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

CRIBS

1. (a) (i) Three out of four of the following:
1. Acid extracted collagen, crosslinking of collagen fibres lead to decelerated degradation rate.
2. Melting of collagen tertiary structures: acceleration of biodegradation rate.
3. Melting of collagen quaternary structures: thromboresistance
4. Commonly used forms (different microstructures), e.g. fibres by electrospinning, sponges by freeze-drying

(ii) Collagen can be used as a hydrogel/ scaffold to support cell growth. For implantation, the collagen quaternary structures should be avoided to prevent throboresistance; thus the collagen would be acid treated and post-crosslinked.
Collagen can also be used as a scaffold for wound addressing applications. Here, the collagen quaternary should be retained to impose thrombosis.

(b) (The answer to this question 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 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 outer boundary of the body, as well. Over the next few hours and days, the site of injury is "***cleaned***" of infectious agents and small particles that have been introduced, 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 to form a blood clot.
Primary haemostasis. This happens within seconds of injury and involves the binding of **platelets**, spilling out of injured blood vessels, to collagen exposed on tissue surfaces.
Upon binding to collagen, the platelets become activated, and the membrane changes following activation allow additional platelets to bind, forming an aggregate. Collagen is absent from the inner surface of endothelial cells, so that platelet aggregation within intact, healthy blood vessels is generally avoided. Platelet aggregation occludes the wound and slows the escape of blood from the wound, but does not stop it entirely. Platelet activation and aggregation also provide triggers for the next step in the process: activation of the coagulation cascade.
Secondary haemostasis (a.k.a. **coagulation**). This happens within minutes of injury and involves the formation of a dense meshwork of an insoluble protein called **fibrin** in between the platelets. The resultant meshwork is fine enough to stop the loss of blood and adds some mechanical strength to the forming blood clot. Together with the platelet aggregate, it also entraps other blood cells. 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. In turn, prothrombin is activated by two other molecules, Factor V and Factor X – but only if they, themselves, are activated by yet more enzymes.
The ultimate trigger that initiates the coagulation cascade, like the trigger for platelet aggregation, is the injury itself, which enables several components of blood and tissues, which are normally kept separate, to meet. One example is the binding of "tissue factor", a protein abundant in tissues but absent from blood and endothelia, to Factor VII, an enzyme precursor in blood.

Cleaning the wound: 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 engulf and kill bacteria and remove particulate matter and dead cell remnants. 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.

A key feature of the inflammatory process is the widening of blood vessels (**vasodilatation**), which facilitates access of phagocytes and complement proteins to the area. The endothelial lining cells of the engorged blood vessels become activated, allowing phagocytes to attach and migrate across into the tissue, and the boundaries between them become permeable, allowing the leakage of blood proteins into the tissue, with water following a gradient of osmotic pressure. This accumulation of protein and fluid at sites of inflammation is called an inflammatory **exudate**.

Repair

After blood loss from the wound has been controlled by haemostasis, and bacteria 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 after they have successfully cleared any remaining bacteria. 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. 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. The fibroblasts also replace the lost extracellular matrix of the dermis, secreting copious amounts of ***collagen*** and other matrix proteins. The production of collagen may be excessive and leave the repaired tissue looking different and functioning less well than healthy tissue, forming a ***scar***. The overproduction of collagen in response to injury is referred to as **fibrosis**, and occurs frequently not only in mechanical injuries, but also in chronic diseases that lead to tissue damage and in some circumstances in response to biomaterials.

In summary:

Processes activated in response to injury include:

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

Comments: *In Part (a)(ii), a significant number of candidates didn't provide details on the processing method, instead focused entirely on the application. In Part (b), marks were lost when descriptions of the tissue healing and wound repair lacked information on the process, timescales or the cells involved.*

2. (a) (i) A medical device refers to a range of physical forms i.e. a machine, apparatus, material, appliance etc. Showing this understanding through examples is also acceptable. It should be noted that a device refers to a range of different actions such as diagnosis, prevention, monitoring, treatment. It is important to note the principal action is not as a drug or pharmaceutical agent. A medical device can cover a range of categories, such as in-vitro diagnostics, active medical devices, active implantable medical devices, custom-made devices, accessories intended to be used with the device and software with a medical purpose.

