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

Cribs

Question 1

(a) (The answer to Question 1a is more detailed than the candidate would have time to complete. The candidates do not have to write as much to get full marks. The summary given at the end of the question summarises the key points that would result in full marks.)

The body's response to injury can be divided into three successive phases, which occur on different time scales and serve different functions. First, within a few minutes, blood loss is limited by "***plugging***" the wound through the process of **haemostasis**. The end result is a temporary "patch" that partially restores the integrity of the wound. Over the next few hours and days, the site of injury is "***cleaned***" of debris and of dead cells left in the wake of the injury, by the process of **inflammation**. Finally, the tissue damage is ***repaired***, to the extent possible, over the subsequent days, a process that may continue for weeks or months. The three phases overlap in time and their cellular and molecular components interact with each other in a complex, orchestrated fashion to bring about wound healing.

Plugging the wound: Haemostasis

This process is triggered by the disruption of the layer of **endothelial cells** lining the blood vessel walls during the injury. This allows contact between constituents of tissue and blood that are normally kept separate. As a result, two key components of the blood, platelets and fibrinogen, accumulate and aggregate within the injury and on the biomaterial to form a blood clot. This happens within minutes of injury and involves the formation of a dense meshwork of an insoluble protein called **fibrin** in between the platelets. Fibrin is formed from the soluble precursor, **fibrinogen**, which has extensions at both ends that keep fibrinogen soluble and that need to be cleaved off to allow fibrin to aggregate. The cleavage is accomplished by an enzyme called **thrombin**, which is also found in normal blood as an inactive precursor, prothrombin.

Inflammation

Inflammation is initiated within minutes after injury, but usually becomes noticeable within about an hour. The main role of inflammation is to bring to the site of injury specialised blood cells that remove debris. Cells that can do this are called **phagocytes** ("eating cells"). The clearance of bacteria is also aided by soluble molecules found in blood, which comprise the **complement** system. Even following the introduction of a biomaterial under sterile theatre conditions the activation of inflammatory processes is observed and phagocytes are observed to populate the repair tissue.

Repair

After blood loss from the wound has been controlled by haemostasis, and debris removed by phagocytes during inflammation, there remains the task of restoring the tissue to its previous state, as much as possible. Repair processes begin during the later stages of inflammation and continue for weeks, or even months, thereafter.

The macrophages, recruited during chronic inflammation, continue to be abundant at the site of injury and assume key roles in wound repair. One important role of macrophages is to orchestrate, using messenger molecules, the repair activities required of tissue-resident cells. As the other cells start to re-grow the tissue, the macrophages clear the clot of fibrin and platelets, a process that uses their phagocytic activity to a new purpose and is referred to as **debridement**.

New tissue needs to be filled in to replace that lost to the injury and this will happen on the surface of the biomaterial. If repair has happened in concert with the local cell types then tissue specific integration will occur e.g. osteoblast deposition of bone on orthopaedic implants. For example in skin, fibroblasts in the dermis, as well as Keratinocytes in the basal layer of the epidermis, migrate sideways into the area of the injury and divide to restore the cell content of the tissue. If conditions are not right, i.e. chronic inflammation fibroblasts can overproduce collagen leading to fibrosis and inappropriate implant integration.

In summary:
Processes activated in response to biomaterial implantation include:

- Haemostasis (secs-mins)
  - Cells: Platelets
  - Proteins: Fibrin
  - Function: Plug the wound
- Inflammation (hours-days)
  - Cells: Phagocytes (macrophages and neutrophils)
  - Proteins: Complement
  - Function: remove debris, blood clot
- Repair (days-weeks)
  - Cells: tissue specific cells, fibroblasts, endothelial cells, macrophages
  - Proteins: Collagen and other ECM biomolecules
  - Function: Rebuild tissue
  - Repair and Integration may be unsuccessful. Alternative outcomes include scarring (fibrosis) and chronic inflammation.

(b) 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.

Choice of cell

A patient's 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 softer 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

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.

