ENGINEERING TRIPOS PART IIA
Module 3G5: Biomaterials **Principal Accessor: AE Markaki**

CRIBS

1 (a) (i) At the nanoscale level, bone is a composite of 30% (dry weight) mineralised collagen fibrils surrounded by 70% calcium phosphate. At the micron scale, the collagen fibres are arranged either as a block of randomly oriented woven fibrils (woven bone) or wrapped into densely packed, concentric lamellar structures known as osteons (lamellar bone). Osteons are cylindrical structures whose long axis lies along the long axis of the bone. At the millimeter scale, there two types of bone: cortical bone, which is dense (5-10% porous) and comes as tightly packed lamellar or woven bone; and trabecular bone, which comes as a highly porous solid (50 to 90% porosity). The latter consists of a network of trabeculae. Cortical bone (compact) is found in the shaft of long bones and forms the outer shell around cancellous bone at the ends of bones.

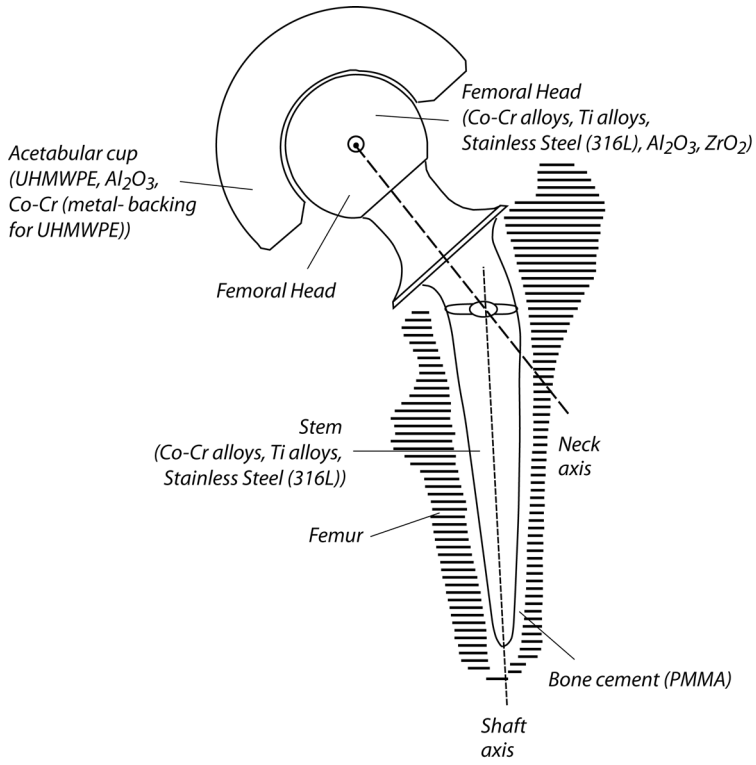
(b) It has been long accepted that bone is an active material, it responds to stresses and strains. This concept was first proposed by Wolf in 1892. Wolff's law states that healthy bone will adapt to the loading regime to which it is subjected. It was Frost though who suggested that bone formation (called modelling) and resorption (remodelling) are triggered at threshold strains using the Mechanostat theory. If bone strains fall below a lower boundary remodelling threshold (~0.05-0.1 millistrains), bone mass is removed in order to bring the strains back to the adapted range, where bone formation and resorption are in equilibrium. Conversely, if strains exceed a modelling threshold (~1 millistrain), bone mass is added to bring the strains back to the adapted range.

(c) The stress shielding effect arises because prostheses are typically stiffer than the surrounding bone (cortical bone is about 7-27 GPa). A stiffer implant inhibits the bone from being strained. As mentioned above, when strains fall below a remodelling threshold value, the equilibrium between bone-making and bone-removal is displaced towards the latter, producing bone loss around the implant.

Typically, ceramics have higher Young's moduli than metals hence the stress shielding effect is stronger. In terms of mechanical performance, metals are tougher and resistant to fatigue crack propagation but are less wear resistant compared to ceramics. Ceramics, on the other hand, are too brittle. In terms of biological performance, ceramics provide a more appropriate environment because of their chemical inertness compared to metals. One possibility to improve the biological response of metals is to coat them with hydroxyapatite, which is similar in composition with the mineral component of bone. It is also possible to incorporate in the coating bone-stimulating biological factors such as bone morphogenetic proteins.

