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Review on engineering biomaterials in tissue engineering application

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Abstract

Biomaterials are the materials used to restore or replace functions in body tissues and are continuously or occasionally in contact with body fluids. Today, biomaterials are being used for wound dressing to soft and heard tissue repairs. In this review, we present a brief elucidation of biomaterials that are being developed for engineering tissues including biocompatible metals, ceramics, polymers and hydrogels. Surgical implant made up of stainless steel, titanium alloys are some of the widely accepted metallic alloys in tissue engineering treatment. Along with these metals, bioceramics made up of hydroxyapatite (HA) and Tricalcium phosphate (TCP) are analogous to the inorganic constituents of hard tissues of vertebrates. In recent years, hydrophilic biodegradable fibers with nanometer range have attracted great attention due to their non-immunogenic, non-toxic and bioresorbable nature.

Keywords: Biomaterials, Regenerative Medicine, Medical Implants.

INTRODUCTION

Regenerative medicine is a new multidisciplinary approach to restore the damaged tissue or organs in the body, either by stem cells or progenitor cells transplantation or by encouraging endogenous precursor cells. The main essence of tissue engineering/ regenerative medicine portray the use of cells together with natural or synthetic extracellular materials in developing implants (Scaffold) to restore the damaged organs or tissues. It is evident that selection of scaffold is vital to facilitate the cells to behave in required manner to produce tissues or organs of our interest [1]. The ideas of using biological substitutes to repair or replace damaged tissues were discussed even in earlier records. As per "The Book of Genesis" Adam was the first donor to donate a rib to fashion Eve [2]. Physicians in antique India developed the skin grafts for cosmetic surgeries as early as 800 B.C. However, Tissue engineering had to wait until progress in the modern surgery. Figure 1 illustrates the transplantation milestones in the western world.

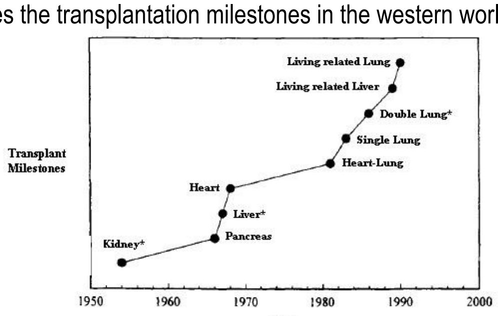

Fig 1. Major breakthroughs in organ transplantation in western world. * Denotes this transplantation is first time in this world. (Source: Fox R. C & Swazey, 1992)

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Significance of Biomaterials in Regenerative Medicine

Till to date, organ or tissue transplantation is like 'robbing Peter to pay Paul'. That is, tissue defects due to accidents, diseases, trauma and congenital defects are repaired by transferring healthy donor organs or tissues as an autograft, isograft, allograft and xenograft [3]. Although these solutions have resulted in great pace in increasing patient survival and quality of life, there are inherent limitations. In case of autograft, volume of donor tissue that can be harvested is dependent on blood supply and the need to avoid visceral injuries. Allografts and xenografts own the risks of immune rejection and transmission of infectious disease [4]. In addition to these limitations, there is presently a growing donor availability crisis which is well explained in Figure2.

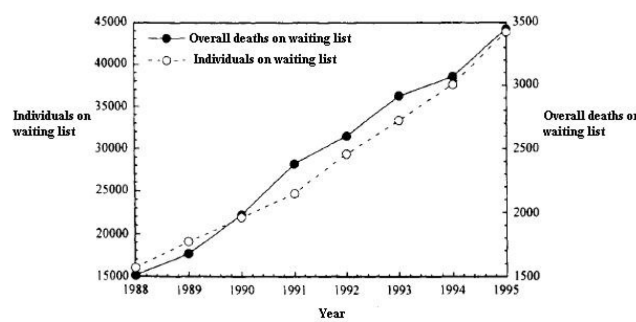

Fig 2. Overall deaths occurring while patients waiting for organ transplantation. (Source: 1996 Annual Report of the U. S. Scientific Registry for Transplant Recipients and the Organ Procurement)

Shortage of tissues and organs availability for transplantation led to the emergence of discipline biomaterials in tissue engineering. A well-designed three-dimensional scaffold is one of the primary tools to guide tissue formation *in vitro* and *in vivo.* Boundaries in medicine are changing rapidly from utilizing synthetic implants and tissue grafts to a tissue engineering approach that uses bio-degradable porous material scaffolds integrated with biological cells or molecules to regenerate tissues or organs [5]. In this regard, the selection of scaffold material is very important to facilitate the cells to behave in the desired manner to generate tissues and organs of our requirement.

