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

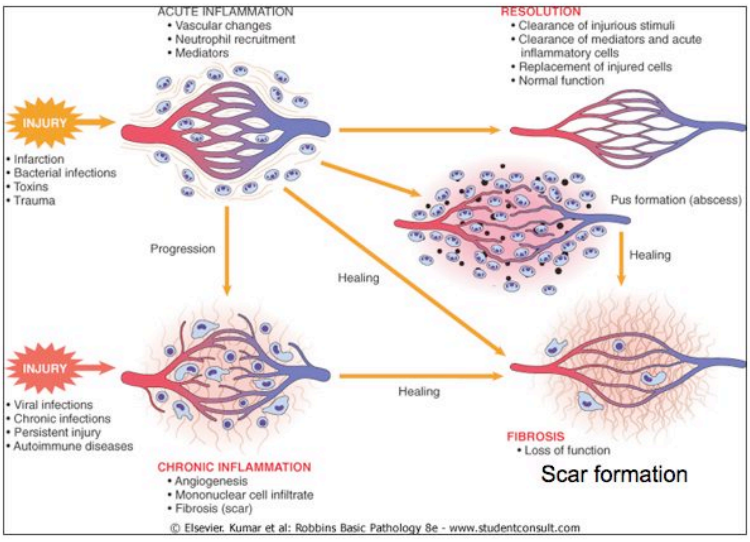
CRIBS
Question 1
(The answer to Question 1 is more detailed than the candidate would have time to complete. The candidates do not have to write as much to get full marks.)
(a) The body's response to injury can be divided into three successive phases, which occur on different time scales and serve different 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.
(i) 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 (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.
(ii) 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**.
(iii) 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**.

In summary:Processes activated in response to injury include:

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

(b) The ideal outcome of all this repair activity is the full ***resolution*** of inflammation with complete removal of bacteria and dead cells and full restoration of the tissue to its previous, functional state. This is not an infrequent occurrence. During bacterial pneumonia, for example, lobes of the lung may fill up completely with neutrophils, pus and bacteria, but be completely cleared weeks later. However, resolution is by no means the only possible outcome. The injured area may be left with an abundance of pus, comprising dead neutrophils and bacteria, which may be slow to be cleared by macrophages, accumulating locally as an ***abscess***. If bacteria are not removed effectively, or if other processes perpetuate the initial tissue damage, inflammation may become ***chronic***, and persist indefinitely alongside repair processes e.g. response to orthopaedic wear debris. This often results in ***Fibrosis***, the replacement of normal tissue by scar tissue with excess collagen and few functional cells. It may arise either due to imperfections during the attempts of the tissue to repair the initial injury; the scar tissue may then continue to be re-modelled by macrophages and fibroblasts and a more nearly normal tissue structure restored gradually over weeks and months. Alternatively, fibrosis may accompany chronic inflammation or wall off an abscess or foreign material persisting at the site of injury. If the regenerative capacity of the tissue becomes sufficiently exhausted, fibrotic changes may become irreversible, and the normal function of the tissue lost permanently.



2 (a) Combining bioactive molecules with a biomaterial therefore provides an opportunity to influence the success of the host response and ameliorate unwanted tissue remodelling such as fibrosis.
The most obvious way to combine bioactive with a biomaterial is to simply coat it onto the implant surface. Many of these active molecules are water soluble and would hence be quickly lost from the surface and therefore strategies have been developed to trap the therapeutics at the surface and slowly release them to exert their effect. Active agents are entrapped in an inert hydrophilic/hydrophobic polymer matrix and controlled drug elution from days to months is achieved through the use of polymer formulation (blend). These include combinations of Polyurethanes, Polyvinyl pyrrolidones (PVP), Methacrylates (HEMA, PMMA), Cellulose esters and related copolymers. Other variables that influence drug solubility include: drug-to-polymer ratio and coating thickness. In diffusion-based systems, the polymer and drug can be homogenously mixed with the drug passing from the polymer matrix into the external environment. The release rate generally decreases since the drug has a progressively greater distance to travel. Examples of applications where drug or biomolecule coatings have been used include: Thrombosis - Anti-thrombogenic agents (Heparin complexes) e.g. Expanded Polytetrafluoroethylene Vascular Graft; Infection - Antimicrobial agents (including antibiotics and germicidals) e.g. silver and chlorhexidine impregnated surgical patch; Tissue Overgrowth - Anti-cancer agents (paclitaxel targeted cancer treatments) and loosening - Scarring Agents e.g. paclitaxel stent.