(d) The most common cause for hip replacement is osteoarthritis. In osteoarthritis, there is a breakdown in the cartilage covering the ends of bones where they meet to form a joint. As the cartilage wears away, the bones become exposed and rub against each other. The second cause is known as avascular necrosis. In this condition, there is cellular death of the femoral head due to interruption of the blood supply. Without blood, this leads to collapse of the femoral head and degeneration of the joint.

A total hip prosthesis comprises an acetabular component and a femoral component – see schematic below. The acetabular component is usually made of UHMWPE (or Al_2O_3) and fixed in place with PMMA cement. UHMWPE is sometimes backed up with a metal cup (usually Co-Cr) which provides better X-ray visibility. The femoral component (stem and femoral head) is commonly made of Ti-6Al-4V, 316L or Co-Cr alloys. They are chosen because of their mechanical properties (respectable strain tolerance, strength and toughness). Often the stem is coated with HA or porous Ti, Co-Cr coatings (bead-sintered or fibre/wire based ones) which allow bone in-growth as a means of fixation. Femoral heads can also be made of Al_2O_3 and ZrO_2. Femoral heads need to have a low coefficient of friction and high wear resistance. An advantage of using a ceramic instead of metal for the head is that it is harder and can be made smoother and more wear resistant.



(e) Implants are subjected to bending and torsional moments around the intersection point of shaft and neck axes of the implant – see schematic above. There is also wear resulting from articulation of the femoral head component against the acetabular cup component. In addition, cement can deteriorate through fatigue and biological processes producing wear debris which can cause osteolysis (bone loss). Furthermore, if the bonding between the implant and surrounding bone is weak, it is very likely that wear debris will form at the interface and have deleterious effects. Ensuring that the interfacial bond remains strong, so that interfacial shear displacements do not occur and wear debris is not created, is an important objective.

Comments: *Parts (a), (b) and (d) were generally well answered by the candidates, marks were lost because of lack of clear descriptions of the concepts. In Part (c), several candidates stated that metals have higher Young's modulus than ceramics and several didn't comment on the relative mechanical and biological response. In Part (e), a large number of candidates provided incomplete information on mechanical loading and wear.*

2 (a) (i) A good answer would list 6 important considerations, a very good answer would give a clear description of each. An excellent answer would link at least 2 of the answers back to the device described in the question.
Considerations may include:

1. The scale of manufacturing and throughput is important, to understand what cycle time is required. For example, this product is relatively high throughput at approx. 30,000 units per day and so it is likely that it would be more economical to use a process that can manage large scale sterilisation and rapid cycle times.
2. The required mechanical performance of materials or their chemical, physical or biological properties must be well understood as they may change when exposed to sterilisation. This product has been made from polymers and may become more brittle upon sterilisation with some techniques, depending on the polymer.
3. The importance of geometry is key to understand. It was noted that the dimensions were precisely defined. However, for example, these may be distorted if a technique provides too much heat.
4. It is important to consider if the material can be sterilised through its bulk, and if the packaging process can also be sterile (primary and secondary packaging).
5. It is important that the process sterilises without leaving any toxic residue or residue that will lead to biocompatibility issues. As an implantable, this is especially important.
6. It needs to be straight forward to measure and validate the sterilisation process.

(ii) A good answer would list **1 positive and 1 negative** for each technique, a very good answer would give a clear description of each. An excellent answer would link at least 3 of the points back to the device described in the question.
Steam: Negatives include the high temperature, that may lead to distortion of polymeric parts, the likelihood that the steam will contain contaminants, that materials will absorb the moisture, and there are limited packaging choices that will allow the part to be sterilised within its packaging. The positives include its inexpensive capital cost, ease of control, lack of toxic residues and fast speed.
Comments relating to product may include: This was carefully designed in terms of geometry and mechanical properties, so the steam may lead to distortion of the shape, which could have an influence.
Gamma radiation: Negatives include the discoloration or degradation of some polymers, the large capital outlay if you decide to have an in-house sterilisation unit and the need for isotope containment. Positives include the fact the gamma radiation can penetrate through the full product in most cases. It leaves no residue at all.
Comments relating to product may include: The polymer properties are noted as important and so any degradation must be understood in case it has an influence on the final behaviour.
Ethylene oxide: Negatives include the need to quarantine the product for 1-2 weeks after sterilisation to allow the residue to dissipate. There are many process variables that need to be controlled carefully, i.e. pressure, temperature, time, humidity, ethylene oxide concentration. Positives include that it has very good compatibility with a broad range of materials. If the packaging is carefully chosen, it can sterilise through the packaging.

