numerous configurations.

Polyphosphazenes: Polyphosphazenes are consisting of an inorganic backbone of alternating phosphorus & nitrogen atoms. Each phosphorus atom in the backbone is substituted for by two organic side groups, giving a wide range of polymer properties and this flexibility of polyphosphazenes have made them suitable for both hard and soft tissue engineering (Heta,

2011) like bone tissue, blood vessels, or tissue regeneration in the periodental cavity e.t.c. So by selecting proper side groups, polyphosphazene scaffolds with required properties and degradation rates can be fabricated (A.K. Andrianov, 2009). By altering the organic substituents, physico-chemical properties, mechanical properties, biocompatibility, degradation rates with nontoxic degradation products can be tailored to a great extent (Heta, 2011).

Polyurethane (PUs): PUs, containing the urethane (-NH-CO-O-) linkage, is typically produced by adding an isocyanate to a hydroxy group (Hetal, 2011). Segmented polyurethanes are block co-polymers comprised of macropolyols made soft segments linked together by diisocyanates and chain extenders made hard segments (Qizhi, 2012). Polyurethanes have been used for many years in biomedical applications owing to their tissue specific biocompatibility, excellent mechanical properties, biodegradability (Lamba, 1998 & Santerre, 2005) & good processability. Their biological mechanical properties and degradation rates can be tuned (Heta, 2011) by modyfing the structure of soft or hard segments (Qizhi, 2012). Generally a high content of soft segments enhance the degradation rates (Pinchuk, 1094). Unlike the PGA, PLA, & PLGA the degradation behaviour of polyurethanes demonstrated no significant pH change in the microenvironment of their degradation products (Qizhi, 2012). But the degradation products could be toxic when aromatic diisocyanates are used which could be removed by replacing aliphatic diisocyanates (Lamba, 1998).

Polyanhydrides: Polyanhydrides are a class of hydrophobic, surface eroding polymers (GUO BaoLin, 2014) consisting of anhydride bonds that connects repeat units of the polymer backbone chain. Polyanhydrides can be synthesized by melt condensation or solution polymerization and can be manipulated to meet desirable characteristics (GUO BaoLin, 2014). Aliphatic polyanhydrides have crystalline structure with melting temperature between50-90°C. Unsaturated polyanhydrides are highly crystalline where aromatic polyanhydrides are very hydrophobic (melting temperature>100°C), degrade slowly in the physiological environment and generates relatively insoluble degradation products. Anhydrides are useful as scaffold for functional soft tissue substitutes but modest Young's modulus for entangled polyanhydrides network has limited their application in load-bearing environment (Hetal, 2011). However, this shortcommings can be overcome by forming cross-linked networks with incorporated imides which has significant mechanical properties such as compressive strength which are in the intermediate rang of cortical and trabecular human bone (Hetal, 2011). Polyanhydrides are biocompatible and in vivo, degrades into non-toxic diacid monomers that can be eliminated from the body as metabolites (GUO BaoLin, 2014). Hydrolytic degradation can be controlled by manipulating

the polymer composition i.e. by adding hydrophilic monomer (such as sebacic acid) to the hydrophobic diacid building blocks of polyanhydrides.

Poly (ethylene glycol) (PEG): PEG, the most commercially important polyethers, also known as polyethylene oxide (PEO) or polyoxyethylene (POE) depending on its molecular weight, refer to an oligomer or polymer of ethylene oxide (Amoabediny, ISBN:978-953-307-609-6). PEG has some critical properties like good biocompatibility, non-immunogenicity, & resistance to protein adsorption and cell adhesion (Amoabediny, ISBN:978-953-307-609-6) for which it has been an important type of hydrophilic polymers in biomedical applications including bioconjugation, surface modification, drug delivery, and tissue engineering (Junmin, 2010). For producing hydrophilic PEG hydrogels, PEG must be cross-linked and three major cross-linking methods are free radical polymerization of PEG acrylates, radiation of linear or branched PEG polymers, & specific chemical reactions such as condensation, Michael-type addition, Click chemistry,, native chemical legation and enzymatic reaction (Junmin, 2010). The most common approach for making PEG hydrogel with biocompatibility and non-toxicity is photopolymerization under mild conditions in the presence of cells and bioactive agents & is regarded as an advantageous method to fabricate hydrogel scaffolds in situ with spatial and temporal control (Amoabediny, ISBN:978-953-307-609-6 & Junmin, 2010). To meet the diverse needs in tissue engineering, bioactive molecules such as cell adhesion ligands, enzyme-sensitive peptides & growth factors have been incorporated into PEG hydrogels, to simulate one or more ECM biofunctions like cell adhesion, proteolytic degredation and growth factor-binding (Amoabediny, ISBN:978-953-307-609-6 & Junmin, 2010). Photodegradation, in contrast to hydrolytic degradation and enzyme-sensitive degradation, allows precise spatial and temporal control over degradation and release.