(ii) The important concept to convey is that bioethics is the study of ethics applied to biosystems. However, a stronger answer should also include some additional detail to show a full understanding. For example, it is helpful to identify that "Ethics" is a system of moral values, for a broad population or society or that bioethics concerns the moral, legal, political, and social issues raised by medicine, biomedical research, clinical care and life sciences technologies.

(iii) A full answer will explain three key concepts that in the Nuremberg code, consent is discussed in terms of:

1. Communication of relevant information by the clinician and its comprehension by the patient (disclosure);
2. The patient has the ability to understand the information and to appreciate consequences (capacity);
3. The patient has the right to come to a decision freely (voluntariness).

The precise terms are not required as long as these key concepts are conveyed.

(b) (i) Standards are documented agreements containing technical specifications or other precise criteria to be used consistently as rules, guidelines or definitions of characteristics, to ensure that materials, products, process and services are fit for their purpose. They are established by consensus and approved by a recognised body that provides for common and repeated use. This is important for medical device manufacturing and standards cover how to apply quality systems: methods, facilities, controls used by the manufacturer in the design, manufacture, packaging, labelling, storage, installation, servicing and post-market handling of medical devices.

Three roles were discussed in the lectures, namely:

- To assure that products continuously meet the technical and quality specs or in order to meet customer requirements.
- To obtain these results at the lowest cost.
- To show conformity to regulations.

(ii) An excellent answer would include 4 brief descriptions and why each was introduced. There may be some repeated reasons as to why each was introduced and this is absolutely fine if accurate. Examples include:

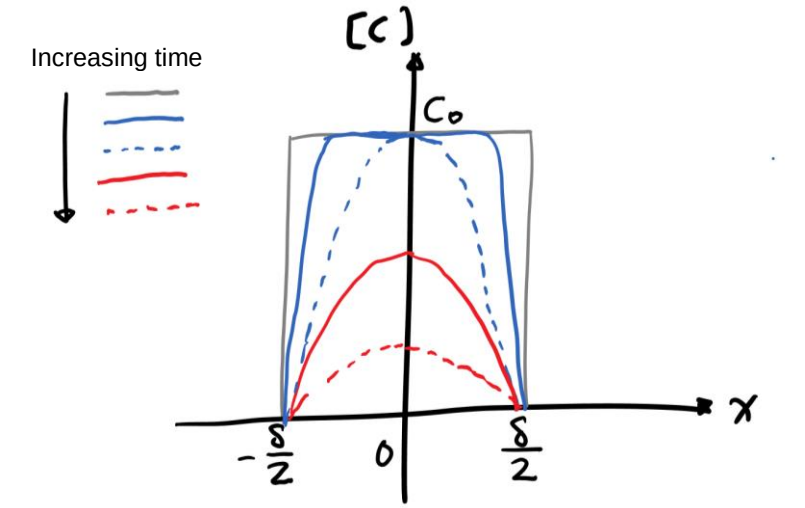
- There is a greater emphasis in new regulations on obtaining clinical trial information. Not requiring trials due to "equivalence" will be much more rigorously interpreted (e.g. Class III can no longer use this rule). This is both to provide more confidence regarding safety (e.g. report by FDA about poor safety due to previous approach) and also to take a step towards harmonisation.
- Class III medical device manufacturers must create a summary of safety and clinical performance in a way that can be understood by patients. This is again to improve confidence in safety.
- Implants for aesthetic purposes are now also covered by the regulations. This is again to improve confidence after PIP and other scandals.
- The regulations have tried to take a step towards harmonisation with FDA. Harmonisation will be important for reducing the time and cost for product development, making it easier to bring new medical devices to market.
- There needs to be a unique identifier for each device and linked information. this is to improve market surveillance and traceability.
- There now needs to be a qualified person in each organisation responsible for regulatory compliance. This is again to improve confidence in devices.
- The legislation approach was changed from that of Directives (where the Member State can define how best to implement the requirements into their own legislation, and are given significant time to adopt) to Regulations (where the legislation is directly applicable immediately in all Member States).

