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Tailor-made intelligent polymers for biomedical applications

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16.1 Introduction

The introduction of resorbable, synthetic suture materials represented important progress in surgery in the early 1970s. Those sutures consisted of poly(α-hydroxyacid)s like polyglycolide and the copolyesters of L,L-dilactide and diglycolide.[1,2] Originally commercialized by American Cyanamid, Ethion Inc. and Davis & Geck, these aliphatic polyesters have been successfully applied until today and have become a well-accepted standard. Due to the time-consuming and cost-intensive process for the approval of novel biomedical devices by federal administration, only a few further biodegradable polymers have reached the market since then. Polyanhydrides are an example of a group of materials which has been introduced to clinics during the 1980s.[3] Based on a polyanhydride matrix, implantable drug delivery systems like Gliadel™ (Guilford Pharmaceutical Co., Baltimore) and Septicin™ (Abbott Laboratories, Illinois) have been developed. Gliadel™ is applied for the treatment of brain cancer (glioblastoma multiforme). Gliadel™ pellets are used to fill cavities caused by surgical treatment of brain tumours and in addition to combat remaining tumour cells. With Septicin™ implants, it is possible to cure chronic bone infections.

To be degradable, a biomaterial needs bonds which are cleavable under physiological conditions. In the case of the aliphatic polyesters mentioned above, as well as the polyanhydrides, these are hydrolysable bonds. There are two mechanisms for hydrolytic degradation – bulk degradation and surface erosion.[1] Both mechanisms differ in the ratio between the rate of diffusion of water in the polymer matrix and the rate of hydrolysis of the cleavable bond. If the rate of diffusion is higher than the rate of hydrolysis, a water uptake of a few per cent, typically 1–3 wt.%, can be observed. The hydrolysable bonds within the bulk will be degraded almost homogeneously. This mechanism is called bulk degradation. For hydrophobic polymers, the rate of diffusion of water into the polymer matrix can be significantly lower than the rate of
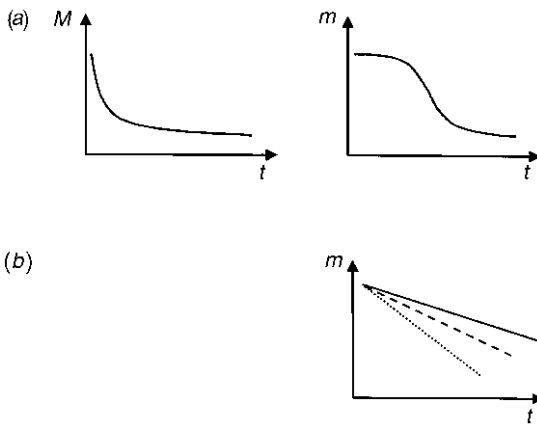
16.1 General schematic representation of the degradation behaviour of degradable polymers: (a) bulk degradation, (b) surface erosion.

cleavage of the hydrolysable bonds. Here the degradation is taking place only within a thin surface layer of the implant. In the case of surface erosion the degradation rate therefore depends on the surface area of a device. While polyanhydrides show surface erosion, polyhydroxyacids undergo bulk degradation. A general scheme of the degradation behaviour for both mechanisms is given in Fig. 16.1.

The number of potential applications for biodegradable implant materials is increasing constantly. One reason can be seen in the growing confidence of clinicians in the concept of degradable biomaterials based on the successful application of the established polymers. Another motivation can be found in completely new therapeutic methods, which have been developed taking advantage of the concept of biodegradable polymers such as tissue engineering.[4,5] Tissue engineering is an interdisciplinary approach aiming at the generation of new functional living tissue, which can be transplanted into a patient in terms of reconstructive surgery. This new tissue should be fabricated using living cells associated to a degradable porous scaffold. The scaffold should determine the three-dimensional shape of the resulting tissue, and should be degraded while the cells are growing and replacing the artificial structures.

The requirements for an implant material are determined by the respective application. The key properties of degradable biomaterials are their mechanical properties, degradation rate and behaviour, their functionality and their biocompatibility. For each application, a specific set of the properties mentioned is needed. With the growing number of potential applications, the number of required materials with specific combinations of properties is also increasing. As the variability of properties is limited for those

biomaterials established, a new generation of biodegradable implant materials is demanded.

In the following section, promising candidates for the next generation of degradable biomaterials will be introduced. This class of biomaterials allows variation of macroscopic properties within a wide range by only small changes in the chemical structure. As an additional functionality, these new materials show shape memory properties.

16.2   Fundamental aspects of shape memory materials

Shape memory materials are able to memorize a second, permanent shape besides their actual, temporary shape. After application of an external stimulus, e.g. an increase in temperature, such a material can be transferred into its memorized, permanent shape. The process of programming and restoring a shape can be repeated several times. This behaviour is called the thermally induced 'one-way' shape memory effect.

The shape memory effect has been reported for different materials, such as metallic alloys,[6–10] ceramics,[11,12] and glasses[11], polymers[18–30] and gels[13–17].

Shape memory alloys (e.g. CuZnAl-, FeNiAl-, TiNi-alloys) are already being used in biomedicine as cardiovascular stents, guidewires and orthodontic wires. The shape memory effect of these materials is based on a martensitic phase transformation.

Several types of shape memory gels are described in the literature.[13–17] Two different concepts are explained below. In the first system the shape memory effect is 'one-way' and originates from the chemical structure of the polymer network. The other system is an example of a reversible 'two-way' shape memory effect. However, this effect is being achieved by the design of the gel specimen as a bilayer system.

The first system can be prepared by a radical copolymerization of stearyl acrylate and acrylic acid with N,N-methylenebisacrylamide as a crosslinker.[16] Due to the intermolecular aggregation of the stearyl acrylate side chains, a crystalline lamellar structure can be observed in the dry as well as the swollen state in DMSO at room temperature. The swelling ratio of a gel film grows with increasing temperature up to 47 °C corresponding to the melting point of the stearyl side chains. This crystallizable side chain is the physical cross-link which can be used to fix a temporary shape. The permanent shape is being determined by the covalent cross-links of the polymer network. In this way, a thermally induced one-way shape memory effect can be programmed.

A reversible shape memory effect can be achieved using modulated gel technology.[14] These gels consist of two components, typically in the form of layers. The first component is not sensitive to an external stimulus (substrate

element), while the second part is responsive to a selected stimulus (control element). The design of the gel specimen is optimized in such a way that a small change of the control element causes a large movement of the substrate element. An example of such a system is a partially interpenetrating system. The non-responsive part consists of a polyacrylamide gel. The control element is an interpenetrating network of the same polyacrylamide gel with a crosslinked poly(N-isopropylacrylamide) (NIPA). It is a specific property of the ionic NIPA gel (containing a small amount of sodium acrylate) to drastically shrink at temperatures higher than $37\,^{\circ}\mathrm{C}$. Since this change in volume of the control component is reversible, the shape memory effect is also reversible.

