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Biomaterials: defining the mechanical properties of natural tissues and selection of replacement materials

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This article reviews the problems which are encountered in defining the mechanical properties of natural tissues, and in replacing them with synthetic materials in the human body. It describes how death, ageing, degeneration, pathology and individual variability influence the properties of natural tissues. Experimental problems arise from degradation and testing conditions; these are illustrated by the properties of the nucleus pulposus of the intervertebral disc. Replacement of natural tissues by graft materials and the products of tissue engineering is then described. Synthetic replacement materials should be biocompatible, *i.e.* they should not cause adverse reactions in the human body. However, polymers which hydrolyse in the body fluids may be useful for implants which are intended to have a limited life or for controlled release of drugs. Synthetic implant materials may attempt to mimic natural tissues but there may be a problem of attaching them to the surrounding tissue. Artificial ligaments provide an example of implants of this kind. Total hip replacement is used to illustrate the successful use of conventional engineering materials. Finally, safety issues are described; an implant material must be biocompatible, have the required mechanical strength, be sterile and should be incorporated into a device so that its performance in the living patient can be monitored.

This review is concerned with the requirements for any synthetic materials which are to be used in the human body for medical treatment. The term 'biomaterial' may be used to describe the tissues which provide natural examples of engineering materials or, more commonly, to describe their synthetic replacements. Synthetic materials may be used for many different purposes: to replace parts of the body and to make products such as contact lenses, wound dressings, catheters (used to supply or drain fluids), *etc.* The human body may have adverse effects on the performance of these materials and, conversely, synthetic materials may interact adversely with the body. Many physical and chemical studies which purport to have biomedical implications are of limited practical value because the special requirements of medical devices have not been appreciated. This review is concerned with factors which need to be considered if biomaterials are to be used in the human body and not with scientific topics which are unlikely to have much impact for the development of useful products.

The first section considers the mechanical properties of natural tissues. This is important because synthetic materials which are used in replacement tissues or organs within the human body must be able to withstand comparable mechanical forces. This requirement applies not only to replacements for mechanical structures, such as joints (like the hip and knee) and the ligaments (which join bones together), but also to membranes, catheters, *etc.* which have a wide range of functions but must still be able to withstand the forces to which they are subjected during insertion and normal use. Determination of their mechanical properties is essential if natural tissues are to be replaced with materials which will perform their required function without failing. In addition, natural tissues may also be used, sometimes after chemical modification, as replacement materials.

The next section is concerned with synthetic materials: these may be composites which are intended to mimic natural tissues or conventional engineering materials. Biological tissues are natural examples of fibre-reinforced composite materials. 'Biomimetics' exploits the principles exploited by natural biomaterials to fabricate new materials which may have natural or synthetic components.[1] Both the biomimetic and the conventional engineering materials approach to replacing tissues have their problems which are covered in this section. The article concludes with a brief summary of the current state of the subject.

Natural tissues

Composition

The physical properties of biological tissues are determined by the composition and structure of the material surrounding their cells, the 'extracellular matrix'.[2] The matrix contains proteoglycans, which attract water, and collagen fibres which provide tensile reinforcement. Proteoglycans consist of a protein core with sulfated glycosaminoglycan (GAG) side-chains covalently attached. GAGs are alternating copolymers of the form $-(AB)_n-$ where AB is a disaccharide.[2,3] Fig. 1 shows the repeating disaccharides for three GAGs: chondroitin 6-sulfate and keratan sulfate (which commonly occur in extracelluar matrix proteoglycans) and heparin (a natural blood anticoagulant). GAGs are polyanions and so attract water by the Donnan effect.[2,4]

Collagen fibres provide tensile reinforcing for biological tissues and consist of rod-like collagen molecules which are aligned with their long axes parallel. In many tissues the fibres are not straight but have a 'crimped' appearance when examined in the scanning electron microscope [Fig. 2(a)].[5] Their mechanical stiffness and strength depend on the formation of covalent cross-links between the molecules in a fibre.[6] These cross-links are possible because a collagen molecule is a polypeptide which contains lysine and 5-hydroxylysine residues; as a result, some of the side-chains on the polymer backbone have the structure shown in Fig. 3(a) and (b). In addition, the enzyme lysyl oxidase catalyses the conversion of some hydroxylysine residues into hydroxyallysine residues, as shown in Fig. 3(c). In cartilage and bone collagen, a hydroxyallysine residue on one molecule reacts with a hydroxlysine residue on another to form a keto-amine cross-link, as shown in Fig. 3(d); in tendon collagen a Schiff base cross-link is formed between an allysine residue and a hydroxylysine residue. Many tissues also contain elastic fibres which have a lower tensile stiffness than collagen. Bones and teeth are stiffer

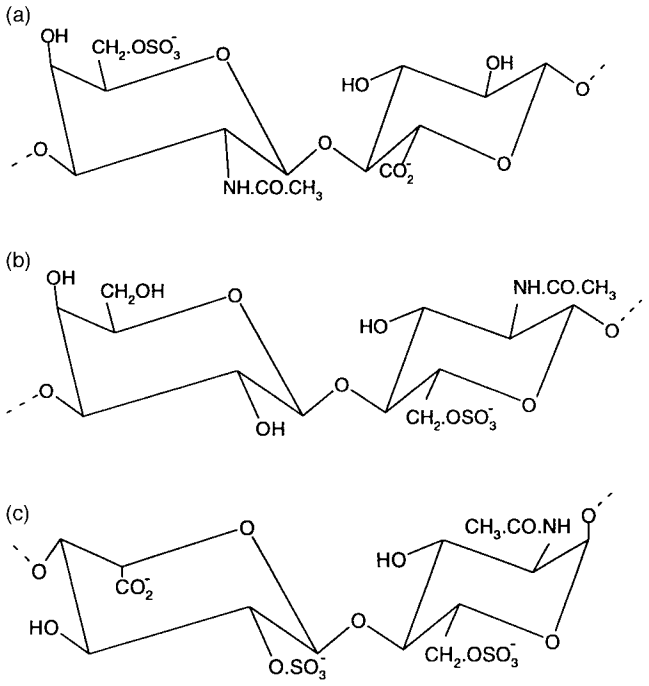

Fig. 1 Structural formulae of the disaccharide repeat unit (AB) in three GAGs: (a) chondroitin 6-sulfate, (b) keratan sulfate and (c) heparin.

