10.5005/jp-journals-10028-1018
SYMPOSIUM

Biomaterials in Regenerative Medicine

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ABSTRACT

Limitations with the conventional methods have bought biomaterials to the forefront for the repair and restoration of tissue functions. Recent advances in the area of biomaterials have revolutionized the field of tissue engineering and regenerative medicine. According to the nature of polymers they are divided into different classes and each one has found applicability in the area of regenerative medicine. Each class of biomaterials has a set of properties which makes them appropriate for a specific application. The most important property is the behavior of biomaterials when implanted *in vivo*. It should not elicit any immune rejection reactions neither should its byproducts be toxic to animal tissue. Any type of the biomaterial can be fabricated into a three-dimensional scaffold which can be used as housing for the initial growth and proliferation of the specific cell type. In addition to the conventional methods of scaffold fabrication few contemporary methods include 'hydrogels' and 'cryogels'. These matrices possess interconnected porous network which facilitates the cell migration and proliferation. These gel matrices can be fabricated from both natural and synthetic polymers and have shown applicability in different areas of tissue engineering. Biomaterials have shown applicability as cardiovascular implants, orthopedic implants, dental implants, etc. Furthermore, recent advances in the regenerative medicine have shown that in addition to the use of autologous and allogenic sources, stem cells can prove to be a very good alternative. Stem cells interaction with biomaterials has shown applicability in the regenerative medicine and thus can have an immense potential in future.

Keywords: Biomaterials, Regenerative medicine, Tissue engineering, Implants, Polymers.

How to cite this article: Bhat S, Kumar A. Biomaterials in Regenerative Medicine. J Postgrad Med Edu Res 2012;46(2): 81-89.

Source of support: Nil

Conflict of interest: None declared

INTRODUCTION TO BIOMATERIALS

The advances of biomaterials as a science dates back to approximately 50 years and the study of biomaterials is called as biomaterial science. Considering the wide explicabilities of biomaterials different authors have put forward diverse definitions to it. In an introductory lecture on Biomaterials by Jeong-Yeol Yoon (http://www.u.arizona.edu/~trouard/courses/bme516/biomater_lec1.pdf)[1] various definitions of biomaterials have been proposed by various authors. In general it can be 'any material natural or synthetic that comprises whole or a part of living structure or a biomedical device which performs, amplifies or replaces the function that has been lost by either accident or injury'. According to Park and Lakes 'biomaterial is any synthetic material which is used to replace part of a living system or to function in intimate contact with the living tissue'. In accordance to Clemson University Advisory Board for Biomaterials 'it is a material which is synthetically and pharmacologically inert and is designed to be implanted within or incorporated with living system'. As proposed by Dee et al these 'are the materials that constitutes part of medical implants, extracorporeal devices and disposables those have been utilized in medicine, surgery, dentistry and veterinary medicine as well as in every aspect of patient health care'. As stated by National Institute of Health (NIH) 'it is any substance (other than drug) or a combination of substances, synthetic or natural in origin which can be used for any period of time as a whole or as a part of a system which treats, augments, or replaces any tissue, organ or function of the body'. According to Williams (1987), 'a biomaterial is a material used in implants or medical device, intended to interact with biological systems'.[2] Talking from the perspective of the 'bioengineering' which is the application of concepts and methods of physical science and mathematics in an engineering approach toward solving problems in repair and reconstruction of lost, damaged or deceased tissue and any material that can be used for this purpose can be regarded as a biomaterial.[2] Examples of biomaterials include the common medical devices like substitute heart valves, artificial hips and knee joints, dental implants, fracture fixtures, skin regeneration templates, dialyzers to support ailing kidneys, etc. Any material that can be used for these medical applications must possess some specific properties and most basic criteria are related to the material biocompatibility. So, any material that is biocompatible with the host tissue can be considered as a biomaterial.[3] Field of biomaterials has evolved in many ways like its capacity to study the various aspects like molecular biology and cell biology at the implant host tissue interface which further gives a detailed idea about the material biocompatibility. Biomaterials have also evolved in terms of their applicability and are now also being used as carriers to deliver small and large bioactive molecules. These delivery molecules or systems can be targeted to a specific tissue for its regeneration.[4]