The shape memory effect of polymers, e.g. heat-shrinkable films or tubes,[18] is not a specific bulk property, but results from the polymers' structure and morphology. The effect is persistent in many polymers, which might differ significantly in their chemical composition. However, only a few shape memory polymer systems have been described in the literature. One example is segmented polyurethanes.[19–30] The thermal transition, which triggers the shape memory effect, can be a glass transition[21–26] as well as a melting point.[20–27] Segmented polyurethanes have found some applications, e.g. as chokes in cars. However, they are not suitable as degradable biomaterials for two reasons. On the one hand, the urethane bonds of their hard segments are hardly hydrolysable. On the other hand, the degradation products would be highly toxic low molecular weight aromatic compounds.

16.3 Concept of biodegradable shape memory polymers

Biodegradable, stimuli-sensitive polymers have great potential in minimal invasive surgery. Degradable implants can be brought into the body through a small incision in a compressed or stretched temporary shape. Upon heating up to body temperature, they switch back to their memorized shape. Repeat surgery for the removal of the implant is not required, since the materials will degrade after a predetermined implantation time period.

Structural concepts for tissue-compatible and biodegradable polymers, thermoplastic elastomers,[31] and thermosets[32] with shape memory capabilities will be introduced. Their thermal and mechanical properties and degradation behaviour will be explained. An important precondition for the shape memory effect of polymers is elasticity. An elastic polymeric material consists of flexible segments, so-called network chains, which are connected via netpoints or junctions. The permanent shape of such a polymer is determined by the netpoints. The network chains take a coil-like conformation in unloaded condition. If the polymer is stretched, the network chains become extended
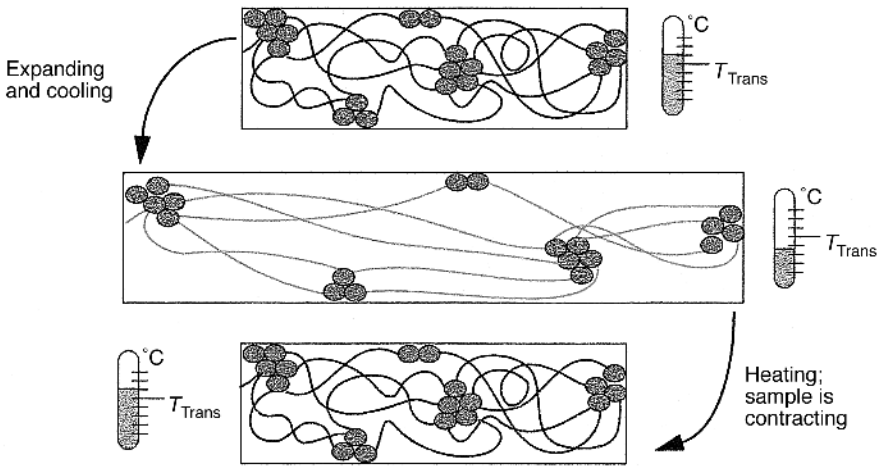
16.2 General schematic representation of the shape memory effect.

and oriented. In the case of an ideal entropy elastic material, the original shape is recovered after the stress is released.

An elastic polymer gains a shape memory functionality if the deformation of the material can be stabilized in the temperature range which is assigned for the specific application. This can be realized by using the network chains as a kind of molecular switch. For this purpose it should be possible to vary the segment flexibility as a function of temperature. Ideally, this process should be reversible. One way to obtain a switch functionality is given by the introduction of a thermal transition of the network chains at a temperature T_{trans}. Above T_{trans}, the segments are flexible, while below the transition temperature, the flexibility of the network chains can be limited to a certain extent. Below a glass transition temperature T_g, the flexibility of the network is frozen. If the thermal transition is a melting point, the network may become partially crystalline at temperatures below the melting temperature T_m. The so-formed crystalline domains prevent the segment chains from spontaneously recovering a coil-like conformation. The process of programming a temporary shape and the recovery of a permanent shape is shown in Fig. 16.2. Above T_{trans}, the segments are flexible and the polymer can be deformed elastically. The programmed shape is fixed by cooling the material to a temperature below T_{trans}. Upon heating above T_{trans}, the permanent shape can be recovered.

For biomedical applications, a thermal transition of the segment chains in the range between room and body temperature is of great interest. Suitable segments for degradable shape memory polymers can be found by regarding
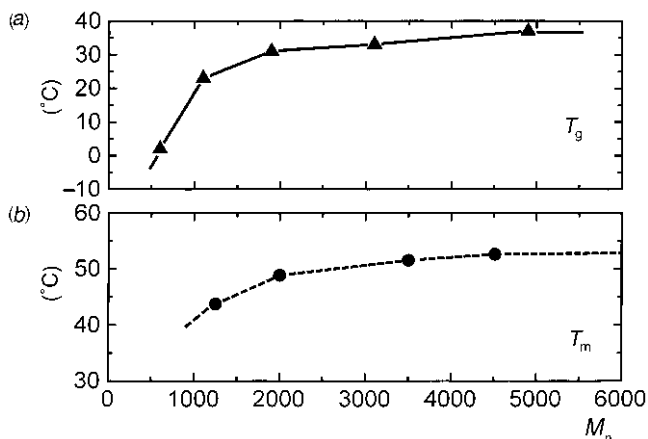
16.3 Dependence of thermal transition on molecular weight: (a) oligo[glycolide-co-(L-Kwsir-lactide)]diol having a glycolate content of 15 mol %, (b) oligo(ε-caprolactone)diol.

the thermal properties of well-established degradable biomaterials. From this assortment, two promising candidates can be extracted: poly(ε-caprolactone), which has a T_m of 61 °C, and the amorphous copolyesters of diglycolide and dilactides, showing glass transition temperatures T_g in the range from 35 °C to 55 °C. A fine-tuning of the respective thermal transition can be managed by variation of the molecular weight and the comonomer ratio (see Fig. 16.3).

Appropriate macrodiols are produced via ring opening polymerization of cyclic diesters or lactones initialized by low molecular weight diols (see Fig. 16.4).[33] The sequence structure of cooligomers can be influenced by application of a transesterification catalyst. The molecular weight of the oligomers can be controlled by the monomer/initiator ratio. Typically, the molecular masses Mn being obtained are between 500 g mol^{-1} and 10 000 g mol^{-1}. The net points can either be of physical or chemical nature. In the case of physical crosslinks, e.g. crystallizable segments with $T_m \gg T_{trans}$, the resulting polymer represents a thermoplastic elastomer. These materials can be melt processed, e.g. by extrusion or mould injection. Here, the permanent shape can be changed several times. In contrast, the permanent shape of a covalently crosslinked polymer network cannot be changed after the crosslinking process.