Poly (propylene fumarate) (PPF): PPF is unsaturated linear polyester, undergoes hydrolytic degradation to fumaric acid & propylene glycol which are biocompatible and readily removed from the host body (Qizhi, 2012 & Leng, 2010). The degradation time and mechanical properties can be controlled by varying the PPF molecular weight and other components for PPF-based composites & that's why preservation of the double bonds and control of molecular weight during synthesis of PPF are critical issues (Qizhi, 2012 & Leng, 2010). PPF is used in 3D scaffolds for guided tissue regeneration & a substrate for osteoblast cell cultures (Leng, 2010).

Polypyrrole (PPy): PPy is a conductive polymer formed by polymerization of pyrrole. PPy is thoroughly investigated among other conductive polymers for biomedical applications due to its high electrical

conductivity, flexible preparation method, ease of surface modification, ion exchange property, excellent environmental stability, & both in vivo and in vitro biocompatibility (Anca-Dana Bendrea. J. of Biomaterials applications, Vol. 00-2011). Polypyrrole's electronic properties are imparted by their conjugated structure of alternating c=c double bonds and c-c single bonds (C.B.Gumera, 2009). Its conductivity to allow signal transduction in nerve cells is one of the major advantages of polypyrrole, in nerve repair applications (Hetal, 2011). Though they are biocompatible (similar ti PLGA), but not cell adhesive and biodegradable which has been limited their application in nerve repair (C.B.Gumera, 2009). Cell adhesion behaviour has been improved by surface modification techniques. During the polymer synthesis process various biomolecules can be incorporated into polypyrrole as biodopants (Anca-Dana Bendrea. J. of Biomaterials applications, Vol. 00-2011). Both electrical and biological activity of the resultant polypyrrole surface is affected by the various biomolecular based dopants. Researchers demonstrated that, cell responses can be modulated in terms of adhesion, growth, proliferation & differentiation by careful selection of the dopant anion (such as Cl^-, Br^-, or NO^{3-}) (Anca-Dana Bendrea. J. of Biomaterials applications, Vol. 00-2011). Most widely used biodopants were generally ECM derived components such as HE, HA, CSA, laminin-derived peptides, collagen, & other biomolecules like ATP, DNA, dermatan sulfate, NT3, NGF, BDNF, poly (L-lysine) were also entrapped in films, membranes or fibers of conjugated polymers (Anca-Dana Bendrea. J. of Biomaterials applications, Vol. 00-2011 & C.B.Gumera, 2009). Erodible and biodegradable polypyrrole polymers have been synthesized to alter the permanent nature of polypyrroles. PPy monomers, β-substituted, were chemically or electrochemically polymerized which showed in vitro biocompatibility by supporting the attachment and proliferation of mesenchymal progenitor cells where β-substituted polypyrroles were erroded on a pH-dependent manner and was controllable according to the composition of co-polymer (C.B.Gumera, 2009).

Composites: Researchers have already made many composites: synthetic polymers with natural polymers, synthetic polymers with bioceramics, polymers with metals, metals with ceramics, e.t.c. For load-bearing applications novel metal-polymer- ceramic composites have also been proposed (Kelly, 2009). Composites are necessary to obtain optimal biological, structural, mechanical and chemical properties of scaffolds. For example, in bone tissue engineering, bioceramics /polymers are commonly used composites. As native bone consists of a naturally occuring polymer and biological apatite, it might seem logical to use bioceramics /polymers composites. Also there are some other factors, sometimes biocompatibility and biodegradability of ceramics are found insufficient. Moreover, ceramics

are very brittle, and too stiff, while the polymers are found to be biocompatible and biodegradable with low mechanical strength (Budi Arifvianto, 2014). So this biological and mechanical mismatch can be overcome by blending the ceramics with natural or synthetic polymers. Some polymers like PGA, PCL, PLA degrades by hydrolysis, forming acidic products, & consequently lowering the local pH (Fergal, 2011). The massive release of acidic degradation products can cause strong inflammatory reactions which can avoid by incorporating CaPs or bioglass, because their basic degradation could buffer the acidic by-products of polymers and thus contributing to stabilize the pH of the environment surrounded by polymer. This is another important reason for proposing the composites (Qizhi, 2012).