HISTORY OF BIOMATERIALS

The introduction of 'nonbiological materials' now called as 'biomaterials' for the benefit of human health was noted

far back in the prehistory. The Mayan people fashioned nacre teeth from the sea shells around 600 AD and apparently achieved what is now referred to as bone integration. Additionally, linen sutures were used by the early Egyptians for surgical purposes. Idea of the construction of artificial heart and organ perfusion dates back to 4th century BC, however, no one could construct such apparatus during that time. Concept of contact lenses dates back to year 1508 with the thoughts of Leobnardo Do Vinci. The first hip replacement was performed in year 1891 by a German surgeon Theodor Gluck, who used cemented ivory ball as an implant. Furthermore, improvements with the technology developed chrome-based alloys and stainless steel implants with better mechanical properties. Also, experimentation with the dental implants dates back to 1809, when Maggiolo implanted a gold postanchor into the fresh extraction socket. Moreover, attempts to construct the dialysis unit for kidney ailments dates back to year 1901 when John Jacob attempted to remove toxins from the patient's blood. However, major advances in kidney dialysis were made by Dr Scribner during 1921 to 2003 at the University of Washington. Thomas Cornin and Frank Gerow at the University of Texas invented the first silicon breast implant during early 1960's. Furthermore, many variants of this device have been tried over the years. First fully implantable pacemaker was developed by the fusion of engineering and medical concepts by Wilson Greatbatch an engineer by profession and WM Chardack a cardiologist in the year 1959.

CLASSES OF BIOMATERIALS

Biomaterials can be divided into three major classes as follows:

1. Polymers (synthetic and natural);
2. Metals and
3. Ceramics (e.g. carbons, glass-ceramics and glasses)

Polymers

Polymeric biomaterials have many other applications in addition to tissue regeneration or in regenerative medicine. Some examples include poly (methyl methacrylate) which has been used as bone cements, poly (glycolic acid) as degradable surgical sutures, poly (glycolic-co-lactic acid) as bone screws or poly (vinyl siloxane) as dental implants. Furthermore, polymer like poly (hydroxyethyl methacrylate) (PHEMA) is being used as soft contact lenses. Polymers used as biomaterials can either be natural or synthetic and both have shown applicability in the field of regenerative medicine. These polymeric classes consist of large number of small repeating units. Synthetic polymeric biomaterials being used in the area of regenerative medicine include; silicone rubber (SR), polyethylene (PE), polypropylene (PP), poly (ethylene terephthalate) (PET), polytetrafluoroethylene (PTFE), poly (methyl methacrylate) (PMMA), poly (vinyl chloride) (PVC), PHEMA, poly (ethylene glycol) (PEG), etc. PMMA which is a hydrophobic polymer has shown applicability as bone cement for orthopedic applications. Due to the excellent light transmittance of PMMA it has proved to be a good material for intraocular lenses (IOL's) and as hard contact lenses. For the fabrication of the soft contact lenses poly (HEMA) is cross-linked with ethylene glycol dimethacrylate (EGDMA). Cross linking helps to retain the dimensional stability of the lens. Additionally polymers like polymethacrylic acid in small quantities are also incorporated into contact lens formulations to improve the wetability. Polymers like polyacrylic acids are being used as dental cements and also these polymers have shown applicability as mucoadhesive additives in mucosal drug delivery formulations. Polypropylene (PP) is a isotactic crystalline polymer with high rigidity and good tensile strength and has thus found applicability as surgical sutures and in hernia repair. Polyvinyl alcohol (PVA) is being used as tubing and blood storage bags in the area of biomedical science. These tubing even include blood transfusion tubes, dialysis tubing, etc. Biodegradable polymer like PLGA is used as resorbable surgical sutures, drug delivery systems and in orthopedic applications as fixation devices. Furthermore, its intrinsic usage is enhanced as the degradation products of PLGA are lactic acid and glycolic acid which are as such nontoxic for the host. Copolymers from various monomeric units represent an important class of material design which has a significant applicability in the area of biomedical science. A copolymer of tetrafluoroethylene with small amount of hexafluoro-proplylene (FEP Teflon) is used as a tubing connector and as catheters.