Important characteristics to be adjusted are the mechanical properties of the polymers in their permanent and temporary shape, the thermal transition temperature T_{trans}, the rate and the mechanism of the degradation process, and the shape memory properties.
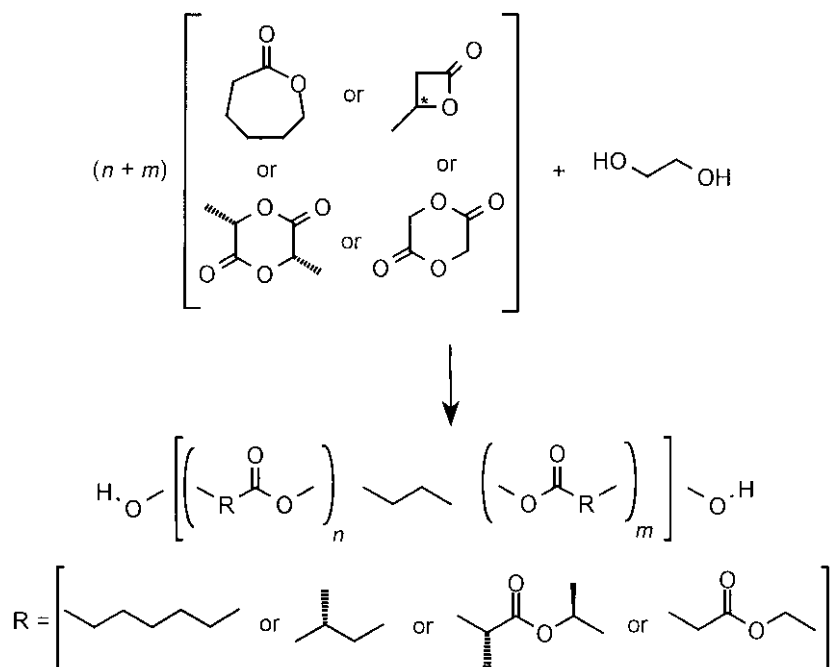
16.4 Synthesis of macrodiols via ring-opening polymerization of lactones or cyclic diesters.[33] Courtesy of Wiley-VCH.

16.4 Degradable thermoplastic elastomers having shape memory properties

16.4.1 Synthesis

In order to synthesize biodegradable multiblock copolymers, the oligoesterdiols and cooligoester diols are linked by bifunctional junction units, e.g. diisocyanates, diacidchlorids or phosgene (see Fig. 16.5). High molecular weight polymers in the range of $M_w = 100\,000\,\mathrm{g\,mol^{-1}}$ need to be obtained in order to get the desired mechanical properties. The resulting thermoplastic copolyesterurethanes are tough and show high elongations at break ε_R. These linear multiblock copolymers are phase segregated and consist of crystallizable hard segments (T_m) and amorphous switching segments ($T_{trans} = T_g$), e.g. poly[(L-lactide)-co-glycolide] with a glycolate content of 15 mol %. The permanent shape of these materials is obtained by melting the polymer followed by cooling to a temperature $T_m > T > T_{trans}$. The shape memory polymer can now be brought
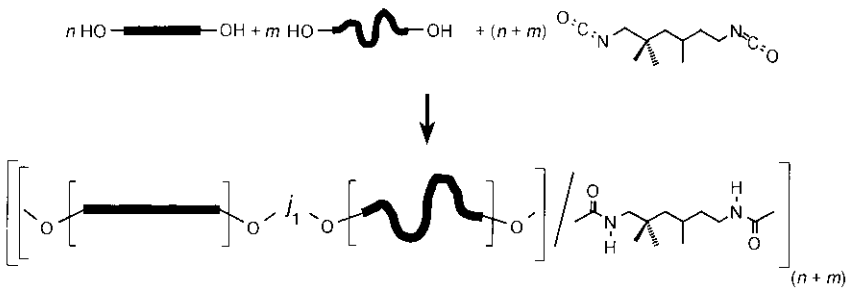
16.5 Synthesis of multiblock copolymers via polyaddition reaction.
Courtesy of Wiley-VCH.

into its temporary shape, which is being fixed by cooling below T_{trans}. The permanent shape can be recovered by heating the material above T_{trans}.

## 16.4.2 Thermomechanical properties of thermoplastic elastomers

The shape memory effect can be determined quantitatively by cyclic thermo-mechanical tests. These measurements are performed in a tensile tester equipped with a thermo-chamber. At a temperature above T_{trans}, a bone-shaped sample is fixed between two clamps and stretched. If the maximum elongation has been reached, the sample is cooled down to a temperature below T_{trans}. The clamps then return to their initial distance. The sample reacts with bending. After reheating to a temperature above T_{trans} but below T_m of the hard segment, the next cycle can be started. Figure 16.6 shows an example for the result of such a cyclic thermomechanical test.

16.4.3 Degradability

As shown in Fig. 16.7, accelerated hydrolytic degradation experiments with different copolyester-urethanes in buffer solution of pH 7 at 70 °C showed that these materials are hydrolytically degradable. The degradation rate varies within a wide range. In contrast to the degradation behaviour of several polyhydroxyacids, mass loss of the investigated shape memory polymers starts early and shows linear behaviour during the whole degradation period.

16.4.4 Toxicity testing

In a first set of experiments, the multiblock-copolymers proved to be non-toxic. The CAM (chorioallantoic membrane) test is a sensitive test for cell toxicity. It is performed by placing a sterilized sample of the polymer on the
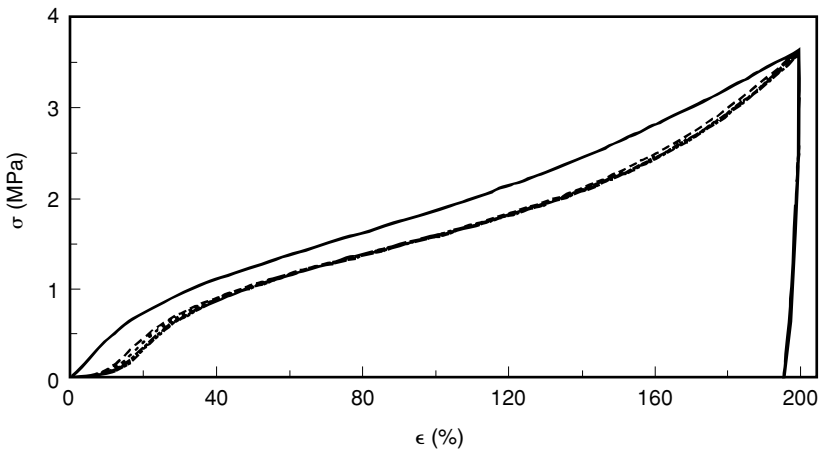
16.6 Cyclic thermomechanical testing of a polymer with an oligo[glycolide-*co*-(L-lactide)] as switching segment with $T_g = 35\,°C$; hard segment content: 22 w/w %; (Cycles: $n = 1$ ———; $n = 2$ ------; $n = 3$ ······; $n = 4$ ·-·-·-; $n = 5$ ·-··-··).
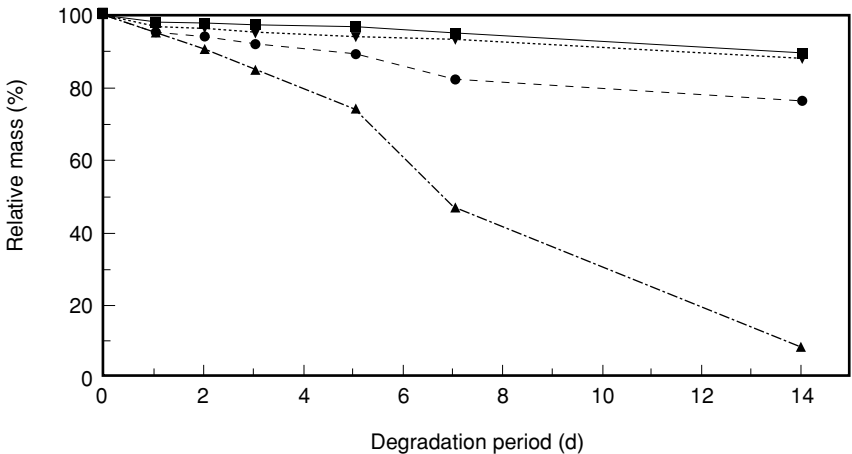
16.7 Hydrolytic degradation of shape memory polymers at 70 °C in buffered solution of pH 7: loss in relative sample mass (■ PDC27; ▼ PDC31; ● PDC40; ▲ PDL30).