(c) In this example, the product has a polymer hub, an adhesive (material not specified), a metal and a thin polymer coating. These are all very different materials and material formats and the answer should recognise the importance of studying the effect of steam sterilisation on the functionality of the product.
The adjective "appropriate" should guide towards an answer about whether it is suitable for these materials. This would mean thinking about each of the materials above and identifying the effect that steam sterilisation has on each. Will the thermal treatment affect the hub, leading to any warping or change in mechanical properties? Will the adhesive be resistant to the thermal and humid treatment? Will the sterilisation be sufficient inside the needle? Will the treatment effect the thin polymer film, either leading to degradation or a change in its functional behaviour?
There may be a reference to ordering tests for hardness, melt temperature, crystallinity of polymers and other properties.
In terms of effectiveness and ensuring the duration is sufficient to sterilise, an excellent answer would note that:
It is important to validate the sterilisation technique according to the international standard. There are standards for medical device manufacturing but also for characterising the sterilising agent and the development, validation and routine control of the process.
The validation would have to happen with the final manufacturing conditions and in the actual packaging configuration.
You would find the most resistant organism for steam sterilisation (not expected to remember the name of this spore) and use this as a control substance to test the sterilisation is effective.
A brief description may also include (a) identification of bioburden, (b) identification of sterility assurance level, (c) a fractional-run sterilisation (or a description that conveys the concept), (d) an extrapolation to the duration required.
There are chemical indicators specifically for steam sterilisation that change colour when the surface has experienced the required conditions. Alternatively, test indicators could be included that contain the viable microorganisms with a defined resistance.

Comments: *All parts generally well answered by the candidates, marks were lost because of lack of clear descriptions of the concepts involved. In Part (c), a large number of candidates focused on the sterilisation process and didn't discuss the effect of steam sterilisation on the hub and coating.*

3 (a) 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.
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.
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.

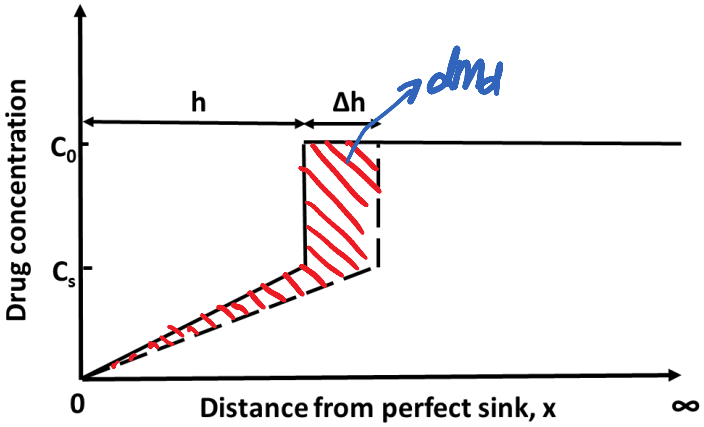
(b) (i)



- Tissue acts as a perfect sink for drugs
- Slab configuration, the dimensions in areas >> thickness (δ)
- 1D diffusion profile across the thickness
- $C(x,0) = C_0$, $C(\pm\delta/2,t)=0$, $dC/dx(0,t) = 0$

(ii) The relationship only holds for initial time points; since eventually, the bulk concentration of the drug will drop below C_s.

The shaded area (equivalent to dm_d) is obtained from subtraction of two trapezia.