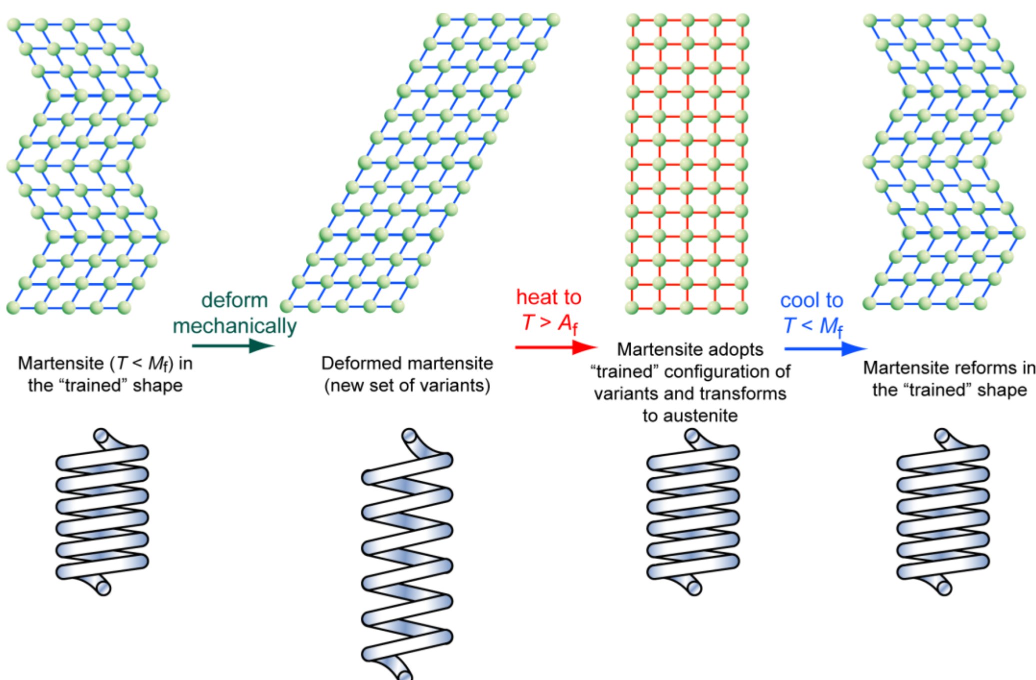
Vasculogenesis

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.

Examiner's comments: A straightforward question, well-answered by most candidates. Marks were lost when descriptions of the wound healing stages lacked detail. Most candidates provided correct timescales, but some answers lacked information on the process or the cells involved. Part (b) was answered less well. Many candidates provided general comments and didn't focus on the choice of scaffold, cells and culture conditions.

Question 2

(a) (i) Shape memory and superelastic stents come premounted onto a delivery system and constrained by a sheath or a restraining membrane. The stent is moved into place. Once it is in the treatment area, the sheath is pulled back, allowing the stent to expand to its predetermined (expanded) diameter.



The shape memory effect involves martensitic transformations stimulated by changes of temperature. They also involve a new concept - that of material being "trained" to have a preferred shape. This involves heating the specimen (while constrained into a certain shape) to high temperature (well above A_f), holding it at this temperature for a short period and then cooling it quickly to ambient temperature. Stress relaxation occurs during holding and then, during cooling (in the constrained shape), the austenite-martensite transformation takes place in such a way as to minimise the overall

shape change. There is subsequently a tendency for the specimen to adopt its "trained" shape, in which transformation between parent and martensitic phases takes place readily.
The schematic below shows how a shape memory alloy can recover a "trained" shape, after being subjected to a large (apparently plastic) strain while in the martensitic state. This is done by simply heating the specimen, so as to stimulate transformation of the martensite to austenite. This takes place (in the absence of applied stress) by the martensite first reverting to its "trained" set of variants and then transforming.
The absence of a delivery balloon results in self-expanding stents being more flexible allowing longer length to be delivered through vessels which exhibit relatively high curvature.