chorioallantoic membrane of a fertilized chicken egg for two days. After incubation, the growth of blood vessels around the polymer sample is observed. In case of a non-toxic polymer, the blood vessels remain unchanged and their development is not restricted. In case of incompatibility, the sample causes changes in the number and shape of blood vessels, and the formation of a thrombus might occur (see Fig. 16.8).
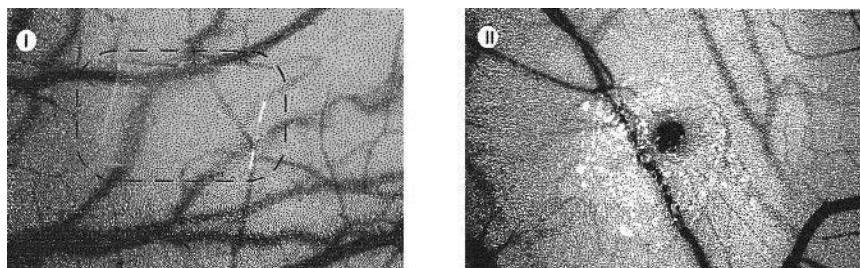
16.8 CAM test after 48 h incubation time: (a) multiblock copolymer, edge length of the sample 1–2 mm, (b) control experiment: incompatible sample causes thrombus (dark spot).[1] Courtesy of Wiley-VCH.
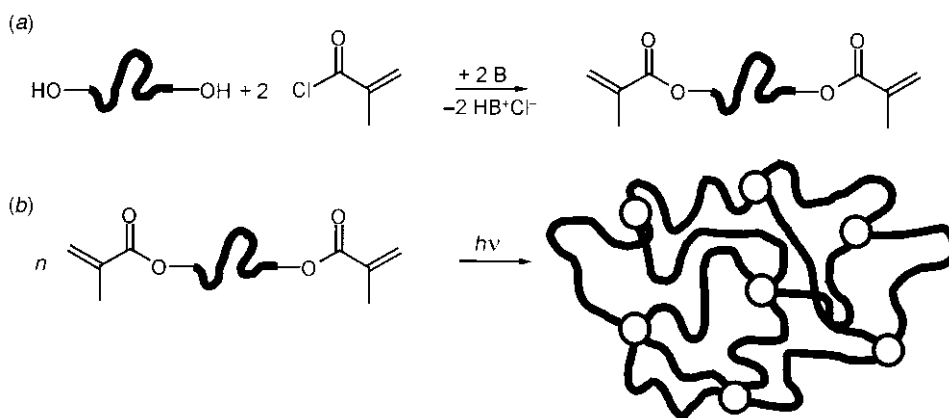
16.9 Synthesis of polymer networks with shape memory properties: (a) synthesis of the dimethyacrylate macromonomers, (b) cross-linking of the macromonomers.

16.5 Degradable polymer networks having shape memory properties

Based on the same switching segments as mentioned for the thermoplastic elastomers, a group of shape memory polymer networks can be prepared. Instead of crystallizable hard segments, covalent cross-links are introduced. For this purpose, the macrodiols can be turned into macrodimethacrylates, which can be cross-linked by photocuring. An example for the synthesis of biodegradable shape memory polymer networks is shown in Fig. 16.9. A potential educt is poly(ε-caprolactone) dimethacrylate with molecular weights between 1000 and 10 000. By copolymerization with n-butylacrylate, Ab-networks can be obtained.[32] The permanent shape of these polyester networks is fixed via photocuring. The thermo-mechanical properties of the network

can be tuned by the choice of the molecular weight of the respective macrodimethacrylates. The temporary shape can be formed by deformation of the sample under temporary heating above T_m.

16.6   Conclusion and outlook

Biodegradable shape memory polymers are candidates for the next promising generation of implant materials. The fact that these materials belong to a polymer system allows the adjustment of certain properties in a wide range, e.g. mechanical properties and degradation behaviour. Today, such materials can be synthesized in a kilogram scale.

In contrast to metallic shape memory alloys like NiTi-alloys, the polymers presented here combine the features of degradability and high elasticities, with elongations at break up to 1500%. Furthermore, shape memory polymers can be programmed much faster, allowing the individual adaptation of an implant to the patient's needs during surgery. Compared to hydrogels, these materials exhibit much higher mechanical strength.

From the point of view of economy and costs in healthcare systems, biodegradable shape memory polymers have two major advantages. Implants based on these materials can be brought into the body by minimally invasive methods, e.g. belly button surgery, allowing more careful treatment of patients; in addition, repeat surgery for the removal of the implant can be circumvented. The high potential of shape memory polymers for biomedical applications will therefore have a decisive influence on the way in which medical devices are designed in the future.

Acknowledgements

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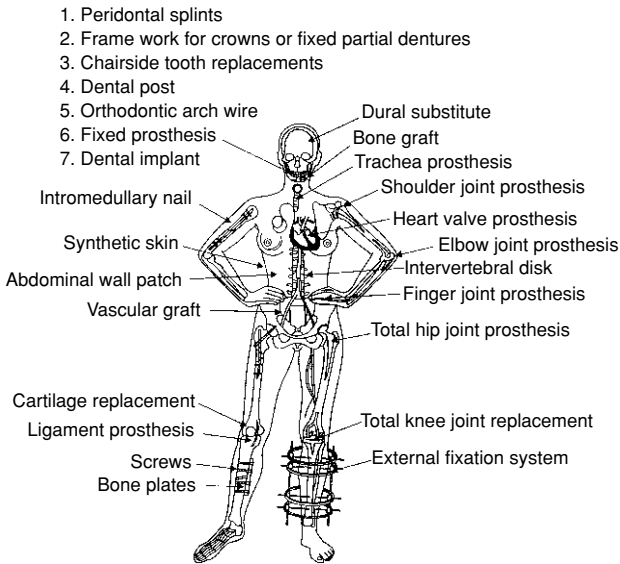25 Ito K, Abe K, Li H L, Ujihira Y, Ishikawa N and Hayashi S, 'Variation of free

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Textile scaffolds in tissue engineering