In addition to synthetic polymers, natural polymers also have shown applications in the area of biomedical science and regenerative medicine. Although several naturally occurring polymeric materials can be fabricated into the potential tissue engineering scaffolds, commonly investigated materials include alginate, collagen, hyaluronic acid, fibrin gels, etc.[5] Alginate consists of repeating monosaccharide units, i.e. L-guluronic acid and D-mannuronic acid. Exposure of these polysaccharides to calcium ions results in the formation of a three-dimensional gel. This gel can be used for the encapsulation of growth factors or specific cell type. Alginate has shown applicability in the area of cartilage regeneration, as chondrocytes are reported to maintain their phenotype in the alginate gels. Alginate gels also have shown immense applicability for the storage of chondrocytes, as cells can survive for up to

8 months in these gels.[6] Natural polymer like collagen has shown a huge potential in the area of skin regeneration as it is a natural component of human skin. Bovine collagen combined with cellular components like human autologous keratinocytes and fibroblasts has shown good results when applied to severe burn victims.[7] Collagen is also being used in the form of bilayered artificial skin for the purpose of skin regeneration.[8] This template is acellular and is composed of collagen and glycosaminoglycans which is commercially available as integra artificial skin or integra.[9,10] Other types of skin regeneration templates, e.g. matriderm uses bovine dermal collagen type I, III and elastin.[11] Additionally natural polymer like hyaluronan as such lacks desirable characteristics to be used as a scaffold for tissue regeneration. Few limitations include high water solubility and fast clearance time from the host tissue.[12] Few limitations can be answered by chemically modifying its structure to increase its stability. One example of such modified hyaluronan derivatives HYAFF 11; it is formed from the complete esterification of all free carboxylic groups with benzyl alcohol. This modified form has been utilized as a degradable scaffold biomaterial for tissue regeneration. These scaffolds have shown considerable promise for cartilage repair.[12,13] Fibrin is a natural component of animal skin and plays a major role during the process of wound healing. So fibrin in the form of a biomaterial has a potential to be utilized as a scaffold for tissue regeneration. Major disadvantage with fibrin gels is their fast biodegradation which occurs due to the phenomenon of fibrinolysis.[13] So modifications with its structure have resulted in the gels those remain intact for longer periods of time and these gels are commercially available as Tissucol.[5] Such modified fibrin gels have been used for the development of extracellular matrix by chondrocytes. Other natural polymers those has shown potential to be used as scaffolding materials for regenerative medicine include cellulose, agarose, chitosan, gelatin, etc.

Metals

Metallic implants have a significant economic impact in the biomaterials field. Examples of metallic implants include steels 316, 316L, vitallium, silver, tantalum, cobalt, F-75 and alloys of Ti, Cr + Co, Cr + Co + Mo, etc. Metallic implants have certain disadvantages like low biocompatibility, susceptibility to corrosion under physiological environment and large variations in the mechanical properties from the biological tissue.[14] Metallic implants have a enormous applicability in the area of biomedical science as they are used as staples, plaques and wires. They are also being used as tooth implants, penis implants and can be used as mesh for facial reconstruction.[15]

Ceramics

Ceramics, glasses and glass-ceramics comes under the broad range of inorganic/nonmetallic composites. This class has shown applicability as eye glasses, diagnostics instruments, chemical ware, thermometers, etc. In general, ceramics show characteristics like high biocompatibility, high resistance to corrosion, low electrical and thermal conductivity which makes them suitable as implants.[16] Ceramics like hydroxyapatite (HAp) has reported to play an important role in the formation of new bone tissue. Other examples of ceramics used as biomaterials include aluminum oxide, calcium aluminates, titanium oxides, calcium phosphate, carbon, bioglass, etc. Disadvantages associated with ceramics include low impact resistance, processing and fabrication difficulties, etc. Major applications of ceramics in biomedical area include as dental parts, coatings, bone fillings, ontological implants, medical tools and implants, etc. Furthermore ceramic composites, i.e. metals with ceramic coatings or materials coated with carbon have found applicability in the area of biomedical science. Their high biocompatibility and resistance to corrosion make them appropriate implanting devices. But their disadvantages like the lack of consistency and difficulties in the fabrication process limits their explicabilities to the area of biomaterials. Their major biomedical applications include heart valve implants, knee implants, hip implants, etc.[17,18]

PROPERTIES OF BIOMATERIALS

Properties of the biomaterials depend on the class to which it belongs. But, key properties include surface properties, corrosion, mechanical properties and degradation.