Whilst coating materials with bioactive agents is one strategy to influence the biological response, perhaps a more exciting opportunity is to make bioactive molecules and motifs an integral part of biomaterial design. Many of our tissues and organs can be considered to be essentially a hydrogel mass and as we know, intrinsic repair is mediated through the formation of a hydrogel (fibrin based network) provisional matrix. It is therefore of little surprise that engineered hydrogels have received considerable interest. For example, protein motifs that play a role in cell adhesion or matrix elasticity can be expressed in bacteria, purified and added to a polymer backbone to create scaffold building blocks. These elements can then be combined through self assembly or chemical cross linking to create a hydrogel network to facilitate tissue repair. An example of this approach is a crosslinked gel that both traps a soluble drug to influence cell activity and also includes within its structure Vascular Endothelial Growth Factor (VEGF), a powerful regulator of angiogenesis. When VEGF is incorporated into a fibrin matrix but allowed to diffuse freely, angiogenesis occurs both in the matrix and elsewhere. When an engineered form of VEGF is covalently coupled to the fibrin matrix, cells migrate into the matrix, cleave the coupled growth factor, and release it on demand, resulting in a much more localized response.

(b) Nanoparticles are particularly attractive in the development of novel biomaterials since they offer the opportunity to package a number of molecular components (drug/biologic/targeting molecule/reporter) in a highly controlled and durable way. In particular, bioactive molecules and drugs have the potential to be released in a time dependent manner and their action localised to the cells and tissues where needed. This means that nanoparticles may be able to influence and fine tune the tissue response to an implanted material. In addition, the next generation of nanoparticles aim to not only deliver drugs to the target cells but also feedback the state of the disease or injury and the success of the intervention (tissue integration and repair).

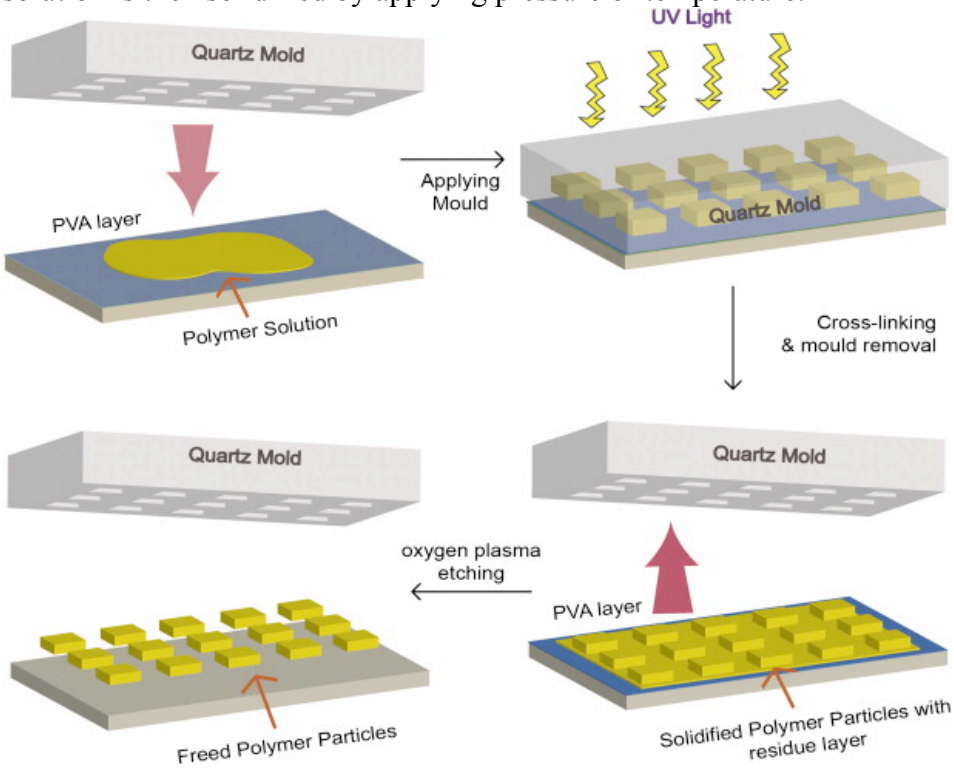
(c) *Description of any 2 of the methods described below will be sufficient to get full marks* - Conventional nanoparticle fabrication method include *nanoprecipitation* where polymer dissolved in organic solvent is added to an aqueous solution in a dropwise manner under constant agitation. Nanoparticles containing drugs form instantaneously as the polymer diffuses to the aqueous phase. Layer-by-layer assembly is performed using the solid form of a drug as the core. A polymer layer is first adsorbed onto the drug colloidal template by incubating in polymer solution and transferred to