and able to withstand compressive loads because they also contain a mineral which resembles impure, poorly crystalline hydroxyapatite [$Ca_5(PO_4)_3OH$; abbreviated to HAP].

Biological properties

Individual variability, degeneration, pathology and ageing all influence the mechanical properties of a tissue.[2] It is not then clear whether information on the physical properties of a tissue from one individual are appropriate for designing a replacement material for another individual. Degeneration causes a problem in that we all suffer minor injuries which may be repaired by scar tissue which is mechanically stiffer than the original tissue. It may be difficult to distinguish the results of degeneration, arising from normal 'wear and tear', from pathology. Pathology can completely change the properties of the natural tissue, *e.g.*, in type V Ehlers–Danlos syndrome, the heart valves are abnormally floppy because of abnormal collagen fibre formation. Increased 'wear and tear' and pathological changes may be associated with ageing and so it is difficult to distinguish them from the normal ageing process. During maturation and ageing of bone and cartilage, the keto-amine cross-links between collagen molecules react with a further hydroxylysine residue to form a pyridinium cross-link, as shown in Fig. 3(e).[6] An alternative form of age-related cross-linking in these tissues involves a lysine, instead of a hydroxylysine, residue. In tendon collagen, the Schiff base cross-link may react with a histidine residue on a further collagen molecule, during ageing. During cartilage ageing there is also a change in the sulfated GAG composition: the ratio of chondroitin sulfate [Fig. 1(a)] to keratan sulfate [Fig. 1(b)] increases. However, it is not possible to simply allow for ageing changes because individuals age at different rates.

Cells are responsible for making and repairing the extracellular matrix. Some cells, *e.g.* muscle cells, have active mechanical properties, *i.e.* they are able to exert a tensile force on their surroundings without being first deformed by an external force. For example, smooth muscle cells enable the walls of a living blood vessel to change their dimensions.[7] The energy for this process is derived from the hydrolysis of adenosine triphosphate.

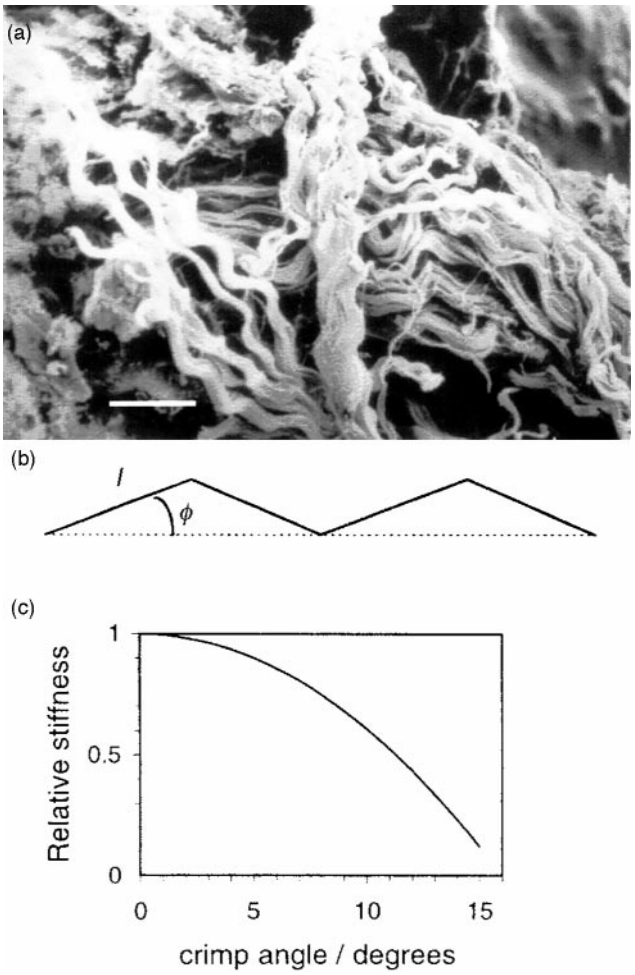

Fig. 2 Crimped structure of a collagen fibre: (a) scanning electron micrograph of collagen fibres in a ligament (the scale bar represents a distance of 40 μm); (b) schematic diagram of a crimped fibre in which a crimp arm of length l makes a crimp angle, ϕ with the axis of the fibre; (c) the effect of crimp angle on the relative stiffness of a tissue containing crimped fibres. In (c) 'relative stiffness' is defined as the Young's modulus of the tissue divided by the Young's modulus of the material of the fibre; the curve was plotted using eqn. (4), setting ϕ_o equal to a reasonable value of 15°.