Essential Features of biomaterials

a) It must be biocompatible, avoid transplant rejection by host immune system.
b) It must have excellent surface properties to allow the cells to adhere, proliferate and migrate in to the scaffold.
c) Scaffold material must possess controlled biodegradability to aid reconstruction of new tissues.
d) The implant designed must exhibit sufficient mechanical properties to maintain the structure and function of the implant immediately after transplantation.
e) Scaffolds must hold the structural and functional properties of extra cellular matrix which have the ability to transfer biomolecular signals between cells.

Classes of Biomaterials

In general, materials used in regenerative medicine are classified in to, Class I materials those that do not directly in contact with the tissues; Class II materials are those that contact occasionally to the tissue; and Class III materials are those that are constantly in contact with the tissues [6]. Class III materials are also entitled as biomaterials and are further divided into 3 categories according to their biological interface with the body tissue as well as immune system. Bio-inert materials which do not produce any immunological reactions but retain their structure in the body. Bioactive materials demonstrate biological function and finally, biodegradable that are dissolved in body and replaced by regenerated tissues [7].

Table 1. Classification of Materials in Tissue engineering applications

Class I	Do not directly contact the body tissue		
Class II	Contact intermittently to the tissues		
Class III	Biomaterials	Bioinert	No Immunological reaction
		Bioactive	Mimic the natural tissue
		Biodegradable	Completely absorbed in the tissues

Biodegradable biomaterials have the potential to induce the biological dysfunction *in vivo*. The interface between the material and tissue is the key area where the biological disturbances are created. To restore the tissue defects, the *in vivo* and *in vitro* studies of the released substances from material surface are indispensible in determining the biological characters of the implant material. The implant material should not inhibit the biological functions of the host tissues [8].

Non Biodegradable Metallic Implants

Metals have quite attractive features in the area of bone tissue engineering including superior fracture toughness, high strength to weight ratio and high ductility [9]. Stainless steel is the first metallic implant that was widely accepted as bone fixation plates, screws, etc since early 19^{th} century. However, metals have some intrinsic problems when used as tissue engineering implants. These metals typically not biologically active, meaning they don't support osteoinduction (stimulate osteoprogenitor cells) and osteoconduction (facilitates out spread of bone cells over the scaffold surface) of the bone cells. To overcome this restraint, surface coating or modification of biocompatible metals presents a way to improve the surface biocompatibility [10]. Another limitation of the current metallic implants is the possible release of toxic metallic ions and particles through corrosion lead to inflammatory cascades and allergic reactions, which reduce the biocompatibility and cause tissue loss [11]. A proper treatment of the implant surface may help to avoid this problem and create a direct bonding with the tissue. Following are the few metals widely used in fabrication of implants for tissue engineering applications.

Magnesium

Magnesium alloys are biocompatible and shown promising features for use in orthopedic implant. [12]. Significant progress on biologically active magnesium stents and orthopedic bones has been achieved in recent years. Furthermore, its elevated biodegradability eliminates the second surgery for the removal of the scaffold. All these facts suggest that Magnesium (Mg) has momentous potential as a load-bearing biomaterial [13]. Recently, Mg-Ca alloys are produced and evaluated *in vitro* and *in vivo* as biodegradable biomaterials for orthopedic applications. However, concerns over the toxicity of dissolved Mg have been raised due to its rapid dissolution in body fluids, but it has been shown that the excess of magnesium is efficiently excreted from the body in urine [14]. If we were able to control the corrosion in Mg and its alloys by ceramic coating or by titanium coating, Mg seems to be more promising for tissue engineering applications.