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17.1 Introduction

Use of textile structures in the medical field is not recent because sutures, which for centuries have been used for the closure of wounds or incisions, are fundamentally textile structures. The emergence of textiles apart from sutures, for various biomedical applications, became of real significance in the early 1950s. Nowadays they are commonly used in various biomedical applications,[1] and are generally referred to as 'medical textiles'. Based on the application, they may be grouped into three broad categories (Table 17.1), namely healthcare and hygiene textiles, extracorporeal devices and surgical textiles.[2] In terms of volume of usage, surgical textiles are much smaller than healthcare and hygiene textiles. However, scientifically, surgical applications are far more challenging. In these applications, the textiles are expected to fulfil a number of requirements, including surface biocompatibility (chemical structure, topography, etc.), mechanical compatibility (elastic modulus, strength, stiffness, etc.), non-toxicity, durability in in vivo (human body environment) conditions and sterilizability. Due to recent advancements in textile engineering and biomedical research, the use of textiles in surgery is growing. They are routinely used to direct, supplement or replace the functions of living tissues of the human body. Soft tissue replacements or implants such as vascular grafts, skin grafts, hernia patches and artificial ligaments are made of textile structures.[3–8] Moreover, polymers reinforced with textiles, called polymer composite materials, are also considered in hard tissue replacements or implants such as dental posts, bone grafts, bone plates, joint replacements, spine rods, intervertebral discs and spine cages.[9,10] Table 17.2 is a partial list of some of the most common implant applications of textiles. Some implant applications are shown schematically in Fig. 17.1. As can be seen from Table 17.2, the implantable textiles are made from a variety of synthetic biomaterials, which are essentially non-living (avital) type. Although the synthetic biomaterials are fairly successful, the profound differences between them and the living tissues of the human body

Table 17.1 Classification of medical textiles

Category	Sub-category	Applications
Healthcare and hygiene textiles		Bedding, protective clothing, surgical gowns, clothes, wipes, etc.
Extracorporeal devices		Artificial kidney, artificial liver, artificial lung, bioreactors, etc.
Surgical textiles	Non-implantable textiles	Wound dressings, plaster casts, bandages, external fracture fixation systems, etc.
	Implantable textiles	Sutures, vascular grafts, ligament and tendon prostheses, bone plates, heart valves, hernia patches, joint replacements, artificial skin, etc.

1. Peridontal splints
2. Frame work for crowns or fixed partial dentures
3. Chairside tooth replacements
4. Dental post
5. Orthodontic arch wire
6. Fixed prosthesis
7. Dental implant

17.1 Schematic illustration of various implants.

lead to problems such as infection, loosening, failure and finally rejection of implants. On the other hand, transplantation (transfer of a tissue from one body to another, or from one location in a body to another) is not always practical due to a shortage of donor tissue, and the risk of rejection and disease transfer. Hence, there is a need to develop biological substitutes (living or vital materials) to avoid these problems. The newly developed field of 'tissue engineering' combines mammalian cells and certain synthetic biodegradable

Table 17.2 Implant applications of textile structures

Application	Materials	Textile structures {monofilament (m), yarn (y), weave (w), briad (b), knit (k), non-woven (n)}
Abdominal wall	Polyester	w
Blood vessel (vascular graft)	Polyester, polytetrafluoroethylene (PTFE), polyurethane	w, k
Bone plant	Carbon, PGA	y, w, b, k
Cartilage	Low density polyethylene, polyester, PTFE, carbon	w, b
Dental bridge	Ultrahighmolecular weight polyethylene (UHMWPE), carbon, glass, aramid	w, n
Dental post	Carbon, glass	y, b
Dural substitute	Polyester, PTFE, polyurethane, collagen	n, w, k
Heart valve (sewing ring)	Polyester	k, w
Intervertebral disc	Polyester, PTFE	w, k
Intramedullary rod	Carbon, glass	y, b
Joint	Polyester, carbon, UHMWPE	w, k
Ligament	Polyester, carbon, glass, aramid	b, w, k
Orthodontic arch wire	Glass	y, b
Skin	Chitin	n, w, k
Spine rod	Carbon	y, b
Suture	Polyester, PTFE, polyamide, polypropylene, polyethylene, collagen, polylactic acid (PLA), polyglycolic acid (PGA)	m, y, b
Tendon	Polyester, PTFE, polyamide, polyethylene, silk	b, w, y

materials (materials that eventually disappear after being introduced into a living tissue or organism) to produce living (vital) synthetic tissue substitutes or replacement tissues.[11-13] It is envisaged that such tissue substitutes will merge seamlessly with the surrounding host tissues, eliminating problems associated with contemporary biomaterials and transplantation. Recognizing the potential of tissue engineering, researchers worldwide are harnessing techniques to produce tissue engineered skin,[14] cartilage,[15] nerve,[16] heart valve[17] and blood vessels.[18,19] It is also envisaged that, using tissue engineering techniques, it will eventually be possible to construct entire replacement organs such as the liver[20] and bladder.[21]

Table 17.3 Various scaffolds used in tissue engineering

Tissue engineered biological substitute	Scaffold materials	Scaffold structures {yarn (y), weave (w), braid (b), knit (k), non-woven (n)}
Bladder[21]	PGA	Textile (n)
Blood vessel[18,19]	Polyester (Dacron); polyurethane; ePTFE; PGA; PLA; PGLA (Vicryl)*	Textile (n, w, b, k)
	Collagen	Textile (k)
Bone[61,62,64]	PGA; PLGA* + hydroxyapatite fibres	Textile (n); Foam
	PLLA	Foam
Cartilage[15,65–68]	PGA; PLLA; PGLA*	Textile (n)
Dental[69]	DL-PLA; PGLA (Vicryl)*	Foam (porous membrane); textile (n)
Heart valve[17]	PGA	Textile (n, w)
Tendon[70]	PGA	Textile (n, y)
Ligament[67,71]	Collagen	Textile (y)
	PGA, PLAGA*	Textile (b, n)
Liver[20,72]	PGA; PLA; PGLA; polyorthoesters; polyanhydride	Foam; textile (n)
	PLGA*	3D Printed
Nerve[16,73,74]	Collagen–glycosaminoglycan	Foam
	PGA	Textile (n)
Skin[14,73]	PGA; PGLA (Vicryl)*; Nylon	Textile (w)
	Collagen–glycosaminoglycan	Foam

*PLAGA, PGLA, PLGA are co-polymers of polyglycolic acid (PGA) and polylactic acid (PLA).
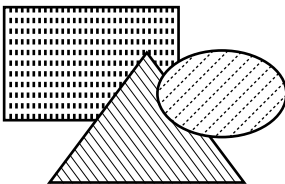
17.2 Knitted fabric scaffold seeded with osteoblastic cells.

The basic concept of tissue engineering is to regenerate or grow new tissues and organs by culturing isolated cells from the tissue or organ of interest on porous biodegradable scaffolds or templates (Fig. 17.2). The scaffold acts as an extracellular matrix for cell adhesion and growth and/or regeneration. An important challenge is to pursuade the cells transplanted onto scaffolds to multiply and produce correct tissue matrices, which can take up and secrete

protein, generate force and resistance, constrain permeability and exhibit other life processes. It has been recognized that the engineering of the scaffold is an important aspect, as it provides the optimal requirements for the survival, proliferation and differentiation of cells, and for the formation of tissue in in vitro or in vivo conditions depending on the intended application. Hence, there has been a multitude of research work carried out in the last decade to design and develop various types of optimum scaffolds for tissue engineering (Table 17.3). They may be broadly categorized into three groups based on the processing methods: (1) foams/sponges,[22–27] (2) three-dimensional (3D) printed substrates/templates[28] and (3) textile structures.[29–39] Textile structures form an important class of porous scaffolds used in tissue engineering.[40–42] This chapter reviews various textile scaffolds from the viewpoint of tissue engineering requirements and possible future developments.