Removal of tissues from the body, for experiments, may change their physical properties.[8] Cells may be damaged, *e.g.* as a result of being deprived of nutrients. *Post mortem* changes are obvious in muscle, which stiffens when the cells die; the result is *rigor mortis*. However, less dramatic changes may occur in other tissues. It is rarely ethical to perform experiments on living human tissues and results from other species may be misleading. It is sometimes possible to keep tissues alive in culture and this may provide the best means of obtaining reliable information on the mechanical properties of living tissue. Finally, many tissues are poroelastic, *i.e.* some of the mechanical energy transmitted to them is dissipated by flow of viscous fluids, such as blood, through their pores.[9] Blood clots when the tissue is excised, with the result that it is no longer able to flow and so the tissue response may be altered.

Mechanical properties

Natural tissues are fibre-reinforced composite materials whose stiffness is given by their Young's modulus (defined as stress, σ, divided by strain, ε).[10] According to the Voigt model, the Young's modulus for a composite, E_v, is given by

$$E_v = V_f E_f + V_m E_m = V_f E_f + (1 - V_f) E_m \quad (1)$$

where E_f and E_m are the Young's moduli of the fibres and

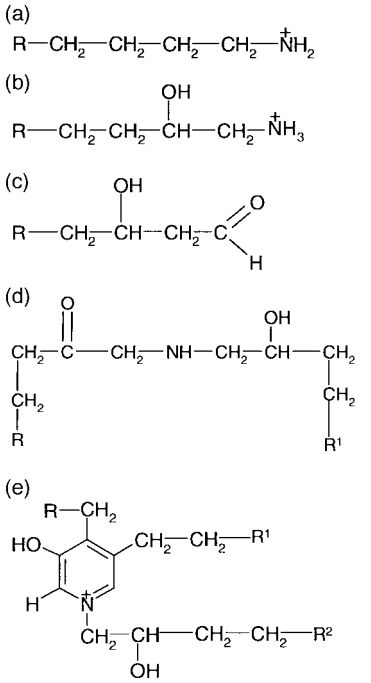

Fig. 3 Cross-linking in cartilage and bone collagen. Collagen molecules contain (a) lysine and (b) 5-hydroxylysine side chains. Lysyl oxidase converts some hydroxylysine residues into (c) hydroxyallysine. Reaction between hydroxlysine and hydroxyallysine produces (d) a keto-amine cross-link. Reaction of the keto-amine cross-link with a further hydroxylysine residue, during maturation of the tissue, produces (e) the pyridinium cross-link; an alternative form of the cross-link involves the reaction of the keto-amine cross-link with a lysine residue.

surrounding material, respectively, while V_f and V_m are their respective volume fractions. Eqn. (1) provides a good model for many tissues which do not contain HAP. Then the fibres are collagen and the surrounding material is the other components of the matrix, so that $E_f \gg E_m$. Then, when V_f is not much less than 1, $E_v \approx V_f E_f$, *i.e.* the stiffness of the matrix is dominated by the stiffness of its collagen fibres. Ageing changes in collagen cross-links, which change the fibre stiffness, can then have a marked effect on the stiffness of the tissue. However, this model cannot be applied to tissues which contain HAP. Tissues like bone consist mostly of collagen and HAP. Then the Young's modulus of the composite, E_r, can be represented by the Reuss model[11] in which

$$1/E_r = V_f/E_f + V_m/E_m$$
$$\Rightarrow \quad E_r = E_f E_m/\{E_m(1-V_m) + E_f V_m\} \qquad (2)$$

where, in this context, the subscript f refers to fibres of collagen and m refers to the surrounding mineral.

The Young's modulus of tissues may also depend on the strain, *i.e.* it is not constant. In many tissues the unstrained fibres are crimped.[5] Increasing the strain involves progressive straightening of the crimp. The collagen fibres are not directly strained until the crimp is straightened. Collagen fibres in ligament and tendon have the crimped structure shown schematically in Fig. 2(b). This structure is defined by the length, l, of the arm of the crimp, and the angle, ϕ, which each arm makes with the axis of the tendon. Since collagen is very stiff, the first stage of tissue strain is mainly straightening the crimp. Straightening the crimp involves a decrease of ϕ from its initial value, ϕ_o, to a final value of zero. The strain, at a given value of ϕ, is then given by

$$\varepsilon = (\cos\phi - \cos\phi_o)/\cos\phi_o \quad \phi_o > \phi \geqslant 0 \qquad (3)$$

Then the stiffness of the tissue, E_t, is related to the stiffness of a fibre, E_f, by

$$E_t = E_f(\cos\phi - \cos\phi_o)/(1-\cos\phi_o) \quad \phi_o > \phi \geqslant 0 \qquad (4)$$

neglecting the contributions of the other components of the matrix. Then the ratio E_t/E_f is a measure of the stiffness of the tissue as compared with the stiffness of the fibre; Fig. 2(c) shows how this ratio depends on ϕ.

The Young's modulus of tissues may also depend on the strain rate, *i.e.* they are viscoelastic.[10] The time-dependent strain, $\varepsilon(t)$, developed in response to a stress, $\sigma(t)$, is given by

$$\sigma(t) = E'\varepsilon + E''(\mathrm{d}\varepsilon/\mathrm{d}t) \qquad (5)$$

where E' is a measure of the elastic properties and E'' is a measure of the viscous properties. If E' and E'' are constant the material is linearly viscoelastic. Then eqn. (5) has a solution of the form