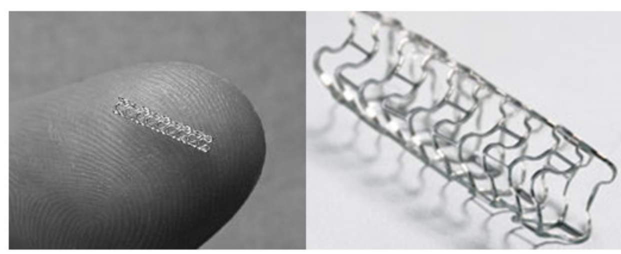

Fig 3. Biodegradable magnesium stents

Titanium and Titanium alloys

Very few metals can be safely implanted into human beings. The implants must be tough enough to support a variety of loads offered to it and also ductile enough to move fluidly. In this regard, an extensive research is carried out on titanium (Ti) and its alloys which are very much biocompatible with a low rate of infection and rejection [15]. In addition, titanium forms a very stable passive layer of $TiO2$ on its surface and provides superior biocompatibility by preventing the corrosion by body fluids [16, 17]. Even if the passive layer is damaged, the layer is immediately rebuilt.

Since 1940, alloys of titanium are being tested for use in orthopedics and continued to gain attention due to their unique properties including high specific strength, light weight, excellent corrosion resistance and biocompatibility [18]. The most efficient Ti alloys being used are "alumina-coated titanium" (Ti-6Al-4V), ASTM F1295 (wrought Ti-6Al-7Nb alloy), ASTM F1713 (wrought Ti-13Nb-13Zr alloy), etc [19]. On the other hand, Ti and its alloys are not ferromagnetic and do not cause harm to the patient on magnetic resonance imaging (MRI) unit. Even though the above discussed metallic scaffolds were appropriate for heard tissue engineering, the bioactivity of these scaffolds was always uncertain. In order to promote these materials as cell carriers in tissue engineering we must coat the metallic scaffolds with biologically active materials like hydroxyapatite (HA) and other growth factors like fetal bovine serum (FBS), etc. (FBS) [20].

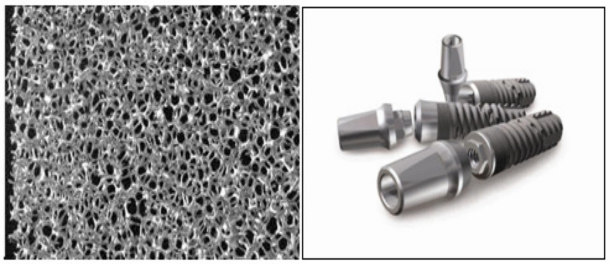

Fig 4. Porous and solid Titanium scaffolds

Nickel- Titanium Alloys (Nitinol)

Nickel titanium alloys have drawn a lot attention due to their mechanical properties like superelasticity or shape memory effects (remember its original shape) [21]. Since the elastic modulus and the compressive strength of Nickel- Titanium alloys (NiTi) are close to that of the bone and due its good biocompatibility, porous NiTi alloys have been used in making spinal inter vertebral spacers used in the treatment of scoliosis [22]. Extensive *in vivo* testing results indicate that NiTi alloys are highly biocompatible, more than stainless steels [23]. Biocompatibility of Nickel titanium alloys and their physical properties suggest that this alloy may offer substantial gains in the orthopedic field.

Fig 5. Metallic Hip made up of Nickel- Titanium Alloy

Biodegradable Implants

To construct an ideal scaffold for tissue engineering, biodegradable implants are indisputable candidates for induction in to the tissues and dissolve away completely forever [24]. Use of biodegradable scaffold has made it easy to sustain the mechanical and structural integrity of the system by gradual replacement of scaffold with newly formed tissues [25]. Currently biodegradable ceramics, polymers, gels and their composites have been used to fabricate scaffolds for tissue engineering applications.