Hydrogels: Hydrogels are 3D networks comprising of highly hydrophilic polymers cross-linked via various chemical bonds and physical interactions which can absorb huge amounts of water (up to 99% (Hikmet Geckil, 2010)) or biological fluids & swell readily without dissolving (El-Sherbiny, 2013). Hydrogels are soft and rubber like in the swollen state, mimic the specific aspects of microenvironments of tissues (Kaji H., 2011). Their highly hydrophilic natures are owing to the presence of hydrophilic moieties like amino, amide, carboxyl & hydroxyl groups distributed along the backbone of the polymer chain (El-Sherbiny, 2013).Various natural and synthetic polymers are used to prepare hydrogels. Synthetic polymers include poly (ethylene oxide) (POE), poly vinyl alcohol (PVA), poly (acrylic acid) (PAA), poly (propylene fumarate-co-ethylene glycol) (P(PF-co-EG)) (Jaenie, 2003), poly (2-hydroxyethyl methacrylate) (PHEMA) (Walker, 1992), poly (N-isopropylacrylamide) (PNIPAAM) (Fujimoto, 2009). And representative naturally derived polymers are agarose, alginate, collagen, fibrin, gelatin, chitosan, hyaluronic acid (Jaenie, 2003) & silk. Hydrogels can be used to engineer almost every tissue in the body such as smooth muscle, bone and cartilage (Mekala, 2012). More recently, hydrogel scaffolds (Figure 3) have gained much attention as a promising material to overcome various tissue engineering challenges such as vascularization, tissue architecture and simultaneous seeding of multiple cells (El-Sherbiny, 2013).

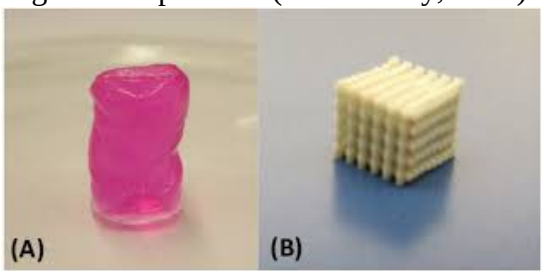

Figure 3: (A) Hydrogel Scaffold, (B) Solid Polymeric Scaffold (Zohreh, 2012)

Conclusion

Over the past few decades, critical improvements have been made in the field of biomaterials, even new and multifunctional biomaterials have been investigated. Though still some drawbacks associated with biomaterials, scaffold fabricating techniques, signaling factors, & cells are challenging the success of tissue engineering, but rapid progress in tissue engineering field and growing demand on regenerative medicine have made the researchers hopeful to be fully successful in the near future.

References:

Fergal J. O'Brien. Materials Today 2011; 14(3):88-95.
Mekala N.K. et al. Recent research in Science and Technology 2012;4(12):5-11.
Flanagan T.C. et al. Biomaterials 2006;27:2233-2246.
B.P. Chan & K.W. Leong. Eur Spine 2008;17(suppl 4):467-479.
Karp J.M., Langer R. Curr Opin Biotechnol.2007; 18(5):454-459.
Berger J. et al. Eur J Pharm Biopharm. 2004; 57(1):19-34.
Skalak R. Fox CF(1993) Tissue Engineering. 1988; 26-29.
Zippel N., Schulze M., Tobiasch E. Recent Patent on Biotech. 2010; 4:1-22.
Min Wang. American Journal of Biochemistry & Biotechnology 2006; 2(2):80-84.
K.M. Kim et al. Tissue engineering: The future of stem cells. Topics in T.E.,2005; V-2.
Brochhausen C. et al. Tissue Engineering, Eberli D. (Ed.). ISBN:978-953-307-079-7.
I.Martin et al. Trends Biotechnol, 2004; 22: p. 80.
nuhs.edu.sg/research/programmatic-research/sm-registered-programms/nus-tissue-engineering.
Wang HM, Chou YT, Wen ZH, Wang CZ, Chen CH et al. PLoS ONE 8(11):10.1371.
J.E. Babensee et al. Adv. Drug. Del. Rev. 1998; 33: p.111.
J.C. Middleton & A.J. Tipton. Biomaterials 2000; 21:2335.
J.Kohn & R.Langer. Biomaterial Sci.(ed. B.Ratner et al.)64;1996. NY, academic press.
M.E. Gomes & R.L. Reis. International material reviews 2004; 49:261-273.
S.L. Edwards et al. AUTEX Research Journal 2004; 4:86-94.
Cherian E. et al. Stem cells,2011, Jaypee Brothers Medical Publishers, India.
Tran N. et al. Biorheology 2003; 40(1-3):411-5.
Knight M.A., Evans G.R., Plast Reconstr Surg 2004; 114:26E-37E.
Kevin L.Ong, Lovald S., Black J., Orthopedic biomaterials in research & practice,2nd edition. 2014,CRC press.