the oppositely charged polymer solution for additional layering. This process is repeated until nanoparticles of desired sizes are formed.

In emulsion-based two-step methods, emulsified oil-in-water droplets containing polymer and drugs are formed in the first step. In the second step, different methods are applied to remove the solvent and precipitate nanoparticles. Using solvent evapoaration methods, the solvent is gradually evaporated under vacuum and high pressure. With the solvent diffusion method the solvent used to prepare the emulsion drops is partially miscible with water. When the emulsion droplets are diluted with water containing stabilizer, organic solvent rapidly diffuses out from the droplets, leading to condensation of the materials within and formation of polymer nanoparticles. With a salting out approach the solvent used to prepare polymer and drug solution is totally miscible with water. Emulsification is conducted with aqueous phase containing high concentration of salt. The saturated aqueous phase prevents solvent from mixing with water. The emulsified droplets are then diluted in water. A sudden drop of salt concentration in continuous phase causes extraction of organic solvent and precipitation of polymer drug nanoparticles.

Microfluidic based systems have also been developed to combine different elements of the drug delivery system. Common types of microfluidics design include flow focusing, T-junction and concentric capillaries. During hydrodynamic flow focusing, precursors self-assemble into nanoparticles when precursor-solvent solution is mixed with buffer, in which the precursor is poorly soluble. The process occurs in three stages involving nucleation of nanoparticles, growth through aggregation and stabilization. A core-shell structure can be achieved through sequential encapsulation which gives a double emulsion after which the shell is solidified to produce a shell layer with a liquid core.

Top down approaches to the creation of drug delivery systems include: *Particle Replication In Non-wetting Template (PRINT)*. A non-wetting PFPE mould with cavities of predesigned patterns is pressed against a polymer solution deposited on another non-wetting surface. The liquid polymer solution is then solidified by applying pressure or temperature.

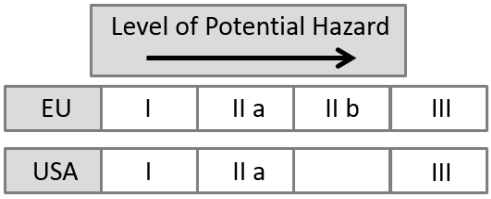


Step-Flash Imprint Lithography (S-FIL). A quartz mold with cavities of predesigned shapes is pressed against a photo-crosslinkable monomer solution on top of a silica wafer. A PVA layer is put beneath the polymer solution for the release of imprinted particles. The monomers are crosslinked by applying UV light. Residual layer from the crosslinking reaction is removed by oxygen plasma etching and particles are freed by dissolving away the PVA layer.

3 (a) (i) Sterilisation is a process used to render a surface or product free from all living organisms. The Sterility Assurance Level (or SAL) is the probability of a single viable microorganism occurring on an item after sterilisation. The sterilisation technique needs to deliver to this accepted level. The generally used SAL is 10^{-6} (one in a million chance that the material or product being sterilised is in fact non-sterile).