Bioceramics

Thousands of years ago people started using ceramic pottery; during last forty years another revolution has accord in the application of ceramics to improve the quality of human health. This revolution was development of specially fabricated bioceramic scaffolds to repair and reconstruction of damaged parts of body. Generally bioceramics are synthesized in crystalline and amorphous forms and they are generally classified into two major groups; calcium phosphates (CP) and others, including yttria (Y2O3)-stabilized tetragonal zirconia (ZrO2) (YTZP), alumina (Al2O3) and some silicate and phosphate families of glasses and crystallized glasses (glass-ceramics) [26]. The most successful class of bioceramics belongs to biodegradable CP group, including hydroxyapatite (HA) and β-tricalcium phosphate (TCP), they are analogous to the inorganic constituents of hard tissues of vertebrates. List of medically applicable ceramics are given in the tabular column 2.

Table 2. Bioceramics with medical application

Activity	Ceramics	Formula
Bioinert	Alumina	Al_2O_3
	Zirconia	ZrO_2
Bioactive	Bioglass	NA_2O-CaO-P_2O_3-SiO
	Hydroxyapatite (high temperature sintered)	$Ca_{10}(PO_4)_6(OH)_2$
Biodegradable	Hydroxyapatite (low temperature sintered)	$Ca_{10}(PO_4)_6(OH)_2$
	Tricalcium Phosphate	$Ca_3(PO_4)_2$
	Soluble calcium aluminate	CaO-Al_2O_3

Hydroxyapatite

Hydroxyapatite (HA) is chemically similar to the mineral constituents of bones and hard tissues in mammals. HA is one among few materials classed under biodegradable ceramics, meaning it supports bone in growth and osteointegration when used in orthopedic, dental and maxillofacial applications [27]. Implanted HA surface binds to the natural apatite through body fluids and this chemical bonding promotes the bone-implant monosystem. Mechanical properties of HA implants can be controlled by varying the synthesis and sintering temperatures. Normally the HA implants were sintered to increase its mechanical properties, but the implants sintered below 800^0C shows lesser mechanical properties but grater biodegradability *in vivo* [28].Because HA is stable under in vivo conditions, it is often recommended to coat over metallic implants (most commonly titanium/titanium alloys and stainless steels) to alter the surface properties and make the implant more biologically active. In this manner the body sees hydroxyapatite material as truly biocompatible happy to accept [29].

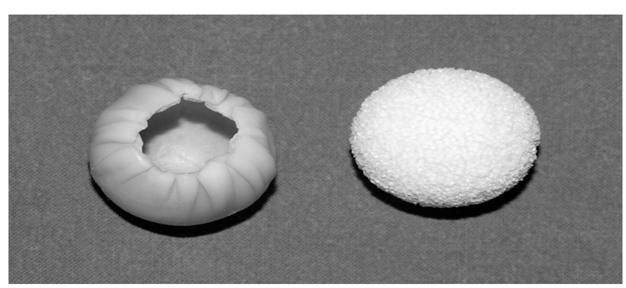

Fig 6. Bioceramic scaffolds made of Hydroxyapatite

β-Tricalcium phosphate

β -Tricalcium phosphate (β-TCP) is calcium salt of phosphoric acid and well known as a biodegradable material demonstrated clinical importance. The porous β-TCP bioceramic scaffolds structurally resemble the cancellous bone, whose porous network could allow tissue to in growth exhibiting good osteoconductive properties. Bone in general consists, by weight of 25% water, 15% organic materials and 60% mineral phases [30]. The mineral phase consists primarily of calcium and phosphate ions, with traces of magnesium, carbonates, etc. as per literature Tricalcium phosphate (TCP) has four polymorphs, *α β γ* and super *α*. The *γ* polymorph is high pressure phase and the super *α* polymorph is observed at temperatures over 1500^0C [31]. Therefore the most frequently available polymorphs in bioceramics are *α* and *β*-TCP and these two forms are interchangeable by varying the sintering temperature.

However, the pure β-TCP porous scaffold shows weak mechanical properties, which limit its application as bone graft [32]. In spite of its limitations β-TCP is preferred over HA due to their excellent bio-absorbability [33]. Application of biphasic composites made up of HA and β-TCP is recommended to fabricate biologically active scaffolds with optimum dissolution rate and other advantages for better performance. However, the biphasic composites cannot be used for load-bearing applications due to their poor mechanical properties [34].