- Shaded area in red is equivalent to dm_d

$$dm_d = \frac{(C_0 + C_0 - C_s)\times(h+dh)}{2} - \frac{(C_0+C_0-C_s)\times h}{2}$$

$$= \frac{(2C_0 - C_s)\times(h+dh-h)}{2} = C_0 dh - \frac{1}{2}C_s dh \quad ①$$

Here, dm_d is calculated as the amount of drug depleted. Thus, from the drug carrier's prospective, its mass is changed by $-dm_d$.

- By applying Fick's Law to the region of 0 to h,

$$J = -D\frac{\partial C}{\partial x} \quad \text{(remember } m_d \text{ is a unit area mass)}$$

$$\frac{-\partial m_d}{\partial t} = J|_{x=0} = -D\frac{\partial C}{\partial x}\Big|_{x=0}$$

$$\frac{-\partial m_d}{\partial t} = -D\frac{C_s - 0}{h-0}$$

$$① \longrightarrow \frac{C_0 dh - \frac{1}{2}C_s dh}{dt} = D\frac{C_s}{h}$$

$$\therefore \frac{(2C_0 - C_s)h}{2DC_s}dh = dt \quad ②$$

- Integrate expression ②: $\int_0^h \frac{(2C_0-C_s)h}{2DC_s}dh = \int_0^t dt$

$$h = \left(\frac{4DC_s t}{2C_0 - C_s}\right)^{\frac{1}{2}} \quad ③$$

Substitute ③ back into: $\frac{dm_d}{dt} = D\frac{C_s}{h}$

$$\frac{dm_d}{dt} = D\frac{C_s}{\left(\frac{4DC_s t}{2C_0 - C_s}\right)^{\frac{1}{2}}} \quad ④$$

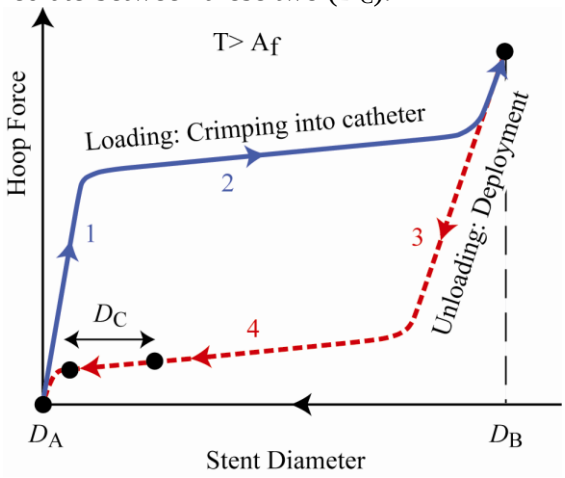
Integrate expression ④: $m_d = \int_0^{m_d} dm_d = \int_0^t D\frac{C_s}{\left(\frac{4DC_s t}{2C_0 - C_s}\right)^{\frac{1}{2}}}dt = \left[DC_s(2C_0 - C_s)\right]^{\frac{1}{2}} t^{\frac{1}{2}}$

(c) Other types include swelling based and chemical erosion based systems. The former typically utilises hydrogel materials to release drugs due to osmotic pressure. For the latter, an example material is the PLA-PGA combination which utilises hydrolysis to degrade the materials to release the drugs.

Comments: *In Part (b)(i), a large number of candidates provided an incomplete drug concentration profile (e.g. missing axis labels). In Part (b)(ii), some candidates resorted to various artifices to obtain the answer.*

4 (a) Superelasticity is the spontaneous reversal of the phase transformation when the load (externally-imposed strain) is removed, and hence recovery of the original shape.