17.2   Ideal scaffold system

Making biological substitutes using the tissue engineering approach fundamentally encompasses several phases, namely: selection of scaffold material; fabrication of scaffold; preparation of scaffold; cell harvest from animal or human patient; cell seeding onto the scaffold; cell proliferation and differentiation; growth of mature tissue; surgical transplantation; and implant adaptation and assimilation. The following describes features of an ideal scaffold system.[43,44] Specific requirements vary from one tissue to another.

- The material used for the scaffold should be biocompatible, not inducing an unfavourable tissue response in the host. The material should be ultra-pure, and easily and reliably reproducible into a variety of sizes and structures.
- In most applications, the support of a scaffold is needed only for a limited time. These temporary scaffolds cannot be removed easily because of tissue grown into the porous structure. Therefore, scaffolds have to be manufactured out of a biodegradable material in which the degradation rate has to be adjusted to match the rate of tissue formation. The scaffold should maintain its volume, structure and mechanical stability long enough to allow adequate formation of tissue inside the scaffold. However, none of the degradation products released should provoke inflammation or toxicity.
- Scaffolds must provide a reproducible microscopic and macroscopic structure with a high surface-area to volume ratio in order to allow a significant amount of cell–surface interaction. The scaffold processing method should not affect the biocompatibility or the desired degradation

behaviour of the material. It should also allow the manufacture of scaffolds with controlled interconnected pore structure, pore size distribution and pore geometry, since these are important factors in tissue growth or regeneration.

- The average pore size and the macroscopic dimensions of a scaffold are important factors which are associated with cell proliferation and nutrition supply, from tissue culture media in vitro and through newly formed blood vessels in vivo, to cells and tissue. The pore size of such scaffolds should be sufficient to allow cells to grow in multiple layers in order to form a three-dimensional tissue. The optimal pore size may be highly variable, depending also on the intended application of the scaffold. For instance, it has been hypothesized that in orthotopic sites, pore sizes below 400 μm lead to bone formation and pore sizes above 400 μm lead to fibrous tissue ingrowth.[45–48] In addition to pore size, porosity, which more reflects the interconnectivity of the scaffold, is also important. High porosity maximizes the volume of tissue ingrowth and minimizes the amount of scaffold material used. It also facilitates transport of nutrients and cellular waste products. Another parameter is the pore morphology, which may be meaningful in favouring the ingrowth of certain cell types.[49]
- Scaffold surface chemistry should be suitable for cell attachment and cell proliferation.
- In certain tissue engineering applications, external electro-mechanical stimulations are often used to promote cell proliferation and tissue development. The scaffold should be able to retain its shape and structure under these electro-mechanical conditions.
- Further, the flexibility of such a scaffold should be close to that of its surrounding tissue so, once the vascularization starts, no extreme change in the mechanical properties between the host tissue and the scaffold can be experienced by the ingrowing tissue. Such forces could be harmful, not only for the vascularization process, but also because they could induce the formation of a different tissue from the desired one.

17.3   Scaffold materials

As most cells are substrate dependent, the scaffold structure as well as the material has control over the cell adhesion and function. The various scaffold materials used in tissue engineering can be grouped into natural and synthetic materials. Collagen, chitin, starch, etc. are a few examples of natural materials (see Table 17.4). Natural materials are isolated from human, animal or plant tissues, which typically result in high costs and large batch-to-batch variations. In addition, these materials exhibit a very limited range of properties and are

Table 17.4 Biodegradable polymers used in tissue engineering

Category	Sub-groups and typical polymers
Natural polymers	Cellulose, starch, chitin, collagen and fibrin[12,43,73,75–74]
Synthetic polymers	Polyesters[24,26,30–32,53,66,75,82,85–98]
	Poly(glycolic acid) (PGA) and copolymers
	Poly(lactic acid) (PLA) and copolymers
	Poly(alkylene succinates)
	Poly(hydroxybutyrate) (PHB)
	Poly(butylene diglycolate)
	Polyanhydrides[99]
	Polyorthoesters[100–102]
	Polyiminocarbonates[103,104]
	Polyphosphoesters[105]
	Polyphosphazenes[106–112]

often difficult to process. Synthetic materials are further classified into degradable[23,50] and non-degradable[51] types. The non-degradable materials, such as polyethylene, polyethyleneterephthalate (PET) and polytetrafluoroethylene (PTFE) may carry a risk of permanent tissue reaction.[33] On the other hand, synthetic biodegradable polymers, such as polyesters, polyanhydrides and polyorthoesters (see Table 17.4) offer control over structure and properties. They can be processed into various shapes and microstructures, such as desired surface area, porosity, pore size and pore size distribution. They can be tailored with degradation times ranging from several days up to years. Their surface properties can be altered to adapt to the biological requirements for cell adhesion, growth and function. Therefore, synthetic biodegradable polymers have been widely investigated in tissue engineering research.[52–54] From the literature (Tables 17.3 and 17.4) it is evident that biodegradable polyester-based materials dominate the tissue engineering applications compared to other biodegradable polymers. This is mainly due to the fact that polyesters of poly(α-hydroxy acids) are used successfully in various implant applications and have already been approved by the US Food and Drug Administration (FDA). Another factor could be the familiar processing and characteristics of these materials to many tissue engineering researchers. However, it is to be noted that the mechanical properties and degradation profiles of these polyesters are insufficient for certain applications. Moreover, certain copolymers may release toxic products during degradation. As tissue engineering applications continue to grow, it is important to find and develop alternative biodegradable polymers that meet the specific requirements of various tissues.

17.4   Textile scaffolds

The need for scaffolds in tissue engineering is undisputed as cells cannot survive on their own and are substrate dependent. However, there is no universal scaffold that meets all the requirements of various tissues, as the optimum tissue engineering conditions vary from tissue to tissue. In other words, the targeted tissue dictates the optimum scaffold design. For example, for hard tissues, such as bone, scaffolds need to have high stiffness in order to maintain the space they are designated to provide the tissue with enough space for growth. If scaffolds are used as a temporary load-bearing device, they should be strong enough to maintain that load for the required time without showing any symptoms of failure. Used in combination with soft tissues, the flexibility and the stiffness of the scaffold have to be within the same order of magnitude as the surrounding tissues in order to prevent the scaffold from either breaking or collapsing and from stress shielding the adjacent tissues. The choice of scaffold for a tissue therefore depends on its characteristics. In addition to the mechanical properties, the optimum design of a scaffold for a specific tissue application requires consideration of microstructural, chemical and biological aspects. It is often difficult to isolate these aspects as they are interdependent and sometimes their effects are unknown. The following sections critically look into some of these aspects of different textile scaffolds.