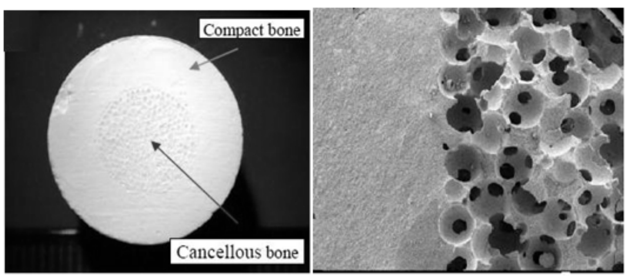

Fig 7. Bioceramic constructs of tricalcium phosphate

Biodegradable Polymers

In recent years, biodegradable fibers which have diameters in nanometer range have attracted great attention, as it is considered that the proper *in vivo* phenotype cannot be consistently achieved if cells are presented with fibers with diameters equal to or greater than the cell size [35]. Biodegradable polymers offer number of advantages over other materials for fabricating scaffolds in regenerative medicine. The key advantages include the ability to tailor mechanical properties and degradation kinetics of polymers to suit various tissue applications. Synthetic polymers are also attractive because they can be fabricated into a variety of shapes with desired pore morphologic features conducive to tissue in-growth. Furthermore, polymers can be designed with chemical functional groups that can induce tissue in-growth [36]. Polymers such as polylactic acid (PLA), polyglycolic acid (PGA), copolymers of PLA and PGA (PLGA), polyanhydrides, polyorthoesters, polycaprolactones, polycarbonates, and polyfumerates have many properties that are well-suited for the purposes of tissue engineering. They are non-immunogenic, non-toxic and bioresorbable [37].

Efforts to find solutions to cure orthopaedic injuries/diseases have elucidated the necessity of developing new polymers that meet a number of demanding requirements. These requirements differ from the ability of scaffold to provide mechanical support during tissue growth and gradually degrade the implant to biocompatible products to more demanding requirements such as the ability to fit in the live cells, growth factors etc and provide osteoconductive and osteoinductive environments [38]. Furthermore, the development of in-situ polymerizable compositions that can function as cell delivery systems in the form of an injectable liquid/paste are becoming increasingly attractive in tissue engineering applications [39]. Many of the currently available degradable polymers do not fulfill all of these requirements and significant chemical changes to their structure may be required if they are to be formulated for such applications.

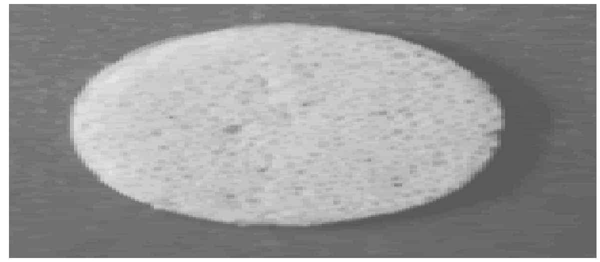

Fig 8. Polylactic acid scaffold

Scaffolds made from synthetic and natural polymers have been investigated extensively in regenerative medicine. This polymer implants have advantages such as the ability to generate desired pore structures, matching size, shape and mechanical properties to suit a variety of applications. However, shaping these scaffolds to fit cavities with complicated geometries, bonding to the bone tissues and incorporating cells and growth factors and requirement of open surgery are a few major disadvantages of this approach. A variety of polymer materials used in fabrication of tissue constructs are given in the tabular column 3.

Table 3. Variety of polymer materials used in tissue engineering applications

	Protein Based	Carbohydrate Based	Synthetic
Natural	Collagen	Hyluronan	
	Fibrin		
Artificial	Gelatine	Polylactic acid	Polymethylmethacrylate
		Polyglycolic acid	Polyethylene
		Chitosan	Polytetrafluoroethylene
		Agarose	Polycarbonate
		Alginate	Polyesterurethane
			Polybutyric acid

Hydrogels

Hydrogels are special class of polymers able to absorb large amounts of water with in the spaces available between polymeric chains. The water holding capacity of the hydrogels arise mainly due to the presence of hydrophilic groups such as amino, carboxyl and hydroxyl groups in the polymer chains [40]. These hydrogels have been used extensively in various biomedical applications like drug delivery, cell carriers, wound management and tissue engineering.