(b) It should be clear from the answer that these ethical principles relate to medical/clinical studies.
The definitions from the lectures are not required verbatim, just the clear description of the underlying principles.
Respect for persons: It is important to note that individuals are able to make their own decisions and that these decisions are respected. If anyone is unable to make decisions autonomously then the requires that the choices of autonomous individuals be respected and that people who are incapable of making their own choices be protected.
Beneficence: This principle focuses on ensuring that if someone participates in a research study, it should only be if the balance of potential benefits and harms is in their favour. While the Belmont Report does not include the principle of Nonmaleficence, and it was not covered in the lectures, it is a standard core principle of bioethics that is similar in nature, i.e. patients should be protected from harm, and this is an acceptable answer.

Justice: This principle is a clear statement that studies and researchers must not exploit vulnerable people. It is also noted that they should not exclude eligible candidates who may benefit from participation in a study, without a good reason.

(c) (i) Medical device classification
An acceptable answer will note that devices are classified based on the device risks and the vulnerability of the human body to the use of the device, i.e. the higher the risk, the higher the classification. A very good answer will note also that there is a graduated system of control (examples may be given to illustrate this) and will note the classifications. An excellent answer will either note that in the EU there is a set of 22 rules to allow the medical device company to assess the classification, or note that in the USA the classification is firstly achieved through comparison and precedence. An outstanding answer may also note that classification is useful to provide reassurance to the patient that the appropriate care is being taken, to ensure the manufacturer can control costs and also ensure the correct precautions are in place, or that classification guides regulatory bodies where they should focus their efforts.

(ii) Biocompatibility of materials
It is important for a full answer to note biocompatibility can be considered in terms of biofunctionality and also biosafety. In terms of biofunctionality, the most common definition is: "Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application" (Williams 1987) i.e. resistance to clotting, antibacterial. This of course does not need to be verbatim, but should be conveyed clearly.
In terms of biosafety: The exclusion of severe deleterious effects of a biomaterial on an organism. Includes cytotoxicity, carcinogenity.

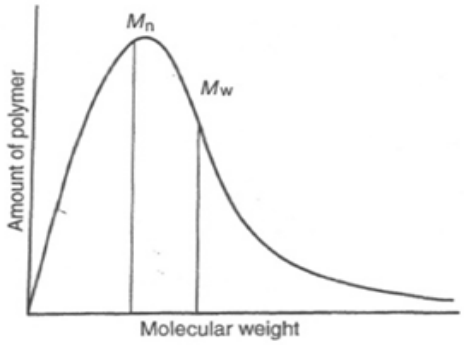
(iii) Parametric release, when sterilising biomaterials
The important point here is that a sterilisation process can be validated through showing control of the process, rather than testing the finished product. This comes about by demonstrating control over the sterilisation process, possibly by using test systems. Example test systems could include a known population of spores with a defined resistance, contained within a test that changes colour to show a pass or fail results, or a chemical change when the appropriate conditions have been met for a sufficient time.

(d) Describe any 4 differences between the US and EU in terms of their regulatory processes when bringing a medical device to market.
Examples include:

- There is very different legislation in place, with the EU using medical device directives that apply to EU member states and the US using FDA regulations and processes.
- The systems for classification are very different, with the EU relying on a set of 22 rules to enable medical technology firms to self-assess the device classification, whereas firms in the USA firstly rely on a database of existing devices which show precedence, and a separate application process if the device is completely new. There are also only three classifications in the USA (I, II, III) and 5 in the EU (I non-sterile, I sterile, IIA, IIB, III)
- In the EU, GMP and compliance to directives is demonstrated by complying with the standard, ISO13485. This is a medical device manufacturing standard that defines the quality management system (QMS) that needs to be put in place, the controls, etc. In the US, this is a different QMS entirely, regulated by the FDA.
- In the EU, the firm making the medical devices are audited by a Notified Body. These are independent, privately run firms. The firm can choose any Notified Body from across the EU. In the US audits are carried out by the FDA only in a much more centralised system.
- In the EU, medical devices are judged during evaluation or trials based on their ***performance*** (the action of the device with reference to its intended use when correctly applied). This does not refer to the final outcome. In the USA, this is instead assessed by ***effectiveness*** (the extent to which the technology does what it is intended to do for a defined population).

Comments: *Generally well answered by the candidates, marks were lost in Parts (i) and (ii) because of lack of comments on the specific medical device.*

3 (a) (i)



Typical molecular weight distribution of a polymer.