(ii) The early complication of stenting involves thrombosis that occurs 1 to 3% of patients within 7-10 days of the procedure. This complication is largely overcome by treating patients with anti-platelet agents such as aspirin. The major long-term complication of stenting is vessel renarrowing (restenosis), known as "Neointimal Hyperplasia", which occurs within 6 months in 30% of patients who are stented. This is attributed to post-implant injury (roughly analogous to a scar forming over an injury) and a foreign body response. The reason for developing drug-eluting coatings is to treat in-stent restenosis, which is due solely to "Neointimal Hyperplasia". This is excessive tissue proliferation due to post-implant arterial injury (roughly analogous to a scar forming over an injury) and foreign body response. To overcome this problem, approaches include drug-eluting coatings.
In drug-eluting stents, drugs are embedded in a polymer matrix that is coated onto the stent. The drug is released into the vessel by diffusion and/or polymer degradation over varying periods of time that can be engineered by the specifics of the polymer-drug system.
The main advantages of drug-eluting stents include:targeted drug delivery to precise area requiring treatment; ongoing delivery through phases of healing; no additional material or procedures required and ongoing delivery through phases of healing

(b) The femoral component (stem and 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). Femoral heads can also be made of ceramics such as Al_2O_3 and ZrO_2. Ceramics offer a low coefficient of friction and high wear resistance.
There are two types of implants fixation. Those involving the use of bone cement ("cemented") and the cementless ones. In the latter, bone-implant attachment is achieved via bone-in-growth into a rough/porous surface.

Cemented prostheses -Eldery patients

Advantages

Cemented implants are generally used for older patients, since the implant can be load-bearing within hours of the operation. Extended rest can lead to secondary problems such as bedsores.

Disadvantages

- Poor cement mixing can lead to poor mechanical properties
- Implant repositioning while the cement is curing
- Cement cures through an exothermic reaction, potentially damaging surrounding tissue
- Cement deteriorates through fatigue and biological processes – production of wear debris which can cause osteolysis (bone loss)
- There are two interfaces to contend with (bone-cement and cement-implant)

Cementless prostheses – Younger patients

Advantages

Cementless implants are normally used in younger patients, since bone requires time to bond to the coated implants. Better bone fixation to implant (direct fixation). Better long-term lifetime performance

Disadvantages

Requires more surgeon skill for implant placement

Bond with bone takes time to develop - immobilisation may be required for 5-10 weeks post operation.

Additional risks depend on the details of the porous /rough surfaces (e.g. spallation of coatings)

Examiner's comments: A very popular question, all parts generally well answered by the candidates except Part (a)(i) on the shape memory effect. Most candidates provided a lot of unnecessary information, and quite a few of them described the superelastic effect instead!

Question 3

(i) Unit molecular weight = 74. Hence n ~ 72,000/74 ~ 973. In the real product, a spread of molecular weight distribution (or a range of chain length) will be present. We use the number average molecular weight (M_n) to calculate the number of repeated units, thus the calculated n indicates the most frequently occurring chain length. Some of the chains can be much longer or shorter than n.

(ii)

M_C is the average molecular chain lengths between the cross-links.

(iii) The equation can be re-written as the following, where X = the average number of cross-links per chain, and M_n is the number average molecular weight of the chain.

$$E \approx \frac{3\rho RT}{\left(\frac{M_n}{X}\right)} = \frac{3\rho RTX}{M_n}$$

With this, we have E is directly proportional to X (or the amount of crosslinks).

E will have a lower limit when X~1, E_{lower} = 3×8.3×300/72000=0.1038 J/cm^3 ~ 0.1 Nm/(0.01 m)^3 ~100 kPa

E will have an upper limit when X = 973, E_{upper} ~97,300 kPa ~97 MPa.

(iv) The rubber elasticity equation is valid for a loose non-interacting network. We can see the Young's modulus is approximately linearly dependent on the amount of crosslinking as the theory predicts. The Young's modulus measured is within the upper and lower limits predicted by the theory.

(v) Skin graft: PDMS can be used as the top cover of a skin graft as a temporary barrier to prevent moisture loose and infection during the initial stages of skin regeneration. Its flexibility, compliance, relative bioinertness, and gas/ moisture permeability have led to its use in this application.

Bone graft: PDMS is not suitable to be used as a bone graft due to its weak mechanical properties (in comparison to bones), and neither it can offer any bio-functionality to the bone regeneration.

Examiner's comments: This was the least popular question on the paper. Parts ii-v were not answered well. Candidates were unclear as to what the average molecular weight of a network chain is, how it relates to crosslinking, and how to determine the upper and lower limits of the Young's modulus using the rubber elasticity equation.