17.4.1 Microstructural aspects

The microstructural aspects of scaffolds includes pore size, porosity, pore size distribution, pore connectivity and reproducibility of pores. These aspects are vital, as they provide the optimal spatial and nutritional conditions for the cells, and determine the successful integration of the natural tissue and the scaffold. For example, Hubbell and Langer[37] showed in their experiments that in animals, the size and alignment of pores in the scaffold greatly influence the amount and rate of vascular and connective tissue growth. Fibrovascular tissues require a pore size greater than 500 µm for rapid vascularization, whereas the optimal porosity for bone bonding materials is considered to be between 70 and 200 µm.[55] In another study,[56] osteoblasts cultured in calcium phosphate ceramic prefer a pore size of 200 µm, and it has been proposed that this pore size possesses a curvature that optimizes the compression and tension of the cell's mechanoreceptors. However, there is concern that optimal pore size in ceramics may not generalize for all scaffold materials.[57] For example, when poly(lactic acid) was implanted in calvarial rat defects, pore sizes of 300–350 µm supported bone ingrowth while smaller sizes did not. Yet, in another study, osteoblasts showed no significant difference in proliferation or function when seeded on poly(lactic glycolic acid) foams with pore sizes of

either 150–300 µm, 300–500 µm or 500–710 µm. The importance of determining optimum values for the specific cells or tissue cannot be underestimated. In another study related to skin tissue engineering, Nerem et al.[18] showed that the endothelial morphology depends on the pattern of the scaffold. The interconnectivity of pores determines the transport of nutrients and waste and thus influences the success of tissue engineering. The reproducibility of scaffolds is also very important as it determines the dimensional stability of the scaffold as well as the consistency of the tissue formation. Table 17.5 compares the various microstructural aspects of foams and textile structures. Owing to the processing techniques employed, in general, each batch of foam will have one particular of porosity. It is possible to tailor the porosity to a certain extent. However, within the same foam, organizing or grading the porosity in a particular fashion may be difficult to achieve with the current processing techniques. On the other hand, textile structures can be tailored to give the required porosity in terms of size, quantity and distribution pattern. For example, in a typical textile scaffold, three levels of porosity can be achieved. The arrangement of fibres in the yarn determines the accessible space for cells. The inter-fibre space (or groove between two adjacent fibres) may be considered as the first level of porosity. In our study[58] it was found that the fibroblasts preferentially organize themselves along the length of the fibres, grouping along the groove created by two adjacent fibres. Figure 17.3 shows SEM pictures of polyethylene terephthalate (PET) fibre yarn before and after seeding with fibroblasts. What is more interesting is that fibroblasts are capable of bridging fibres which are as far as 40 µm apart (Fig. 17.4). The inter-fibre gap or first level of porosity in a textile scaffold can be controlled by changing the number of fibres in the yarn and also the yarn packing density. Further variations in porosity can be achieved by using twisted, untwisted, textured, untextured, continuous or spun yarns.

The gap or open space between the yarns (it is open space inside the loop in the case of knits) forms the second level of porosity. In the case of knitted scaffolds, the porosity can be varied selectively by changing the stitch density and the stitch pattern. In the case of braided scaffolds, porosity can be varied by controlling the bias angle of the interlacing yarns. In the case of woven scaffolds, it is possible to change the porosity by controlling the inter-yarn gaps through a beating action. In our preliminary study[58] involving the seeding of woven, braided and knitted scaffolds with hepatocytes, it was observed that cells attach preferentially at the inter-yarn gaps or pores in the case of woven and braided scaffolds, whereas they clump together on the ridges of curved yarns in the case of knitted scaffolds (Fig. 17.5). It may be noted that woven and braided scaffolds share similar surface topographies formed by the interlacing yarns. Knitted scaffolds, however, comprise curved yarns, which had a significant effect on the behaviour of hepatocytes. The same

Table 17.5 Microstructural aspects of scaffolds

| Fabrication | Foam/sponge | Textile structures | | | |
		Non-woven	Weave	Braid	Knit
Pore size (μm)	0.5–500	10–1000	0.5–1000	0.5–1000	50–1000
Porosity (%)	0–90	40–95	30–90	30–90	40–95
Pore distribution	Random to uniform	Random	Uniform	Uniform	Uniform
Reproducibility of porosity	Poor to good	Poor	Excellent	Excellent	Good to excellent
Pore connectivity	Good	Good	Excellent	Excellent	Excellent
Processability	Good	Good	Excellent	Excellent	Good
Other comments	Current techniques are associated with processing undesirable residues such as solvents, salt particles	Equipment cost is high. Control over porosity is always questionable	Shapes are limited	Limited to tubular or uniform cross-sectional shapes	Limited by the low bending properties of current biodegradable fibres
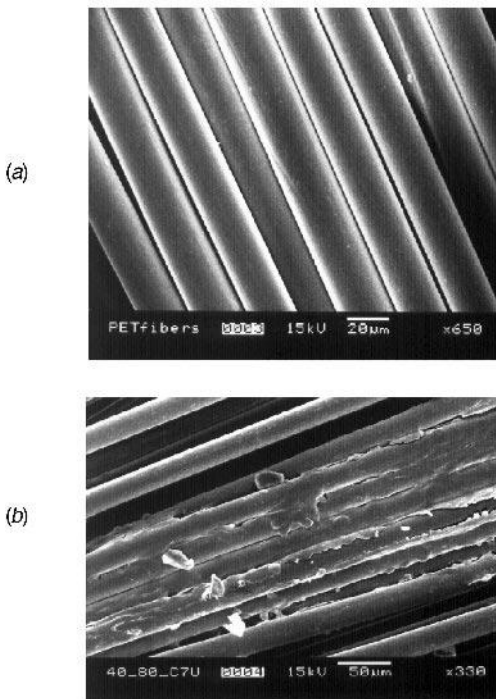
17.3 PET fibre yarn (*a*) before and (*b*) after seeding with fibroblasts (Cos7 kidney cells from SV40-transformed African green monkey).

experiment was repeated with fibroblasts to investigate the effect of cell type. Unlike the hepatocytes, the fibroblasts were found to attach to the ridges of yarns irrespective of the scaffold type. The different behaviour of fibroblasts and hepatocytes may be due to their different cell sizes and shapes. It may be noted that the diameter of fibroblasts ranges from $10\,\mu m$ to $20\,\mu m$, and they flatten out after attachment. The hepatocytes are larger with diameters in the range $15\,\mu m$ to $30\,\mu m$, and they retain their spherical structure even after attachment to the scaffold.

Furthermore, a third kind of porosity can be introduced by subjecting the textile structures to secondary operations such as crimping, folding, rolling, stacking, etc. In other words, the flexibility of microstructural parameters is tremendous in the case of textile scaffolds.