$$\varepsilon(t) = \sigma(t)/E(\omega) \qquad (6)$$

where ω is the angular frequency (in rad s^{-1}) and $E(\omega)$ is defined by

$$E(\omega) = E' + i\omega E'' \qquad (7)$$

Hence, eqn. (6) has the same form as the definition of Young's modulus except that $E(\omega)$ is frequency and, hence, time dependent. Eqn. (6) shows that, in a viscoelastic material, $\varepsilon(t)$ will continue to increase when σ is constant; this phenomenon is called 'creep'. Similarly, $\sigma(t)$ will decrease when ε is constant; this phenomenon is called 'stress relaxation'. The terms E' and $\omega E''$ are called the 'storage' and 'loss' moduli, respectively. In order to determine whether a material is linearly viscoelastic and to characterise $E(\omega)$, it is necessary to repeat measurements over a range of frequencies. Its viscoelastic properties may be characterised by the angle, δ, defined by

$$\delta = \tan^{-1}(\omega E''/E') \qquad (8)$$

which represents the phase lag between the application of a stress and the generation of a strain. Eqn. (6)–(8) may be combined to yield

$$|\varepsilon(t)| = |\sigma(t)|/\{E'(1+\tan^2\delta)^{1/2}\} \qquad (9)$$

When tan δ is equal to zero, the material behaves as an elastic solid and the magnitude of the strain is maximised.

Fracture is a mechanism for a strained tissue to dissipate excess mechanical energy. The energy, W, supplied to a unit volume of tissue is given by

$$W = \int\sigma\mathrm{d}\varepsilon = \int E\varepsilon\mathrm{d}\varepsilon \qquad (10)$$

where the left-hand equality follows directly from the definition of work and the right-hand equality is derived from eqn. (6). The limits of integration are the initial and final strains. In practice, however, it is common to assume that mechanical deformation is quasi-static and to characterise fracture empirically by the stress at which a material fails.

Mechanical testing

Mechanical testing of biological tissues introduces many problems which are encountered infrequently, if at all, when testing synthetic materials. The first problem is the availability of suitable test material, especially in the case of young adult humans. As a result, tissue has often to be collected and stored until there is sufficient for testing. Since biological tissues attract bacteria and moulds which degrade their structure, they need to be stored frozen. Ice crystal formation may then damage tissue structure and specimens have to be frozen as quickly as possible to minimise crystal size.[12]

It is not always clear what the form of the test specimen should be or how it should be tested. Biological tissues cannot

usually be cut into the ideal shapes for tensile tests or into cubes for compression tests.[10] Shaping tissues in this way may change their properties because they are not homogeneous, isotropic materials but are fibre-reinforced composites which have an internal structure. For example, a vertebra of the spine is about four times stiffer when tested intact than when tested as an isolated plug.[13] Furthermore, many tissues in the body naturally merge into other tissues, with no discontinuity, so that one may influence the properties of the other. Finally, it may be difficult to reproduce the loading encountered *in vivo* because the conditions are not precisely known.

The mechanical properties of tissue may depend on temperature, hydration and loading rate. Since natural tissues function at 37 °C, there may be advantages in measuring their properties at this temperature. However, this elevated temperature may lead to dehydration and effect its properties.

Test conditions change the properties of nucleus pulposus, the soft solid which forms the inner region of the intervertebral disc of the spine. Compression squeezes water out of the tissue. Also testing at 37 °C (body temperature) will cause loss of water by evaporation. Any barrier (such as a polymeric film) to prevent water loss would influence the test results because the specimen is very compliant. Surrounding the specimen by fluid would change its properties because its GAGs attract water. Although methods exist for producing solutions which have the same osmotic pressure as the nucleus pulposus[14] they are not applicable here because of the variation of osmotic pressure between samples from different individuals. A different solution would need to be made up for each sample. However, so much nucleus would be required to test the solutions that the remainder of the sample would be too small for mechanical testing. A further problem arises because the nucleus pulposus is viscoelastic;[15] tan δ is greater than zero and, as expected from eqn. (8), is frequency dependent. Therefore, the mechanical properties of a specimen have to be characterised at a range of loading frequencies. Testing at a range of frequencies appears to show that the E' is slightly frequency dependent, as shown in Fig. 4, but a repeat experiment shows that the original properties are not recovered and that the changes may simply be induced in the specimen by performing the test procedure. The alternative approach of Fourier transformation of creep or stress relaxation data, which is successful for the intact intervertebral disc[16] will lead to excessive water loss from the isolated nucleus pulposus during the time-scale of the experiment.

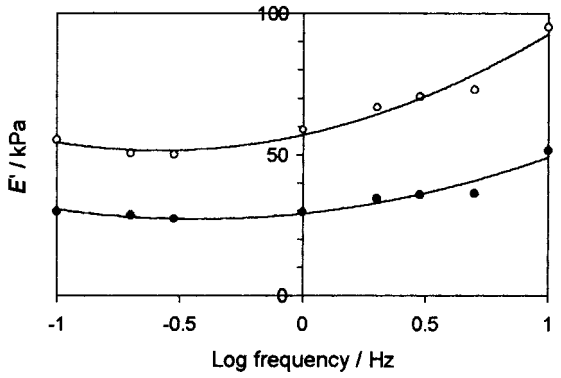

Fig. 4 Effect of repeated testing on the mechanical properties of the nucleus pulposus of the intervertebral disc. The storage modulus, E', is plotted against the frequency for a series of cyclic compression tests. The test sequence was repeated twice. The data from the first sequence are represented by filled circles; open circles are used for the second sequence. Although the modulus appears to increase slightly with increasing frequency, comparison of the first and second sequences shows that a progressive increase can be attributed to the conditions of the test.