Question 4

(a) (i) Basic chemistry—which backbone (choice of polymer class)** most important

Side chains that are hydrophobic (slower) or hydrophilic (faster)

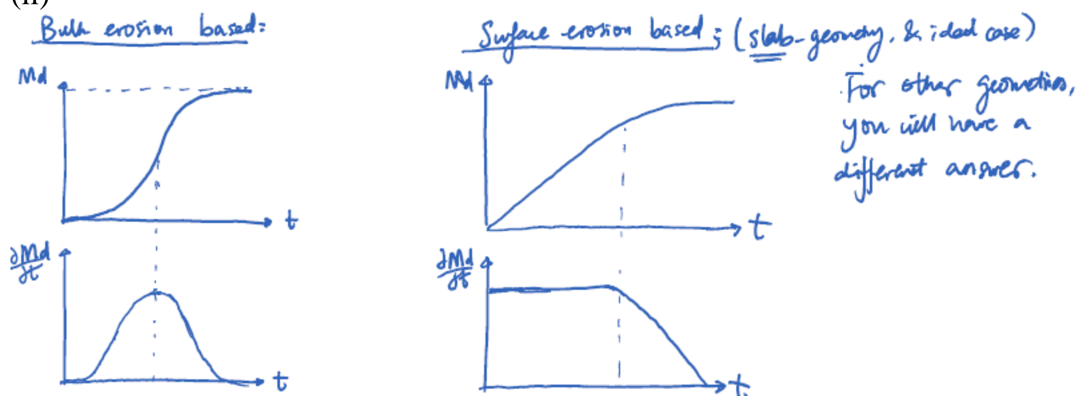
Crystallinity: packing

Glassy versus rubbery state of the polymer—rubbery state, faster reaction

Geometry of the implant/material: surface area to volume ratio, implant or coating thickness. This controls the motion of water into the material, and also determines the mechanism of dissolution: surface versus bulk erosion.

Porosity

(ii)



(iii) Surface erosion as shown by the initial periods of dMd/dt~ constant. For the design consideration, one can talk about the hydrolysis half-life of the selected material, and also incorporate the factors stated in (i) above to tune the rate of erosion, and thus the rate of drug release. In order for surface erosion to take place, the characteristic thickness of the patch has also to be greater than the critical thickness *Wc* for surface *vs.* bulk erosion.

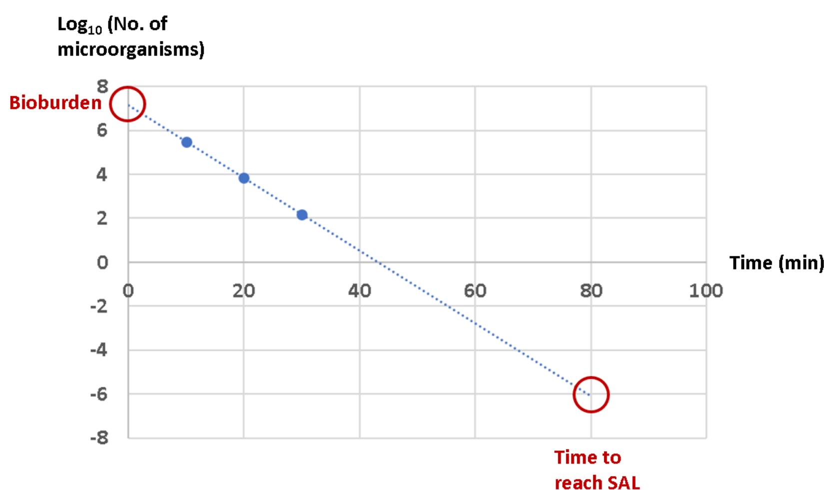
(b) (i) Sterilisation is a process used to render a surface or product free from all living organisms. Microorganisms include bacteria, viruses, fungi (moulds, yeasts) and bacterial spores. Sterilisation of medical devices normally has to be completed to a Sterility Assurance Level of 10^{-6} (1 in a million chance of product being non-sterile). Because elimination of all living organisms is difficult to demonstrate, sterility is described in terms of probability of a number of micro-organisms capable of surviving. Sterilisation usually focuses on disrupting the protein structure (denaturing) or ionisation of nucleic acids. Manufacturers have to validate successful sterilisation by complying with appropriate standards (e.g. ISO 14937).
The sterilisation techniques may include: Steam (in autoclave), gamma radiation, ethylene oxide, X-ray radiation, E-beam radiation, pulsed light, hydrogen peroxide vapour (or gas plasma).
One technique should be included in more detail and include 2-3 points on advantages and 2-3 points on disadvantages as well as a note on the mode of action.
Steam - autoclave: Saturated vapour brought to 121 – 125°C, usually 15 – 30 minutes. Energy transfer during condensation. Destroys structural components essential to replication. Denatures proteins (unfolds and coagulates).
Adv: Cheap, good efficacy, easy to control/monitor, lack of toxic residue, fast.
Disadv: High temperature/pressure, distortion, may contain contaminants, moisture absorption, limits range of packaging materials
Gamma radiation: Exposure to Co 60 approx. 25 kGy. High energy leads to sterilisation by ionisation of nucleic acids leading to cell death.
Adv: Complete product penetration, process in shipping package, immediate product release, no residue, reliable, controlled, economical
Disadv: Some plastics degrade, discolouration of some products, large capital outlay, isotope containment
Ethylene oxide (EO): Exposure to 600-800 mg/L EO at 40 – 50°C, usually 2-16 hours, sterilisation by alkylation, proteins are attacked and lose structure (hence function).
Adv: Good efficacy, process in shipping packaging, good compatibility.
Disadv: Usually quarantined 7-14 days, many process variables (vacuum, P, T, t, RH, concentration)., residues, increasing regulations / environment (maximum ppm remaining on components)
Other points that may be raised:

- X-ray radiation: Excellent penetration, can work at pallet level, unlike gamma radiation the source can be switched on and off, also no ozone build up (machine-generated radiation).

- E-beam radiation: No radioactive isotopes, in-line processing, rapid dose rate, no toxic rate, unsuitable for thick or densely packed materials, complex dosimetry, product can heat up.
- Pulsed light: Broad spectrum, including short UV, effective at killing all microorganisms but direct exposure required. Very inexpensive technique.
- Hydrogen peroxide gass plasma: The vapour is sporicidal and the activity is enhanced in combination with the plasma. Plasma breaks components down to water and oxygen. This is surface active only and needs very close monitoring in the workplace because of the toxic nature of the vapour.

(ii) Table 1 will need to be updated to include log (based 10) of the number of microorganisms. These can then be graphed against time to show the trend in decreasing numbers of microorganisms with sterilisation. Assuming a linear response, the bioburden can be found by extrapolating to time = 0 min. The time required to reach SAL can be found by extrapolating to -6 on the y-axis. The question asks the sterilisation time recommended and so a safety factor could be added to this time.
The bioburden will be close to $10^{7.1}$, and the time required to reach SAL is close to 80 min with a final recommended time of 88 - 96 min (10 - 20% safety factor).

Time (min)	No. of microorganisms	Log_{10} (No. of microorganisms)
10	3×10^5	5.477
20	6.7×10^3	3.826
30	1.5×10^2	2.176



Some answers may use a mathematical extrapolation because they know it is linear. This is also fine if the method is made clear.

(iii)

- Sufficiently broad antimicrobial efficacy
- Can you prove the compatibility of the technique with the materials (mechanical, chemical properties unchanged)
- Are there methods available to monitor and confirm that the sterilization process has been adequately applied to a load?
- Is the packaging suitable for this change in technique also?
- Can the sterilization process be shown to be in compliance to standards
- What effect will the change in sterilisation process have on the certification of the medical device. Will the product need to undergo approval again, provide additional clinical evaluation/investigation, etc.?

- Is the move safe for staff to use with minimal risks
- Is the cycle time appropriate for this product (i.e. can you respond to orders with sufficient speed)?
- What is the most economical (based on throughput, in-house control, etc.)

The candidate may also include some specific positive and negative points about these two techniques to show their understanding. For example, gamma radiation requires a very large capital outlay and instead may require working with a third party, also some polymers degrade under the ionising radiation. Ethylene oxide is potentially explosive, highly toxic and requires a 1-2 week post-sterilisation quarantine to ensure toxic residue is removed.

Examiner's comments: A popular question, well-answered by most candidates. In Part (a)ii several candidates didn't sketch the correct drug delivery profiles. Only a few candidates elaborated on the design considerations for the polymer matrix in Part (a)iii. Part (b) was answered well, marks were lost because of lack of detail.