Surface Properties

As interactions between biomaterial and host tissue is a surface phenomenon, so surface properties of an implant material/biomaterial is of great importance. Material surface is the termination of normal three-dimensional structure of a particular biomaterial. Ceramics are typically electrical and thermal insulators. The strong ionic and covalent bonds make them hard and brittle. Furthermore, thermal behavior of polymeric biomaterials is influenced by the factors like composition of the backbone and side groups. A change in the polymer composition or structure that increases the relative movement of chains in turn increases the strength and decreases the plasticity of the material.

Corrosion

Metallic implants are subjected to the phenomenon of corrosion under physiological conditions. During the

process of corrosion, metallic biomaterials have a tendency to release the ions which reduces the biocompatibility of the implant and can also jeopardize the fate of the implant. So metallic implants like 316L stainless steel performs satisfactorily in short term applications but are susceptible to corrosion when implanted for longer periods of time.

Degradation

Other classes of biomaterials like ceramics and polymers do not undergo corrosion, but they are susceptible to the degradation under physiological conditions. An ideal biomaterial should possess a regulated rate of degradation, which should match with the formation of the neotissue. During the process of degradation, byproducts released from the implant may induce adverse local and systemic host reactions. This release is a concern for materials like bone cements, poly (vinyl alcohol), etc. Among the biodegradable polymers poly (lactic acid) and poly (glycolic acid) or their copolymers degrade majorly into lactic acid which is removed by the normal metabolic processes of the body.

Mechanical Properties

Mechanical properties of an implant depend on several factors like fabrication process and the type of metal used. Mechanical properties of any biomaterial implant should be at par with the native tissue. So for bone implants they should possess modulus that is of higher magnitude than that of the bone. Metallic implants like stainless steel or Co-Cr alloys have Young's modulus in the range of 190 to 253 GPa. But biomaterials like ceramics and glasses are brittle and have poor tensile properties. Among the biomedical ceramics, alumina has the highest mechanical properties, but its tensile properties are still lower than those of metallic implants. Furthermore, ceramics like calcium phosphate and bioactive glasses are unsuitable as load bearing implants due to their inferior mechanical properties. In case of polymeric biomaterials, mechanical properties depend on the composition and structure of the macromolecular chains. Compared to the metals and ceramics polymers have much lower mechanical strength and moduli. So polymers are generally not used in biomedical applications that are meant for bearing the heavy loads. But out of the polymers ultra-high-molecular weight polyethylene is an exception and is thus used as a bearing surface in hip and knee replacements.

EVALUATION OF BIOMATERIALS FOR CLINICAL USE

For any biomaterial to be used for regenerative medicine, the first perquisite is to evaluate its biocompatibility under *in vitro* conditions and then *in vivo*. Evaluation of the material under *in vitro* conditions provides a rapid and inexpensive approach on the interaction of biomaterial with a particular cell type. But results obtained from *in vitro* experiment may not be relevant, if a biomaterial is meant for the *in vivo* implantation. So, lab animals are used for testing the *in vivo* biocompatibility of a newly designed biomaterial. First important step is the selection of model system which offers a similar anatomy and physiology to that of humans.

In vitro Analysis for the Determination of Biocompatibility

For any new polymeric biomaterial or implant device to be utilized for the regenerative medicine, first important assessment is to check its 'cytotoxicity' on the model cell line. Term cytotoxicity refers to any toxic effect (death, alteration in cellular permeability, etc.) induced by the material at the cellular level. Any biomaterial can be tagged as 'toxic' if it kills the cells either directly or indirectly. So any biomaterial intended to be used as a medical implant should be initially assayed for its cytotoxicity in the *in vitro* conditions. After the cytotoxicity profile has been analyzed, then more specific tests are performed to assess the overall biocompatibility of the implant. During the assay methods, test material may be placed directly on the cells or may be extracted in a solution that is then placed on the cells. Based on how the assay is performed there are three major methods to analyze the biocompatibility of any material i.e. direct contact, agar diffusion and elution. Out of all these method direct contact method is majorly used in the area of tissue engineering and regenerative medicine. This assay is done by taking a near confluent monolayer of L-929 cells (mammalian fibroblast cells). Culture media is removed and fresh media is added to the plate. Specimen (biomaterial or implant) is placed carefully in the culture plates and incubated for 24 hours at 37ºC under humid atmosphere. Following the incubation, cell line is stained with cytochemical stains, e.g. hematoxyline and eosin or by live fluorescent stains, e.g. fluorescein diacetate (FDA). Toxicity is evaluated by the absence of stained cells under and around the specimen. Other direct contact assay is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT assay). In this assay cells are allowed to grow on the biomaterial for few days. On the day of the assay, media is removed from the wells followed by the addition of MTT reagent. This assay involves the conversion of tetrazolium salt MTT by viable proliferating cells to an insoluble product, purple formazan after 4 hours of incubation time. These formazan crystals can be solubilized in an organic solvent like DMSO and the resultant colored

complex can be quantified by checking the optical density spectrophotometrically at 570 nm.