(ii) Polyethylene monomer weight = $12\times2 + 1\times4 = 28$ g mol^{-1}

Mean degree of polymerisation, $n \sim M_n/28 = 4\times10^6/28 \sim 1.43\times10^5$

$\text{PDI} = M_w/M_n \rightarrow M_w = \text{PDI}\cdot M_n = 1.2\times4\times10^6 = 4.8\times10^6$ g mol^{-1}.

(iii) PE2 is expected to have a higher Young's modulus, better achievable degree of crystallinity, and higher melting point than PE1.
The Young's modulus of a polymer increases approximately linearly with the degree of polymerisation (n) initially, the plateau is at around $n\sim10^5$.
Crystallinity requires ordered chain packing. Polymers with a narrow distribution of molecular weight (i.e. with PDI close 1) will have more similar chain lengths, thus higher probability of resulting in an ordered chain packing.
Melting point of a polymer depends on the chain length/ degree of polymerisation of the crystalline region. PE2 has a longer chain length, thus more thermal energy is required to overcome the inter-chain van der Waals forces.

(iv) PE2 can be used as an insert separating the metallic cup and the femur head in a total joint replacement implant. With the high molecular weight of PE2, the material is expected to have good strength, toughness, and wear resistance, providing a smooth interface reducing frictional wear between the cup and the head.

(b) (i) Polytetrafluoroethylene (PTFE) for vascular grafts
Vascular graft requires an inert surface for interaction with the blood components, and PTFE provides the inert, non-adhesive, smooth surface required and it is biocompatible. The most likely PTFE polymer structure is in the linear chain form, which can lead to the formation of PTFE with a high degree of crystallinity. Vascular grafts require good strength, toughness and fatigue resistance to withstand the blood pressure over the graft's lifetime (~10 years); it also requires good wear resistance against the blood flow. These mechanical properties required will be improved with polymer crystallinity.

ii) PGA (90%)/PLA (10%) co-polymer for resorbable sutures
PGA-PLA with a co-polymer ratio of 90:10 is a hydrolysable (or biodegradable) polymer giving a half-life of approximately 3 weeks. The biodegraded bi-products can be re-absorbed by the body without inducing toxicity, and the degradation life time is tuned to match the surgical wound healing time for most cases. The PGA (90%)/PLA (10%) also gives a higher crystallinity, and better mechanical property than the PLA-rich co-polymer equivalent. Good mechanical property is required for suture applications.

iii) Poly(anhydrides) made into wafers containing chemotherapeutic agents for treatment of brain tumour
Poly(anhydrides) is a hydrolysable polymer with a short degradation half-life (in the order of days). The biodegraded bi-products can be re-absorbed by the body without inducing toxicity, and the degradation life time allows the drugs to be controlled released into the tumour surrounding region over the time scale of days. Since it is a drug delivery device, more emphasis is placed on the predictability of controlled release rather than the mechanical property. An amorphous structure of the polymer is used.

iv) Collagen (Type-I) fibre scaffold as a haemostatic dressing

The haemostatic dressing would consist of crystalline collagen-I fibres. These crystalline fibres are arises from the quaternary structure of collagen I (i.e. the banding structure). When platelets (the cells involved in blood clotting), are in contact with the collagen quaternary structure in the collagen fibres, they form clots. This thus gives rise to the haemostatic property of the collagen fibre scaffold. Melting of banded structure prevents clotting and down-regulates the inflammatory response.

Comments: *In Part (a)(i), a few candidates added incorrect axis labels for the molecular weight distribution graph. Several candidates didn't use the polydispersity index for commenting on the achievable degree of crystallinity in Part (a)(iii). In Part (b) marks were lost because of lack of details.*

4 (a) Drugs alone can suffer from low solubility, poor stability, short circulation time, rapid clearance from the body, and non-specific toxicity. The three main functions of a biomaterial drug delivery system include controlled release, targeted delivery and solubility enhancement.
For controlled release, drug efficacy can be enhanced by maintaining the concentration within the therapeutic window (effective dose). Polymer carriers loaded with therapeutics enable controlled temporal and spatial release of a drug by controlling drug diffusion, the rate of dissolution, or degradation of the carrier.
For targeted delivery, drug efficacy can be enhanced and toxicity minimized by localization at the organ, tissue, cellular, or organelle level. Targeting can be achieved by coating or conjugating the carrier with affinity reagents such as nucleic acids, peptides, antibodies, or others that bind specific cell receptor proteins, nucleic acids, or polysaccharides.
For solubility enhancement, low drug solubility and stability often reduce the effectiveness of an otherwise promising therapeutic candidate. Drug delivery systems can be formulated to improve the in vivo solubility of lipophilic and hydrophobic drugs by encapsulation in a drug delivery carrier or by conjugation with a polymer.