Natural tissues as implant materials

Natural tissues may be replaced by grafts from the same patient, from a human donor, or from an animal. Examples include heart valve, tendon, ligament, corneal and skin grafts. Skin, tendon and muscle have all been grafted successfully from one site to another in the same patient. Muscle transplantation is used in an experimental procedure called dynamic cardioplasty. Here a muscle graft is wrapped around a failing heart and contractions are stimulated by an electrical pulse, so that the heart continues to beat.[17] Tissue banks provide human graft material instantly. Donors are screened to reduce the risk of transmittable diseases, such as AIDS and hepatitis, and the tissue is stored under sterile conditions.[18] Corneal transplantation provides an example of a successful use of banked tissue from human donors.[19]

Animal tissues may be treated for subsequent implantation into humans. Bioprosthetic heart valves are natural pig heart valves which are treated with glutaraldehyde (pentan-1,5-dial) which cross-links macromolecules, thus preventing them from having many of their biological properties and, as a result, also kills cells.[20] Glutaraldehyde cross-links macromolecules because it is a bifunctional aldehyde. Since it reacts with lysine, hydroylysine and histidine residues, it creates more intermolecular collagen cross-links and so can stiffen tissues. The chemically treated valves may be mounted in a synthetic frame or stent. Alternatively, the valve may be used attached to surrounding tissues which are known as the valve 'root'. Implanting the valve–root combination removes the need for a frame. However, body fluids are supersaturated with calcium and phosphate ions at physiological pH values, which may lead to deposition of HAP or tricalcium phosphate $[Ca_3(PO_4)_2]$, which has a very similar crystal structure, on to synthetic materials. These deposits are likely to make the composite behave more like a Reuss composite in which V_m, of eqn. (2), is increased. Since $E_m \gg E_f$, the increase in V_m leads to an increase the Young's modulus, E_r, of the composite and, hence, a higher stress for a given strain. In practice, the deposits have been found to lead to high mechanical stresses within the valve.[20] Roots and valves from human donors function better than chemically treated pig tissue, but their availability is limited.

Tissue engineering uses living cells to help to make replacement tissues[21,22] from natural materials. It attempts to overcome the problems encountered by using synthetic materials and the problem of availability of graft material. There are three strategies for engineering new tissues. The first is to stimulate new tissue by injecting isolated new cells into the bloodstream or a specific organ of the recipient. This method is most useful for influencing tissues with high metabolic activity, such as the liver. The second strategy is to implant cells encased in a selectively permeable barrier which allows diffusion of nutrients and waste products but prevents the passage of antibodies and immune cells which would lead to rejection of the tissues by the body.[23] Polyacrylonitrile–polyvinyl chloride membranes and alginate gels have been used for this purpose. This approach has been successfully used for treating Parkinson's disease and diabetes mellitus in animal models. However, fibrous tissue overgrowth (see 'Biocompatibility' below) may lead to reduced diffusion of nutrients and waste products and so prevents the cells from functioning properly. The third strategy involves transplanting cells into matrices consisting of natural materials such as collagen or synthetic polymers. These are used to create new tissues which are incorporated into the recipient's host tissues. A problem with all three strategies is to prevent specialised

cells from reverting to undifferentiated cells once they are inside the body of the recipient.

Synthetic materials

Biocompatibility

The natural response of the human body is to treat most foreign materials as being harmful. Despite the inert and non-toxic nature of many biomaterials, adverse reactions such as inflammation, formation of fibrous tissue, blood clotting and infection are frequent and sometimes life-threatening.[23] Formation of a fibrous capsule around an implant is a normal response.[24] For some implants, like artificial ligaments, fibrous capsule formation is not a disadvantage; indeed it has even been claimed that the fibrous tissue provides additional reinforcement. In contrast, a breast implant is supposed to have the same stiffness as the natural tissue. A fibrous capsule can then stiffen the breast, cause pain and change its appearance.[25]

If materials are to be in contact with blood, it is important that they should not induce clot formation. Surface roughness promotes clot formation, so materials in contact with blood should have surfaces which are as smooth as possible. A clot which adheres to a surface is called a thrombus. Metal stents, which are used to dilate the coronary artery, have had their surfaces modified to reduce clot formation.[26] The first stage is to coat the metal with a polymer which contains amine side-chains. Heparin chains are partially fragmented by cleaving a disaccharide linkage shown in Fig. 1(c), to give a chain with an aldehyde group at one end. This aldehyde group reacts with an amine group, on the surface coating, so that heparin chains are attached by keto-amine cross-links resembling that in Fig. 3(d). This surface tends to prevent clot formation because heparin is a natural anticoagulant.

Wear debris from synthetic implants can cause an inflammatory response. An example is provided by particles of polyethylene produced by the wear of total hip prostheses (see 'Total joint replacement' below). In general, smaller particles are engulfed by cells, called macrophages, and the larger wear particles prompt the formation of fibrous tissue. Both result in inflammation.

The hydrolysis of polymers by body fluids is exploited to aid tissue repair and to manufacture devices which are only required for a short time. The advantage is that the material degrades when it is no longer required. Examples of these materials are polymers of lactic acid, glycolic acid and hydroxybutyric acid. These carboxylic acids also contain an OH group so that they form polyesters which hydrolyse to produce the original monomer. Polylactic acid has the further advantage that the monomer is a natural product of metabolism and, therefore, is expected to be biocompatible. Polymers of this kind are regularly used to make resorbable sutures. They also have been used to make plates for repair of bone fractures, the idea being that they are resorbed when the fracture has repaired. In combination with HAP, they have been used to make a bone substitute which is intended to resorb when replaced by real bone in the remodelling process. More recently, porous composites of hydrolysable polymers and HAP have been used for bone replacement; cells are able to penetrate into the material through the pores[27] (see 'Biofilms' below). The material has the strength required for withstanding load but it is intended to be replaced by natural bone. Finally, hydrolysable polymers may be used for controlled release of drugs encapsulated within them.