Bowers et al.[59] investigated the effect of surface roughness. It has been reported that a higher percentage of osteoblast-like cells cultured on commercially pure Ti attached to rougher surfaces than to smooth surfaces. Another study using the same material showed higher osteocalcin content and ALPase activity on smooth, polished surfaces than on rough surfaces.[60] In our
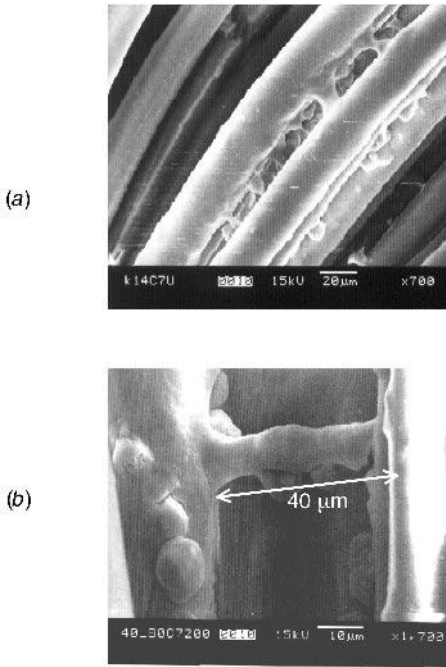
17.4 Fibroblasts bridging adjacent PET fibres.

study[58] involving woven, braid and knitted scaffolds, it was observed that the fibroblasts prefer to attach to the ridges of scaffolds rather than the valleys. This may be due to the cell's attempt to minimize distortion to its cytoskeleton in response to the topography of the scaffold. Further systematic study is needed to fully understand the influence of scaffold topology on tissue engineering.

17.4.2 Mechanical aspects

Similar to the microstructural aspects, the mechanical aspects of scaffolds, such as structural stability, stiffness and strength, have considerable influence on the cellular activity. For example, in tissues like bone, cell shape is influenced by mechanical forces.[11] Cell shape modification takes place as a result of external forces including gravity, and also of internal physical forces. Cell shape modification also depends on the nature (constant or cyclic), type (uniaxial, biaxial, multiaxial, etc.) and magnitude of the mechanical stimulation. The mechanical stimulation also affects the release of soluble signalling factors and the deposition of extracellular matrix constituents. Researchers are
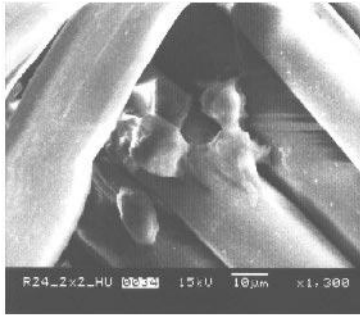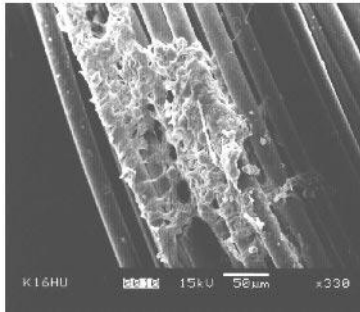
17.5 Hepatocytes (cells from Wistar rats) attached preferentially at (*a*) interyarn gaps in a woven scaffold and (*b*) ridges of curved yarn in a knitted scaffold.

making use of these observations in the case of bone tissue engineering. They are applying external mechanical stimulation to promote tissue formation. Therefore, in bone tissue engineering, the scaffolds are designed to withstand severe physiological loads.[55] In blood vessel applications, the scaffold needs to be strong enough to withstand physiologically relevant pulsatile pressures and at the same time match the compliance or elasticity values of a native blood vessel. The mechanical aspects of various scaffolds are compared in Table 17.6. Among the scaffolds, the woven fabrics are normally rigid and inflexible due to the tight interlacing of yarns. The next stiff and strong scaffold is the braid. Knits, non-woven and foams display the lower end of the mechanical properties. Of all the scaffolds, knits display considerable deformability and good compliance owing to their looped yarn arrangement. Hence, they are suitable for bladder and blood vessel tissue engineering applications. Researchers are using woven scaffolds for tissue engineering of bone and acetabular cups. It is to be noted that the mechanical behaviours of scaffolds can be varied significantly by controlling the various microstructural aspects stated earlier. In other words, both the microstructural and mechanical aspects are

Table 17.6 Mechanical aspects of scaffolds

		Textile structures			
Fabrication	Foam/sponge	Non-woven	Weave	Braid	Knit
Stiffness	Low	Low	High	High	Medium
Strength	Low	Low	High	High	Low
Structural stability	Good	Poor to good	Excellent	Excellent	Poor to good
Drapeability	Poor	Good	Poor	Poor	Excellent
Other comments	Isotropic behaviour	Isotropic behaviour	Anisotropic, with good properties parallel to fibres and poor properties normal to fibres	Anisotropic, with good properties in axial direction and poor properties in transverse direction	The behaviour can be tailored from isotropic to anisotropic

interrelated and it is less meaningful to understand them individually. Further work is necessary to understand how the scaffolds behave in in vitro or in vivo environments, and how they contribute to the growth of tissue.

17.4.3 Other aspects

There is increasing evidence that scaffold surface chemistry influences cellular activity.[61] Boyan et al.[113] showed that osteoblast response varies with the material on which cells are cultured, and attributed this to differences in the surface chemistry, charge density and net polarity of the charge. Some variations have been attributed to the proteins present in the medium that adsorb onto the surface to different degrees or with different structural arrangements.[62] In one study, osteoblasts were cultured on glass modified with the RGD peptide or non-adhesive, scrambled sequence and in the presence or absence of BMP-7.[63] The culture with a combination of RGD substrate and BMP-7 showed a substantial increase in mineralization in 21 days over all other combinations of treatments. Because of its role in both attachment and differentiation, RGD incorporation may contribute greatly to scaffold osteoinductivity and bone regeneration. Technologies for the incorporation of peptides on to the scaffold surfaces are being further perfected.

In our laboratory, a systematic study[58] was made involving unmodified polyethylene terephthalate (PET) textile scaffolds and YIGSR (Tyr–Ile–Gly–Ser–Arg) peptide conjugated PET textile scaffolds. Three types of scaffolds, namely woven, braided and knitted fabrics, were seeded with fibroblasts (Cos7 kidney cells from SV40-transformed African green monkey) and hepatocytes (cells from Wistar rats) separately. All three types of scaffolds indicated a 35% to 46% increase in the number of fibroblasts attached when conjugated with peptide bonds compared to the unmodified scaffolds. However, no appreciable change in the hepatocyte attachments was found with the peptide surface modification of scaffolds. This study clearly indicates that the cellular activity also depends on the source of cells (bone, liver, blood vessels, etc.), number of cell types (pure, co-cultured or mixed cell type cultures), species (e.g. rat, rabbit, chicken, human), sex and age (i.e. embryonic, neonatal or adult). Furthermore, it is generally believed that, depending on the characteristics of the cell culture and culture period used, different reactions may be expected. The current literature clearly indicates that a combination of various factors, such as scaffold material, structure, physical, chemical, mechanical, and biological properties, cell types, in vitro or in vivo conditions, etc., determines the success of tissue engineering.

17.5   Conclusions

Scaffolds play a central role in tissue engineering. Textile structures are particularly attractive to tissue engineering because of their ability to tailor a broad spectrum of scaffolds with a wide range of properties. Preliminary studies clearly demonstrate the suitability of textile scaffolds for tissue engineering purposes. There is no universal scaffold that meets the requirements of the various tissues of the human body. Further systematic study is necessary to design an optimal scaffold for each tissue application.

Acknowledgements

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