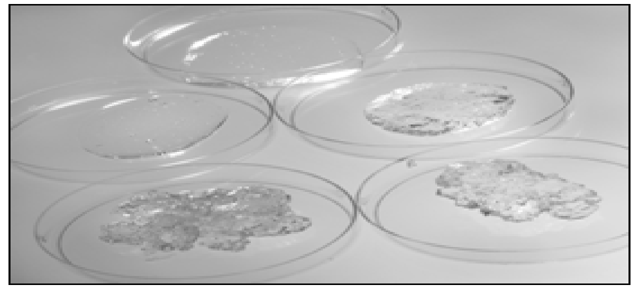

Fig 9. Novel hydrogels for tissue engineering

Cross linked polymers in hydrogels provide a 3-dimensional polymeric network structure to hydrogels. The use of hydrogels for tissue engineering applications dates back to 1960 when Wichterle and Lim developed crosslinked ‘poly hydroxyethyl methacrylate’ (pHEMA) for use in soft contact lenses [41]. A variety of synthetic and naturally hydrogels may be used to synthesize implants for regenerative medicine. Synthetic materials include poly (ethylene oxide) (PEO), poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA),etc are the widely used materials for the scaffold preparation [42]. Recently interest is gained for natural materials like agarose, alginate, chitosan, collagen, fibrin, gelatin, and hyaluronic acid due to their better bioavailability [43].

Hydrogels have many diverse functions in the field of regenerative medicine. They are used as space filling agents, as delivery vehicles for bioactive molecules and as three-dimensional structures that organize cells and present stimuli to direct the formation of a desired tissue [44]. Finally, implants of hydrogel are being used to transplant cells and to engineer almost every tissue in the body including cartilage, bone, and smooth muscle [45].

Application of biologically active hydrogels in tissue engineering has gained much importance due to (a) easy to process the polymers; (b) easy to tailor the properties of the hydrogels; and (c) highly resorbable.

Examples of various hydrogels with tissue engineering applications are provided below: [46]

I. Collagen-coated tissue culture inserts are used for growing three-dimensional corneal implant, tracheal gland cells etc.

II. Poly (lactic-co-glycolic acid) (PLGA) polymer foams are seeded with preadipocytes for the epithelial cell culture of the breast.

III. Porous scaffolding (e.g. filter, swatch of nylon, transwell) coated with fibrillar collagen, ideally type III collagen mixed with fibronectin or with Matrigel are used for the culture of the normal mature liver cells (polyploidy liver cells).

Current status of Biomaterials Research

Over the last two decades the tissue engineering industry and regenerative medicine has continued to evolve and it has become “credibly new sector” [47]. More recently, there are efforts happening to produce artificial extracellular materials that are actually required by cells and tissues for their successful clinical setting [48]. Current research primarily focused on functionality of biomaterials *in vivo*, most of our knowledge in this notion gained by regular observation of clinical symptoms of failed implants. There is a lack of suitable technologies for *in vivo* evaluation of biomaterials in a clinical setting. However, by alterations in existing medical devices, we may expect to see devices that heal in a physiologically normal manner. Such normal healing will pick up the performance of many devices that are now vulnerable due to poor healing [49].

Two areas namely tissue engineering and regenerative medicine, both in good progress to craft ultimate and rational medical devises [50]. Tissue engineering allows replacement of many tissues and organs with functional replacements. Tissue-engineering advances will rest on advances in biodegradable materials, rapid prototyping (RP), stem cell cultures, angiogenesis, and biomimetic strategies for synthesis of extracellular matrix. Regenerative medicine, permitting *in vivo* regeneration of whole organs and tissues will ultimately replace tissue engineering. Here some of the biomaterials applicable at various levels are explained in tabular column 4.