Biofilms

A further biological response to synthetic materials implants is colonisation of their surfaces by bacteria; the resulting layer of bacteria and their products is sometimes called a 'biofilm'.

$$(NH_2)_2CO + H_2O \xrightleftharpoons{\text{urease}} 2NH_3 + CO_2$$

$$2NH_3 + 2H_2O \rightleftharpoons 2NH_4^+ + 2OH^-$$

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^{2-} + 2H^+$$

Fig. 5 Chemical reactions occurring in urine infected with bacteria which produce urease.

Biofilms form because synthetic materials lack the ability of the cells within natural tissues to combat infection. Gristina[28] proposed that, when an implant is inserted in the body, there is a 'race' between the host tissue and bacteria to reach the implant surface and occupy it. He believed that an implant should encourage host tissue ingrowth and so resist bacterial colonisation. An example of tissue ingrowth is provided by bone growing into pores in implants. For example, titanium alloys are usually coated with a natural oxide layer with which bone will grow in contact. If the surface has pores of dimension 1–2 μm, the bone will grow into the pores to give good fixation of the implant.[29] Bone also grows into similar sized pores in HAP coatings on implants.[30]

Biofilms commonly coat the surface of silicone and latex catheters which are used to drain urine from the bladder.[31] Catheter biofilms contain ammonium magnesium phosphate hexahydrate ($NH_4MgPO_4 \cdot 6H_2O$), which occurs naturally as the mineral struvite, and poorly crystalline HAP. These hard deposits can block the device or abrade the surface of the urethra when the catheter is removed or replaced. These deposits are formed because the catheter becomes colonised by bacteria (*e.g. Proteus*) which produce the enzyme urease. Urease catalyses the hydrolysis of urea to form ammonia. Fig. 5 then shows the chemical reactions which occur at the catheter surface. Since carbon dioxide is a weak acid ($pK_{a1} = 6.4$; $pK_{a2} = 10.3$), but ammonia is a relatively strong base ($pK_b = 4.8$) the pH of the urine rises to above the value of 7.2 at which HAP and struvite are precipitated.

A recent approach to minimising biofilm formation on catheters and other devices is by covalent attachment of the phosphoryl choline (PC) group [Fig. 6(a)]. This approach was developed initially to prevent the formation of blood clots on synthetic materials but has since been applied more widely. It was recognised that fundamental physico-chemical approaches to solving the problem were inadequate because of its complexity.[32] The most simple common feature shared by cells which resist clot formation is the presence of PC groups in their membranes. Therefore, methods were developed to attach the PC group to synthetic materials.[33] The first step in these methods is to react choline hydroxide [Fig. 6(b)] with phosphorus oxychloride ($POCl_3$) to produce choline dichlorophosphate [Fig. 6(c)]. In practice, choline hydroxide is so strongly basic that it is neutralised with acetic acid before use. Choline dichlorophosphate is then reacted with dichlorodimethylsilane [$(CH_3)_2SiCl_2$] to synthesise phosphorylcholine dimethylsilyl chloride [Fig. 6(d)]. This compound reacts with the OH group on a polymer side chain to covalently attach the PC group [Fig. 6(e)].

Artificial ligaments

The development of an artificial ligament illustrates many of the principles involved in using synthetic materials to mimic biological tissues. The example chosen here is the anterior cruciate ligament (ACL) of the knee which joins the front of the tibia (shin bone) to the back of the femur (thigh bone) and passes through the centre of the joint. It is commonly ruptured in sports, especially football and skiing, but repairs itself poorly, if at all. As a result, there has been considerable

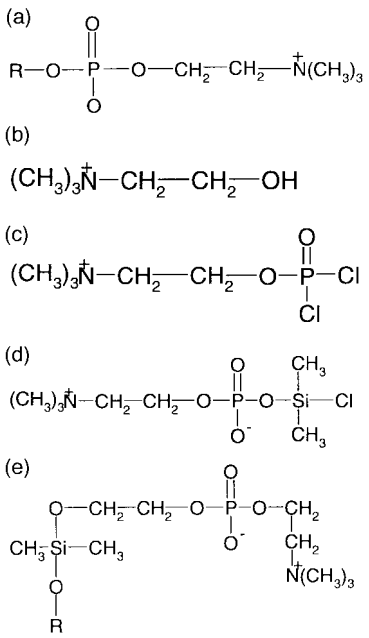

Fig. 6 Phosphoryl choline (PC) immobilised on a surface. This figure shows: (a) the PC group, attached to a group, R; (b) choline hydroxide, the starting material for synthesising PC derivatives; (c) choline dichlorophosphate, which is synthesised from choline hydroxide using $POCl_3$; (d) phosphoryl choline dimethylsilyl choline, which is synthesised from choline dichlorophosphate using $(CH_3)_2SiCl_2$; (e) the result of reacting phosphoryl choline dimethylsilyl choline with an OH group to covalently link the PC group to a synthetic polymer.

interest in materials which could be used for a synthetic ACL.[34] Commercial prosthetic ligaments tend to be braided from polyesters or a mixture of polyester and carbon fibre to produce a material with a comparable stiffness and strength to the natural ligament. The natural ligament fractures at about 1.7 kN and is composed mostly of crimped collagen fibres.