The superelastic effect takes place at $T > A_f$. the material is in the parent phase (austenite). So the stent in the parent phase (austenite) with an original diameter D_A (higher than the relaxed diameter of the vessel) is collapsed to a small diameter D_B. Initially, there is conventional elastic straining (Regime 1), which is then followed by a superelastic plateau whereby the austenite transforms to martensite (Regime 2). The crimped stent is then inserted into a catheter. When deployed from the catheter *in vivo*, initially the stent gets elastically unloaded (Regime 3) followed then by reversal of the phase transformation (Regime 4). The stent is trying to recover its original shape but is constrained from full recovery to D_A by the lumen walls. The stent exerts an outward force on the vessel (trying to expand to its relaxed diameter) and, conversely, the vessel exerts a constrictive force on the stent. A suitable equilibrium diameter is then established with a value intermediate between these two (D_C).



(b) The shape memory effect involves the concept of "training", the material being "trained" to have a preferred shape. This is done by heating the specimen (constrained into the shape concerned) to a relatively high temperature (well above A_f), holding it at this temperature for a short period and then cooling it quickly to room temperature. Stress relaxation occurs during the holding period 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. When a portion of the lattice shears so as to form the martensite phase, there are usually several alternative directions in which it can do this – forming what are often termed different "variants" – so it's possible for groups of variants to be formed which, taken together, have a very similar shape to the original parent material. There is subsequently a tendency for the specimen to adopt its "trained" shape, in which transformation between parent and martensitic phases takes place readily. This takes place (in the absence of applied stress) by the martensite first reverting to its "trained" set of variants and then transforming.
The obvious choice for a cardiovascular stent is to utilize the superelastic effect as it does not rely on changes in temperature. In superelastic stents, the stimulus for the transformation is provided by crimping the stent onto a catheter whereas in shape memory stents, a change in temperature is required for the deformed stent to recover its original shape.

(c) One of the desirable mechanical characteristics for a stent is to have a low axial beam stiffness (high flexibility) before deployment, because this allows the stent to to be pushed through vessels which may have high curvature. Flexibility is one of the key requirements. A low value of axial beam stiffness allows high curvature to be adopted without a large bending moment needing to be applied. However, upon expansion, the stent needs to loose its flexibility and become rigid along its length in order to provide support to the vessel.
The figure shows how the axial stiffness varies with orientation angle for tubes having all members lying at the same angle (a single angle ply) or at a $\pm\theta$ angle ($\pm\theta$ angle-ply). A section of a tube in which there are only members lying at large orientation angles will have a low beam stiffness. Figure 3 shows that high angles (>50˚) lead to very low beam stiffnesses and hence to highly flexible tubes. While both tubes

have low axial stiffnesses at high orientation angles, a $\pm\theta$ angle-ply would be more suitable because a single angle ply will tend to distort under bending.

Stents with high axial beam stiffness before expansion may apply excessive local pressures causing serious damage to the vessel wall. The subsequent repair process is complex with inflammatory and thrombotic pathways being activated. Platelets become adherent to the damaged vessel wall due to loss of the protective endothelium (inner layer of the blood vessels). These changes culminate in recurrence or restenosis also known as Neointimal Hyperplasia, and the need, because of luminal renarrowing, for further intervention.

(d) Other mechanical characteristics of a stent are:

- Relatively high yielding pressure after expansion. Stents need to have sufficient structural strength after plastic deformation to retain the vessel in an expanded condition.
- Low axial contraction ratio. The axial contraction ratio is defined as the ratio of the relative decrease in length divided by the relative increase in radius. Stent wall designs can incorporate features which will tend to reduce the axial contraction ratio, which may be beneficial from the surgical point of view as limited shortening during expansion facilitates stent placement.
- High expansion ratio at fracture. The expansion ratio at fracture of a stent will depend on the influence of the wall design on the distribution of plastic strain during expansion and on the presence of any regions of low ductility.

Comments: *In Part (b), several candidates explained the shape memory effect instead of the concept of "training". In Part (c), only a small number of candidates recognised that a single-angle ply would have very low resistance in bending after full expansion. In Part (d), a number of candidates described functional characteristics rather than mechanical.*