Table 4. List of biomaterials applicable at various levels of regeneration

Tissue/ Organ	Level of Regeneration	Organic Material	Inorganic Material	Composite Materials
Skin	Basically two-dimensional tissue. Culture is relatively straight forward.	Collagen, synthetic biodegradable polymers (polylactic acid, etc.)	--	--
Cartilage	Extracellular matrices have 3-dimensional structures, but there are no blood vessels in cartilage tissues. Cartilage cells are tolerant to low-oxygen, low-nutrient environment, making it relatively easy to perform 3-dementional culture.	Polysaccharides (collagen, chondroitin sulfate), synthetic biodegradable polymers (polylactic acid, etc.)	--	Collagen / polysaccharide, collagen / polysaccharide / hydroxyl apatite
Bone	Extracellular matrices have 3-dimensional structures containing blood vessels. Difficulty in sustaining cellular activity and function in the central porous core.	Collagen, synthetic biodegradable polymers (polylactic acid, etc.)	Calcium phosphate (hydroxyl apatite, tricalcium phosphate.	Biodegradable polymers/calcium phosphate, collagen/calcium phosphate
Liver	Almost no extracellular matrices exist. Regeneration is difficult because of the extensive vascular networks and large blood flow.	Hydrophilic polymer (polyethylene glycol, etc.) and hydrophobic polymers (coating) for culture plates. Temperature-responsive culture plate for regeneration of 2D liver cell sheet.	Apatite porous media used for liver cell culture	--
Capillary blood vessel	Regeneration of capillaries is difficult to regenerate because of the small tubular structure consisting of 3 different layers, but capillaries are necessary for survival of regenerated organs. Vascular endothelial cells are the most common target of tissue engineering.	Patterned culture plates capable of regulating cell adhesion. Hydrogel-cell compositions. Synthetic biodegradable polymer nanofibers used as scaffolds for cell culture.	--	--

DISCUSSION

Now we may recap the information regarding developments in clinical expertise and the engineering quality of medical devices during the last thirty years has led to considerable success and effectiveness of trauma repair and tissue replacement. Today, the medical device industry relies on knowledge of materials science, engineering and medicine to develop sophisticated replacements for natural tissues. Furthermore, it is vital to know the basic biology of the biomaterials, such as *in vivo* degradation, osseous replacement and biocompatibility in order to evaluate their appropriateness for the use in regenerative medicine. Biomaterials of current interest are divided in to two categories based on their working strategy namely, acellular and cellular materials. Materials that can endorse tissue or organ formation without cellular components are called acellular materials and cellular materials need cells embedded in the matrix to guide tissue development. Implants made up of bioinert metals come under cellular materials, and require cell seeding for better performance. Acellular materials include biodegradable polymers, ceramics and hydrogel, which can perform well even in the absence cells. In this regard, acellular materials with good biodegradability are found to be more sophisticated for the current application. Further disadvantageous of metallic implants include need for a second surgical procedure for implant removal and surgery is complicated resulting from the presence of the implant. The intent of biodegradable implants is to offer protected initial fixation strength while allowing degradation and replacement by the host tissue. Therefore, there is no need for implant removal.

When we check biomaterial applications in regenerative medicine, not all biomaterials are implanted within the body and there are several examples of medical devices that are used external to the body but which, nevertheless, come into critical contact with the tissues. We have a large variety of biodegradable implants such as sutures, staples, tacks, anchors and interference screws to treat both external and internal tissue damages. This brief review is to focus on current developments and to provide an insight in biodegradable implant biology in regenerative medicine. It is not astonishing that there are not just a few widely accepted biomaterials in clinical practice but rather a whole range of metals and alloys, ceramic, polymers, hydrogels, composites and natural materials from which selection is made depending on the precise circumstances.

CONCLUSION

This review on biomaterials/medical devices in tissue engineering has attempted to demonstrate the significant progress that has been made with the use of advanced materials in the areas of regenerative medicine. It is clear that, interactions between cells with the scaffolding materials play pivotal role in successful designing of tissue engineering constructs. The nature of the scaffold can directly affect biological response, ultimately influencing the rate and quality of new tissue proliferation. Although a variety of materials have been tested as tissue engineering scaffolds, it is not surprising that there is still a long way to go before we can readily and effectively intervene and correct nature's mistakes.

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