In vivo Analysis for the Determination of Biocompatibility

Main aim of *in vivo* assessment is to determine the biocompatibility of new biomaterial/medical device in a biological environment under physiological conditions.

Animal models for the *in vivo* assessment of medical devices:

Device classification	*Animals*
1. Cardiovascular	
– Heart valves	Sheep
– Vascular grafts	Dog, pig
– Artificial heart	Calf
2. Orthopedic/bone	
– Bone regeneration/ substitutes	Rabbit, dog, pig, mouse, rat
– Total hip/knee joint replacements	Dog, goat
– Vertebral implants	Sheep, goat, baboon
3. Neurological	
– Peripheral nerve regeneration	Rat, cat Nonhuman primates
– Electrical stimulation	Rat, cat, nonhuman primates
4. Ophthalmological	
– Contact lens	Rabbit
– Intraocular lens	Rabbit, monkey

In vivo assessment is done by the following methods:

Implantation

A newly designed biomaterial is implanted surgically or is placed into an implant site in an animal model. Evaluation of the pathological effect is then carried out at both gross and microscopic levels.

Hemocompatibility

This test evaluates the effect of a medical implant on the blood components. According to ISO standards five test categories are indicated for hemocompatability, i.e. thrombosis, coagulation, platelets, hematology and immunology (complement and leukocytes).

Biodegradation

This test determines the amount of degradation during a given period of time, i.e. kinetics of degradation. It is done by the implantation of a biomaterial in experimental animal. This test further gives an idea about the nature of released by products during the process of degradation. An ideal biomaterial should have an optimized rate of degradation and should not be degraded into any toxic byproducts.

NEW CLASSES OF SCAFFOLDS FOR BIOMEDICAL APPLICATIONS

The traditional biomaterials as discussed before can be fabricated into a desired three-dimensional scaffold which acts as a housing for the initial cell growth and proliferation. These biomaterials can be fabricated into scaffolds using different fabrication technologies like:

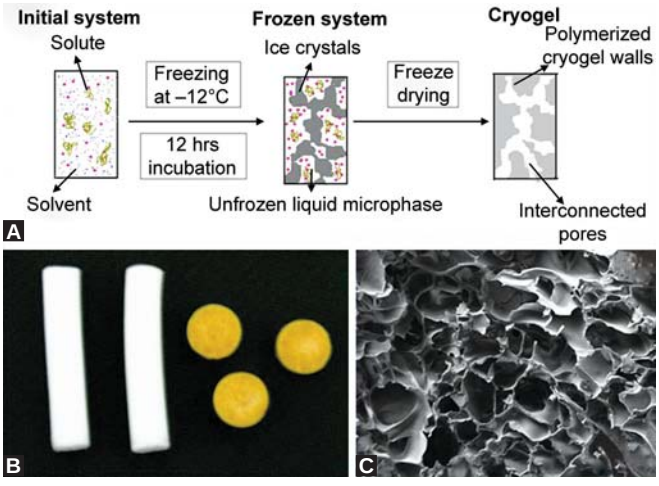
1. Solvent casting in combination with particulate leaching;
2. Fiber networking;
3. Phase separation in combination with freeze drying
4. Solid free form fabrication.[19] But these scaffold fabrication technologies have associated limitations like lack of interconnectivity, desired pore size, etc. and thus new scaffold fabrication technologies are emerging.