Pandit N, Malik R, Philips D, Indian Society of Periodontology 2011; 15:328-337.
http://www.aaas.org/spp/dsp/sfrl/projects/stem/main.html.
Aneta J Mieszawska, David L Kaplan, BMC biology 2010,8:59.
Jin Gao et al. A neuroinductive biomaterial based on dopamine. 103(45):16681-86.
Ruth R. Chen & David J. Mooney. Pharmaceutical Research 2003; 20:1103-1112.
Bose S., Roy M., & Bandyopadhyay A. Trends Biotechnol. 2012; 30(10)546-554.
Hua Ye et al. Materials in medicine 2007; 18:641-648.
Leor J., Amsalem Y., Cohen S., Pharmacology & Therapeutics 2005; 105:151-163.
Garrett Ryan et al. Biomaterials 2006; 27:2651-2670.
XIAO Dong-ming et al. Trans. Nonferrous Met. Soc. China 2012; 22:2554-2561.
Kelly Alvarez et al., Materials 2009; 2:790-832.
Li Y,Xiong J, Wang CS, Hodqson PD, Wen C, Tissue Eng.Part A, 2009;15(10):3151-9.
GP Dinda et al., Metallurgical & Materials Transactions A 2008; 39A:2914-2922
Jansen J.A. et al., J. Control Release 2005; 101:127-136.
Greiner L.C.,Oppenheimer M.S., Dunand D.C., Acta Biomater. 2005; 1:705-716
Christos G Paganias, Indian J Orthop. 2012; 46(5):505-513.
Randall C. Willis, ACS Publications 2004; 7(9).
F. Witte et al., Biomaterials 2007; 28:2163.
M.P. Staiger et al., Biomaterials 27:1728-1734.
A.H. Yusop et al., Int. J. Biomater 2012(2012), article ID:641430, 10pages.
P. Quadbeck et al., Mat. Sci. Forum. 2007; 1005, 534/536.
Renáta Oriňáková et al., Int. J. Electrochem.sci. 2013; 8:12451-12465.
Chou DT, Wells D,Hong D, Lee B, Kuhn H et al.,Acta Biomater 2013;9(10):8593-603.
Shikinami Y, Okuno M. Biomaterials 1999; 20:859
Burg KJL,Porter S, Kellam JF. Biomaterials 2000; 21:2347-2359
Hench LL,Wilson J.(1993)An introduction to bioceramics, World Scientific,Singapore.
Q Chen et al.(Eds. N Ashammakhi et al.)Topics in Tissue Engineering, 2008; Vol 4.
Jarcho M, Clin Orthop Relat Res 1981, 259-278.
Mohamed N. Rahaman et al. Acta Biomater 2011; 7(6):2355-2373.
Yang S, Leong KF, Du Z, Chua CK. Tissue Eng. 2001; 7(6):679-89.