The ABC™ system (Surgicraft, Redditch, UK)[35] provides an example of a synthetic ACL. In order to function satisfactorily, the synthetic ACL must have a comparable stiffness to that of the natural ligament. Similarly, if it is not to fail in normal use, its failure stress must not be less than that of the natural ligament. However, it must be sufficiently small to be inserted into the knee and occupy the same space as the natural ligament. The synthetic ACL is based on a 'unit material' which, in the original version, consisted of four strands of polyester and a single strand of carbon fibre. The polyester strands are woven to impose an initial crimp angle, ϕ_o, on the carbon fibre. The crimp length, l, and the value of ϕ_o are controlled, during manufacture, by the tension in the polyester strands. A complete synthetic ACL consists of 24 strands of unit material and has a diameter of about 5 mm. When a load is applied the polyester braids are strained so that the crimp angle, ϕ, decreases from ϕ_o to a final value of zero. According to eqn. (4) and Fig. 1(c), the stiffness of the composite device then increases with strain. The response of the synthetic ACL to load then closely resembles that of the natural tissue and the load at which it fails is even higher.

The challenge is to attach this synthetic device to bone. This problem does not arise with the natural ACL which merges into the bone tissue. Adhesives do not provide a viable method for joining a braided textile of this size to wet tissue, in a surgical procedure, if the attachment site is to withstand the high tensile and shear forces to which it will be subjected. Therefore some form of mechanical attachment is required. At either end, the strands of unit material are tightly braided. One end is looped around a notched polysulfone button which is thus secured in place as an integral part of the device. The other end is braided into a loop. In order to insert the device, a hole is drilled in the front of the tibia, upwards into the joint space, as shown in Fig. 7. The device is drawn through the hole until the button engages the front face of the tibia. The mid-part then occupies the joint space between the tibia and the femur. The loop at the other end is then attached to the back of the femur so that the points of attachment of the synthetic device are in roughly the same position as those for the natural ACL. A plastic bollard which expands into a drilled hole in the bone holds the loop close to the bone surface. This example shows that attachment of a replacement device inevitably involves designing components from materials which must have the required mechanical properties and be biocompatible.

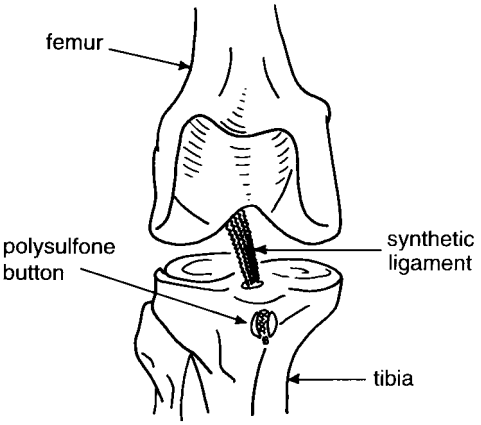

Fig. 7 Attachment of a synthetic anterior cruciate ligament in the knee. This is a front view of the bones of the knee with the patella (knee cap) removed. A hole is drilled upwards and backwards through the front of the tibia. The ligament is pulled through the hole until the polysulfone button engages the front of the tibia. The other end has a loop which is attached to the back of the femur by a bollard.

The performance of the attachment technique in patients is of special concern. Although devices have to be tested under laboratory conditions, the ultimate test is their performance in patients. Laboratory conditions cannot simulate the attachment of a synthetic material to natural tissue in a living person. Initial problems which occurred with the ABC™ system involved multiple splitting, as a result of fatigue, and crushing abrasion where the textile emerged from the hole in the bone.[36] This problem was solved not by modifying the materials of the device but by designing new tools to change the size and shape of the hole.

Total hip replacement

Total hip replacement prostheses provide an example of a device whose components make no attempt to mimic the natural materials. The natural hip [Fig. 8(a)] is a ball and socket joint. It is replaced by sawing off [along the dashed line of Fig. 8(a)] and removing the ball and reaming out the socket. The femoral component of the prosthesis [Fig. 8(b)] is made of a suitable metal (*e.g.* implantable grade stainless steel) and the acetabular component, or 'cup' is made of 'ultra high molecular weight polyethylene' (UHMWPE).[37,38] Articulation of a metal on a polyethylene surface has an acceptably low coefficient of friction so that a reasonable range of movement can be achieved. Total hip and knee replacement are very successful surgical procedures, because of the relative simplicity of the prosthesis design and the reliability of the materials involved.

Fig. 8(b) shows that the components of the prosthesis are held in place by polymethylmethacrylate 'bone cement'. The function of this cement is to fill space so there is no need for good adhesion with either the synthetic materials or the natural tissue. Bone cement is supplied as two phases: a liquid and a

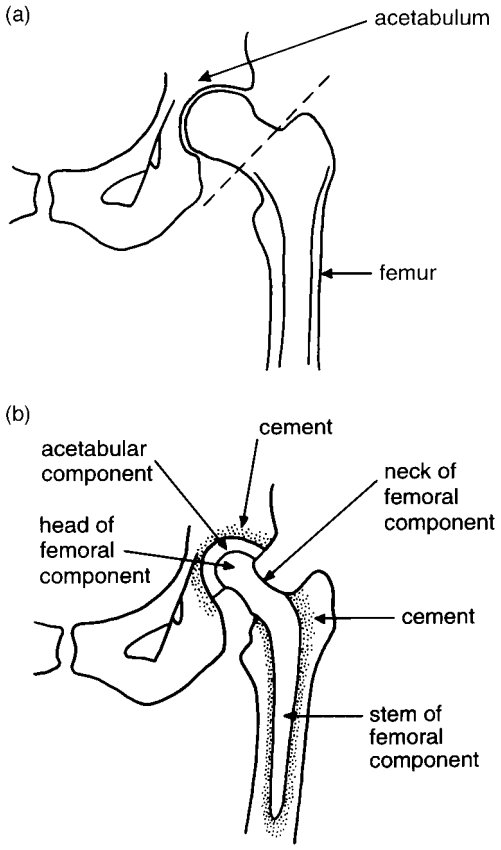

Fig. 8 Total hip replacement. The natural joint is shown in (a). In order to insert the femoral component of the prosthesis, the femur is sawn along the direction shown. The femur and acetabulum are then reamed to accept the femoral and acetabular components of the prosthesis, respectively. The two components are shown, in place, in (b) where they are secured in place by polymethylmethacrylate bone cement.