Hydrogels as Tissue Engineering Scaffolds

Hydrogel is a colloidal gel in which water is the dispersion medium. They have emerged as important scaffolding biomaterials as they resemble the native tissue. Aqueous nature of hydrogels closely resembles the cells in body. They possess a good porosity which allows the nutrient and waste exchange. Both natural and synthetic polymers can be fabricated into hydrogel scaffolds those are then used for the purpose of tissue regeneration.[20] Synthetic hydrogel scaffolds used in the area of regenerative medicine are polyurethanes (PU), poly (ethylene oxide) (PEO), poly (N-isopropylacrylamide) (PNIPAAm), poly (vinyl alcohol) (PVA), poly (acrylic acid) (PAA) and poly (propylene furmarate-co-ethylene glycol) [P(PF-co-EG)]. Naturally derived polymers fabricated into tissue engineering scaffolds include agarose, alginate, chitosan, collagen, fibrin, gelatin and hyaluronic acid (HA).[21]

Cryogels as Tissue Engineering Scaffolds

Cryogels are the gel matrices synthesized by the process of cryogelation which synthesizes the scaffolds at sub-zero temperature from natural or synthetic polymers without the use of inorganic solvents (Fig. 1). Scaffolds generated by the process of cryogelation have advantage over other types of scaffolds. As cryogel scaffolds can be fabricated in diverse formats like disk, sheets and monoliths with variable dimensions. Cryogelation generates the matrices those possess large and interconnected pores. During the process of cryogelation most of the solvent gets frozen while part of the solvent is left unfrozen (unfrozen liquid microphase),



Figs 1A to C: Process of cryogelation (A), digital images of the cryogels in different shapes (monoliths and disks) (B), and scanning electron microscopy image of the cryogel showing interconnected porous network (C) (reproduced with permission from Kumar et al 2011)[23]

where monomeric or polymeric precursors concentrate and undergo chemical reactions. This chemical reaction in the liquid microphase leads to gel formation that is converted into porous scaffold on thawing the frozen part which acts as porogens. Cryogel matrices facilitate the unhindered diffusion of solutes and nutrients due to the presence of interconnected macropores.[22] Cryogels possess continuous interconnected pores up to 200 mm that provide a surface for the proliferation of most of the cell types making these matrices suitable for tissue engineering applications.[23]

The potential of cryogel matrices have been explored widely in the areas of tissue engineering/regenerative medicine, bioseparation and therapeutic protein production, etc. In the area of tissue engineering cryogels have shown potential for the regeneration of cartilage, bone, skin and liver, etc.[24-26] These scaffolds have the potential to be utilized for other biomedical applications which can be explored further.

APPLICATION IN REGENERATIVE MEDICINE

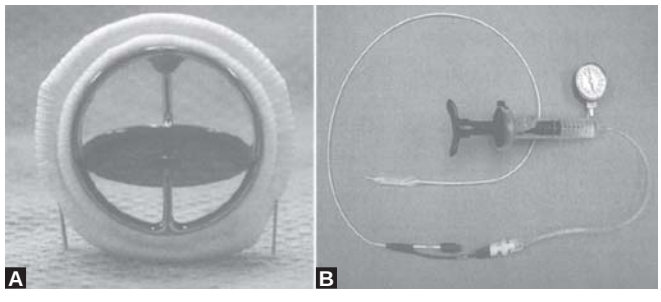
Cardiovascular Devices and Implants

Cardiac replacement valves fall into two general types, i.e. mechanical and biological. Mechanical valves can further be categorized into three subclasses, i.e. caged ball, single tilting disk and bileaflet.[27] Biological valves are divided according to the source of tissue material, e.g. homograft biological valves and heterograft bioprosthetic valve. Homograft biological valves are preserved human aortic valve or pulmonary valves those are surgically placed within the recipient.[28] Heterograft bioprosthetic valves are derived from animal sources like porcine heart valves or bovine pericardial tissue those are formed into valves over a support structure.[27] Additional types of cardiovascular implants are stents and stent grafts. Stents can be divided into two main groups and this classification is based on the method of expansion. First type is balloon-expandable stents those arrive premounted on a balloon angioplasty catheter or can be mounted by the surgeon before the procedure (Figs 2A and B). Next type of stent is self-expanding which comes premounted or sheathed. During the procedure sheath can be pulled back allowing the stent to expand to its predetermined diameter.[29]

Artificial RBC Cell Substitutes

Research work toward the development of red cell substitutes has the applicability in blood replacement therapies, either for perioperative hemodilution or for resuscitation from hemorrhagic blood loss. Unlike true blood, artificial blood is intended for the sole purpose of transporting oxygen and carbon dioxide throughout the body. The ideal artificial blood product should possess the following characteristics; it must be safe to use and compatible within the human body with different blood types. It must be able to transport oxygen throughout the body and release it where it is needed and lastly it must be shelf stable. It should be used as an alternative and/or supplement to homologous and autologous transfusion, or in combination with the use of erythropoietin. Depending on the type of artificial blood that is made, various raw materials are used. The two significantly different products, i.e. perfluorocarbons (PFC) and hemoglobin-based products are under development as blood substitutes. They differ primarily in the way that they carry oxygen and have been used for perfusional protection of organs. PFC products involve a polymerization reaction. PFC is chemically inert compound consisting of fluorine substituted hydrocarbons. Unlike Hb-based substitutes, PFCs have the following advantages:

1. They do not react with oxygen or other gases.
2. Increase the oxygen solubility in the plasma compartment.



Figs 2A and B: (A) A typical heart valve, (B) a balloon catheter and inflation pump (reproduced with permission from Gage and Wagner, 2002)[29]

3. The dissolved oxygen is not subject to the effects of temperature, pH, 2,3-DPG, etc. (thus, the oxygen dissociation curve is linear)
4. Facilitate effortless transfer of oxygen from red cells to the tissue. The final goal of any transfusion service is to create a transfusion system with no side effects and with more effective medical care. Although current system of homologues blood is working well with low cost, acceptable efficacy and relatively less side effects.[30]

Extracorporeal Artificial Organs

These devices are used to process the patient's blood outside the body before it is returned to the circulation. Examples of these devices include gas and heat exchangers, dialyzers, apheresis devices, bioartificial liver, etc. All these devices are designed to optimize the flux of material between body fluids and other fluids separated from each other by a membrane. Bioartficial liver devices are used to support the patients suffering with acute liver failure. These devices either use hepatocytes in bioreactor which provides both exchange and synthetic functions or uses nonliving components which remove toxins accumulated due to liver failure. These devices are used to assist the patient till transplantation. These devices use natural or synthetic polymers like collagen in case of hollow fiber system.[31]

Orthopedic Applications/Dental Implants/ Cartilage Implants

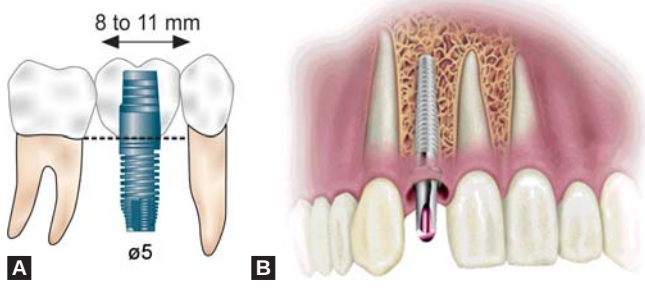
In addition to the metallic implants naturally derived matrices made from hyaluronic acid, chitosan, collagen, fibrinogen, etc. have also been used in bone tissue engineering. But the disadvantage with the use of these matrices is that they are difficult to sterilize and can also elicit immune response in the host. Synthetic polymers like poly(a-hydroxy acid), polypropylene fumarate, polyethyleneglycol, etc. have an advantage that they can be fabricated with desired parameters, but they degrade into toxic components. Wide range of bioactive inorganic materials is showing potential in bone tissue engineering, e.g. bioactive glass, hydroxyapatite, porous coralline, tricalcium phosphate, etc. Further work is being focused on enhancing the bio-functionality of the scaffold by the incorporation of osteinductive cues which can enhance osteoblast proliferation on these scaffolds.[32] Scaffolds fabricated from natural and synthetic polymers are being used in cartilage tissue engineering. Examples of natural polymers include agarose, alginate, chitosan, collagen, fibrin, hyaluronan, etc. and synthetic polymers are poly (a-hydroxy ester), polylactic acid (PLA), polyglycolic acid (PGA) and their copolymers, etc.[24] Dental implants those are designed for the commercial applications can be divided into two categories:

a. Endosteal or endosseous, these implants extend into the bone tissue while.
b. Subperiosteal systems are in contact with the exterior bone surfaces (Figs 3A and B). The endosteal implants, e.g. root forms (cylinders, screws), blades (plates), transossious or staples or endodontic stabilizers are placed into the bone. But superiosteal devices are fitted to the bone surface in the form of customized shapes. Furthermore bone plates are placed into bone under periosteum and are fixed with the help of endosteal screws.[33] Synthetic materials used for root form devices include valuable metals like gold, platinum, iridium and palladium. Other commonly used biomaterials or dental implants include titanium and its alloys, aluminum oxide and surface coatings of hydroxyapetite.