(b) (i) The main function of the co-polymer is for controlled release. The co-polymer is in an amorphous form such that the polymer 'carrier' can degrade spatially homogenously, which will result in homogenous release of the encapsulated drug. An amorphous structure can also result in a more predictable controlled release rate.
(ii)

Drug Delivery System and Polymer Types	Advantages	Limitations
Microparticles		
• Biodegradable polymers • Natural polymers	• Encapsulate a variety of drugs • Sustained release can be achieved	• Burst release possible, may lead to local toxicity
Nanoparticles		
• Biodegradable polymers • Natural polymers	• Stable delivery system • Small size enables enhanced retention and permeation into tissue and tumor	• Non-specific uptake in RES

(c) Fabricating a cell/biomaterial construct for a period of time in vitro has been shown to provide a successful treatment strategy for some applications e.g. skin. The major advantage of this approach is that it ensures that a critical number of cells with the appropriate phenotype (progenitor or differentiated) are delivered juxtaposed to the biomaterial surface. This "head start" on integration means that some of the early steps of wound repair at the implant site are circumvented and that the success of the implant is less dependent upon the recruitment of endogenous cells to facilitate repair and restoration of function. It also means that some of the biological interplay between the cells and scaffold has already been established allowing a more immediate restoration of target tissue functionality.
The weaknesses or limitations of this approach include the normal caveats to a tissue engineered approach including choice of scaffold, cell type, cell seeding approach, bioreactor effectiveness and of course, cost – cell isolation, culture in GM-labs and second clinical procedure mean healthcare economics often become a consideration. The formation of a more "mature" tissue in vitro may mean that while functionally similar to the target tissue it remains difficult to integrate into the surrounding tissue e.g cartilage.

Choice of cell
A patients own cells will often have to be isolated and grown in vitro, requiring an initial hospital intervention. Allogeneic sources continue to be explored but remain less common.
Differentiated or functionally specific cells, e.g. endothelial (blood vessel), lung alveoli epithelial, kidney tubule epithelial, cardiomyocytes. Using differentiated cells means that the function of the repair target is directly replicated but these cells can be difficult to isolate in sufficient numbers (hence limiting the ability to repair certain tissues or organs) and their phenotype can be quickly lost, especially during in vitro culture.
Stem cells include adult stem cell, e.g. mesenchymal stem cells, induced pluripotent stem cell (iPS) and embryonic. These cells clearly retain the ability to differentiate into all or a subset of cell types that may be required to produce a tissue engineered treatment. However, this means that their use must be

carefully monitored so that in those cells that don't directly effect a repair, stem-like state is maintained and/or differentiation to unwanted cell types does not occur.

Choice of scaffold

Materials should contribute actively and controllably to the biological and functional components of the construct. Degradation rates should be matched to the ability of the cells to produce their own ECM; degradation by products should be nontoxic; materials should demonstrate suitable swelling or contractile characteristics. Structural and mechanical properties of biomaterials should be considered based on the required mechanical properties of the construct, ranging from rigid thermoplastic polymers or metals for strength to soft hydrogels for cell compatibility. For example, the mechanical properties of a scaffold used to support mesenchymal stem cells should match their target function – stiff to ensure osteogenesis and bone formation, whilst something more compliant for myotubes and muscle formation. However, the choice of the biomaterial components may be a compromise between biocompatibility, application specific factors and fabrication approach.

Culture conditions

Cell seeding (static v dynamic) and culture (static vs bioreactor) clearly play a significant role in the successful distribution of viable cells throughout the construct. These should aim to perfuse the construct and ensure that nutrients are delivered to all of the structure and that waste products are removed. The bioreactor may also need to be designed to provide specific mechanical and physical cues, e.g. pulsatile flow for blood vessels or hydrostatic pressure loading for cartilage formation or a liquid/air interface for the trachea. However, complex environments may be required to maintain the phenotypes of multiple cell types.

Vasculogenesis

Successful integration of constructs will often necessitate integration with the vascular system. The current inability to create vascular networks in constructs in vitro that can easily be directly anastomosed to the host is a clear limitation. This limits both the size of constructs (too big and cells in the middle would atrophy) and the differentiated phenotype of the tissue (since a period of host vascular invasion is required) to ensure functionality.

Comments: *Parts (a) and b(i) generally well answered by the candidates, marks were lost because of lack of details. Only a few candidates answered fully question (b)(ii). Several candidates didn't elaborate on the strengths and limitations in Part (c).*