solid.[39] The liquid phase consists of methylmethacrylate monomer (MMA) mixed with *N*,*N*-dimethyl-*p*-toluidine; the solid consists of polymethylmethacrylate powder mixed with benzoyl peroxide (PhCOO–OOCPh) and barium sulfate. The two phases are mixed when required. MMA polymerises by a free radical addition process which is initiated by formation of PhCOO• radicals from the benzoyl peroxide; *N*,*N*-dimethyl-*p*-toluidine promotes curing of the resulting PMMA. After about 15 min, the mixture is putty-like and is ready to be used; it cures *in situ*. The barium sulfate increases X-ray attenuation by the cured cement enabling radiographs to be used to examine the cement around the prosthesis.

The main problems encountered in total hip replacement are infection, loosening of either component of the prosthesis and wear of the acetabular component. Infection can occur through the wound created in surgery by bacteria spreading from other parts of the body. A recent approach to this problem is to use cements which contain antibiotics.[40] Diffusion of antibiotic through the cement is intended to lead to controlled release to the site of potential infection over a useful period of time. It has also been proposed to release growth hormones from cement, in the same way, to encourage bone growth around the prosthesis and so minimise loosening.[41] Attempts have been made to prevent loosening by dispensing with cement and manufacturing components which are intended to have a press-fit with the bone. Bone ingrowth may be encouraged by a porous surface (see 'Biofilms' above), often involving an HAP surface layer, in order to improve a more secure fixation. Cementless fixation has the further advantage that it prevents the drop in blood pressure which accompanies the application of cement before it hardens. However, cementless prostheses still loosen because surface coatings are weak in shear and because living bone may be resorbed as well as grow into pores.

Movement of the joint prosthesis can lead to wear of the relatively soft UHMWPE cup. The cellular reaction to wear debris may be implicated in implant loosening. One approach to this problem is to use Co–Cr–Mo–C alloys for both the cup and the head of the femoral component.[42] These alloys are the only metals which can articulate against themselves without micro-welding because their structures enable them to have a very smooth surface finish.

Safety

In order to ensure that an implant is safe it must have the required mechanical strength and be made of a biocompatible material. However, there are other considerations: it must be sterile and it must be possible to monitor the performance of the device in a patient.

Mechanical testing has to be performed to the standards set by the appropriate regulatory authority. For well established devices, such as total hip protheses, there are standards which have to be followed. However, it would be prohibitively expensive to build a succession of prototypes and test them all at each stage of the design process. Finite element methods allow defined loading conditions to be applied to a computer model of the device if the materials properties of its components are known.[43,44] Regions of high stress or with undesirable deformations can then be identified and the design modified with no need to build a prototype.

Sterilisation is the destruction and/or removal of all microorganisms from contaminated material. The traditional method of sterilising a material is by heat. Steam sterilisation is used for some permeable materials. Its main disadvantage is that the combination of water and pressure can cause water absorption within a polymer to such an extent that it degrades due to microcavitation.[45] Studies on titanium have shown that steam autoclaving inhibits cell attachment because it causes changes in the surface oxide layer.[46] Gamma irradiation is an alternative approach which cross-links UHMWPE but may lead to increased wear in practice.[47] Ethylene oxide gas has been used for several years to sterilise many types of implant; however there is concern that it may be carcinogenic[45] so its use is now limited.

Conclusions

The first difficulty in developing synthetic materials is to determine the properties of the natural tissues which they are intended to replace and the forces to which they will be subjected in use. Difficulties in determining the properties of natural tissues arise for two reasons. The first is that they function normally as part of a living system. Their properties may change after death or when they are removed from their natural surroundings. They will also be influenced by biological factors, such as ageing, degeneration, pathology and individual variability. The second difficulty arises from the nature of their physical properties. Most tissues are highly hydrated and may express fluid in experiments, and so their properties may change. Specimen decay may limit the number of experiments required to define their viscoelastic properties. It is not always possible to measure the forces acting on human tissues directly; in some cases such as the ACL in the knee joint (see above), estimates may vary considerably. Even if it were possible to define and mimic the properties of a natural tissue, it is not clear whether a replacement material should necessarily have the same properties.

Tissues may be replaced by grafts from other sites in the patient's body, grafts from donors, the products of tissue engineering or synthetic materials; synthetic materials may

mimic the structures of natural tissues or be effectively conventional engineering solutions using conventional engineering materials. Whatever approach is used to replace a tissue, the method of attaching the replacement is likely to be a problem. At first sight, it might appear that the use of natural tissues or the biomimetic approach is likely to achieve better results. However, the success of total hip replacement, which uses conventional engineering materials, indicates that this is not necessarily the case. Total hip replacement prostheses can be securely fixed in place and use well characterised materials whose behaviour is reasonably predictable. Finally, the ultimate test of the success of any replacement material must be its longterm performance in the human body.

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