Surgical Sutures/Surgical Adhesives

Surgical sutures can be classified as follows:

a. *Natural sutures:* Major examples include catgut suture which is derived from bovine intestines serosa or from the submucosa of sheep or goat intestines. Primary constituent of the catgut suture is collagen.
b. *Synthetic nonabsorbable sutures:* Examples include polysester sutures those are based on poly (ethyl-eneterephthalate) (PET), poly (butylenes terephthalate) (PBT) and polybutester. Other example of synthetic suture is polyamide sutures which includes nylon-6, 6 and nylon-6.
c. *Synthetic absorbable sutures:* All the major absorbable sutures are made from one or more of the five different cyclic monomers, i.e. glycolide (GA), L-lactide (LLA), trimethyl carbonate (TMC), p-dioxanone and ε-caprolactone. Primary mode of degradation for natural materials is enzymolysis while for synthetic materials it is hydrolysis. Adhesive is a general term that covers designations like cement, glue, paste, fixative, etc.



Figs 3A and B: (A) Modern dental implant and (B) a endosseous dental implants (reproduced with permission from http://www.cosmeticdentistry bydesign.com/implants.html)[33]

Examples include cyanoacrylates derived from methyl-2-cyanoacrylate. This liquid monomer polymerizes rapidly even in the presence of moisture and blood. Other examples include protein glues, hydrogels, tooth and bone cements, etc.

INTERACTIONS OF BIOMATERIALS WITH STEM CELLS

Regenerative medicine and tissue engineering intend to generate a functional tissue or organ. Following section of this review discusses about the developments in biomaterials and knowledge of the stem cell biology for their applicability in the area of regenerative medicine.

Stem Cell Niche

During the tissue damage sometimes there is a loss of deeper layers that contains the stem cell niches. In such cases biomaterials could be useful tool for reestablishment of the niche functionality.[34] In comparison to the standard two-dimensional (2-D) culture systems a three-dimensional (3-D) scaffolds based model would facilitate a spatial distribution of the different cells resulting in the structural organization which may resemble the *in vitro* tissue organization.[35]

Bone Tissue

Both autografts and allograft therapies for the bone regeneration have several drawbacks, so alternative approaches are being explored. One such approach is the isolation and expansion of the mesenchymal stem cells (MSC's) from the patient and their seeding onto the porous three-dimensional scaffolds. During the *in vitro* cultivation as the stem cells are exposed to signaling molecules supplied in the media, MSC's differentiate toward osteogenic lineage. This engineered tissue can then be implanted at the defect site to regenerate the new bone as scaffold degrades.[36]

Nervous Tissue

Treatment of the nervous tissue particularly in spinal cord injuries require new medical therapies as axons do not have regenerative capacity in the native environment. Current strategy is the use of nerve autografts, but due to the limitations like donor site morbidity, etc. this strategy does not provide a promising solution for the repair. Alternative therapy consists of use of nerve guidance conduit that could provide a pathway for nerve out-growth and could also promote nerve regeneration. Recently it has been shown that design of surface topography may stimulate stem cell differentiation toward the neural lineage. In this direction hydrogenated amorphous carbon (a-C:H) groove topographies with the width/spacing ridges ranging from 80/40 μm, 40/30 μm, 30/20 μm and depth of 24 nm were used as a single mechanotransducer stimulus to generate neural cells from hBM-MSCs *in vitro*.[37]

Skeletal and Cardiac Muscles

Traumatic injuries can interrupt muscle contraction by causing damage to the skeletal muscles and peripheral nerves. Natural healing may result in scar tissue formation. Therefore, use of three-dimensional scaffolds will trigger muscle cell elongation, orientation, fusion and striation. Electrospun chitosan microfibers have been used as novel biomaterials for muscle repair. Classical porous scaffolds may be inadequate as they do not reproduce typical myocardial environment. Considering the significance of topography in this process, one approach is to mimic the microenvironment of the native tissue. In this direction, microfabricated scaffolds were created with soft lithography technique using bioartificial blend, based on alginate, gelatin and a novel poly (N-isopropyl acrylamide) based copolymer. This scaffold showed anisotropic mechanical properties which resembles the native tissue.[38]

ACKNOWLEDGMENT

The authors would like to acknowledge Department of Biotechnology (DBT), Department of Science and Technology (DST), Ministry of Science and Technology, Government of India for financial support for our projects in this area.

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