Martin RB et al. Biomaterials 1993; 14:341-348.
Bose S. et al. Bone 2011; 48:1282-1290.
Hoppe A, Guldal NS, Boccaccini AR. Biomaterials 2011; 32:2757-2774.
Wilson J et al. J Biomed Mater Res. 1981; 15:805-817.
Hench LL, Polak JM. Science 2002; 295:1014-7.
Chen QZ, Thompson ID, Boccaccini AR. Biomaterials 2006; 27(11):2414-2425.
Huang W et al. J Mater Sci Mater Med 2006; 17:583-596.
Yao A et al. J Am Ceram Soc 2007; 90:303-306.
Amoabediny G et al. 2011,Biomaterials science & eng.,Pignatello R.(Ed), ISBN:978-953-307-609-6, In Tech.
Chunlin Y et al. Bio Drugs 2004; 18:103-119.
Dhandayuthapani B et al. Int. J of Polymer Science, 2011(2011),article ID:290602,19p.
Cuy J. Biomaterials tutorial: Natural polymers. University of Washington Engineered Biomaterials, 2004.
Mohammad Albanna et al. J of mechanical behavior of biomedical materials(Impact factor:2.37),1/2012; DOI:10.1016/j.jmbbm.2012.09.012.
Madihally SV, Matthew HWT. Biomaterials 1999; 20(12):1133-1142.
BouhadirKH, Mooney DJ. Synthesis of Hydrogels: alginate hydrogels.(Eds.Atala A,Lanza RP), Methods of Tissue Engineering, NY, academic press, 2002; 653-662
Sutherland IW et al.(Eds.),Biomaterials: Novel materials from biological sources. NY, Stockton Press; 1991:309-331
Willerth S et al. Advanced Drug Delivery Reviews 2007; 59(4-5):325-338
Viscoelastic characterization of agarose gel scaffolds, Bose corporation,2012
Wnek GE et al. Nano Lett. 2002; 3(2):213-216.
Ahmed TA et al. Tissue Eng. Part B Rev. 2008;14(2):199-215.
Linnes MP et al. Biomaterials 2007; 28(35):5298-306
Wang Z, Zhang Q, Konno M, Saito S. Biopolymers 1993; 33: 703-711.
Wozniak G. Cardiovascular Surgery 11(Suppl 1): 17-21,2003.
Cholewiaski E et al. Tissue Eng. Part a 2009; 15(11):3645-53.
Stoffels JM et al., Cell Mol life Sci 2013; 70(22):4243-53.
Zohreh Izadifar et al. JFB 2012; 3(4):799-838.
Lin YM et al., J Biomater Appl. 2006; 21(2):109-18.
Qizhi Chen et al., Progress in Biomaterials 2012,1:2, doi:10.1186/2194-0517-1-2
Shalaby SW, & Johnson RA, Synthetic absorbable polyesters. Shalaby SW (Ed). Biomedical Polymers: Designed to degrade systems. NY, Hanser Pub,1994; pp1-34
M S Muthu. 2009; 3(4):266-273.
PLGA-PEG block copolymers for drug formulations. 2003; 3(5)

85.Polyscitech.com/PLGA/PLGA.php.
Christian L.E.Nijst et al. Biomacromolecules 2007; 8(10):3067-3073.
Leng J et al. (Eds),Multifunctional polymer nanocomposites, CRC press,
Mosahebi et al.,(2001)Genetic labeling & transplantation of schwann cells to enhance pheripheral nerve regeneration,Royal Free & Uni. College Medical School,Uni of london.
Honghe Z. Carbohydr Res 1997; 302: 97-101.
Sérgio Henrique et al., Mat. Res. 2006, Vol 9, No 1.
Wang Y et al. Mat. Res. Soc. Symp. Proc. 2002, Vol. 724.
K.N. Jayachandran & P.R. Chatterji. Eur. Polym. J. 2000; 36:743-749.
A.Laschewsky et al. Macromol.Chem. Phys. 2001; 202:276-286.
Mabilleau G et al. Biomaterials 2004; 25:5155-5162
Rice MA et al. Biomacromolecules 2006; 7:1968-1975
Hetal Patel et al. Trends Biomater. Artif. Organs, 2011; 25(1):20-29
A.K. Andrianov(Ed.),Polyphosphazenes for biomeducal applications, 2009,462 p
Lamba NMK et al.,(1998)PUs in biomedical applications, CRC press,USA.
Santerre JP et al. Biomaterials 2005; 26:7457-7470
Pinchuk L. J Biomater Sci Polym Ed 1994; 6:225-267
Junmin Zhu. Biomaterials 2010; 31(17):4639-4656
Anca-Dana Bendrea. J. of Biomaterials applications, Vol. 00-2011
Gumera,New mtls & scaffold fabrication method for nerve Tissue Eng. 2009
Budi Arifvianto, & Jie Zhou. Materials 2014; 7(5):3588-3622
Hikmet Geckil et al. Nanomedicine(Lond), 2010; 5(3):469-484
Kaji H. et al. Biochem. Biophys. Acta 2011; 1810:239-250
Jaenie L.Drury, David J. Mooney. Biomaterials 2003; 24:4337-4351
Walker A.S. et al. ASAIO J. 1992; 38:M550-M554
Fujimoto K.L. et al. Biomaterials 2009; 30:4357-4368
El-Sherbiny & Yacoub. Global Cardiology Science and Practice 2013; 38:27pages GUO BaoLin & MA Peter X. Science China 2014; 57(4):490-500