(ii) Bulk erosion: MW decreases. Materials geometry is constant. The solvent we talk about in the lectures is water because of the biological systems most often discussed. Water diffuses through material faster than the degradation of the polymer can occur, so it firstly diffuses in and then the entire geometry starts to hydrolyse, breakdown and erode.
Surface erosion: MW remains constant. Material volume decreases. Diffusion of water through material is at a lower rate than the degradation of the polymer. This means that the polymer starts to hydolyse or chemically degrade on the external surfaces before significant diffusion takes place.

(iii) Medical devices are classified based on the device risks and the vulnerability of the human body to the use of the device, with Class I being the lowest risk and Class III being the highest risk. This may be illustrated with a diagram:



Examples based on the European classification are:

- Class 1: Non-sterile dressings used to aid the healing of a sprain, corrective glasses and frames, stethoscopes for diagnosis, walking aids.
- Class 2a: Antistatic tubing for anaesthesia, arterial blood filter, device to manage the micro-environment of a wound, hearing aids.
- Class 2b: Haemodialysis, insulin pen, tracheal cannula.
- Class 3: Vascular stent, cardiovascular catheters, total joint replacements.

Classification is self-assessed. In the EU this is carried out with reference to 18 rules defined in the Medical Device Directive and Guidance Document. In the US, the FDA uses a product classification database and so the self-assessment first step is to search by precedence.
The first and most important reason for concern is the patient. With an increased risk, the greater reassurance that needs to be given. Secondly, this classification system helps the manufacturer understand the level of control and costs. Thirdly, the classification helps regulatory bodies see which medical devices they should focus their efforts on.

(b) The candidates should identify and explain at least four points relating to: Polymer chemistry (backbone, side chains), tacticity, level of crystallinity, molecular weight, molecular weight distribution, level of cross-linking.
Other valid answers will of course be accepted. Some students may decide to examine the glass transition temperature, which is indicative of certain properties or explore the idea of intermolecular bonding differences, for example.
Molecular weight (MW) should be linked to the chain length (not necessarily linear chains!). Small chains are torn apart easily. Long chains become entangled and are difficult to unwrap with all of the intermolecular forces. The answer may also note points such as modulus increases with MW, which then plateaus (not expected to explain plateau), strength increases with MW, viscosity increases with MW, melting temperature increases with MW, glass transition temperature increases with MW.
Molecular weight distribution is also critical. It is recognised that the molecular weight can drive a change in mechanical properties (above) and so a clear understanding that a change in the number of

different chain lengths present will also lead to different properties. Polydispersity index was covered as an indicator of distribution of lengths.
The level of crystallinity will influence the mechanical properties significantly and so each batch should be within a specified range. Crystallinity leads to higher modulus and strength, and lower extensibility. Linear polymers are most easy to crystallize. Network and branched polymers are typically amorphous and it is possible that the batch was contaminated with different structures.
Tacticity is relevant also as it can change the level of crystallinity due to ease of packing. It was noted that atactic was less likely to pack easily into a crystalline form.
The degree of cross-linking is important because cross-links pull network back to position when undergoing strain. Also the bulk polymer is not softened by heat and has high elasticity.

(c) (i) There are a significant number of steps but they were covered in lectures and the examples class question and so detailed answers are expected.
A. It is important to decide where the product is intended to be marketed. The regulations differ significantly between EU and US (these are the examples discussed in class) and so this is an important consideration. The EU regulations were covered in most detail and are likely to be the ones included in the answer.
B. The appropriate directive needs to be considered from the examples given:

- Active Implantable Medical Devices (90/385/EEC)
- The Medical Devices Directive (93/42/EEC)
- In Vitro Diagnostic Medical Devices (98/79/EC)

C. As referred to in the first question, classification needs to be determined. This question asks for details so if this is chosen as a step, they need to include why classification is important. E.g. greater the risk, the greater the reassurance needs to be given.
D. The Quality Management System needs to be implemented. It will be important for the student to note that the standards (most likely ISO13485) need to be re-examined and followed. Quality system requirements result in Good Manufacturing Practice (GMP), reducing non-conforming products.
E. This is a significant change to a Class III product. A Design Dossier needs to be produced and clinical evaluation will be needed. (Evaluation: assessment and analysis of clinical data to verify the clinical safety and performance of the device.) The student does not need to provide a decision if new trials will be needed but should note that it is highly likely. It is likely they will also note here that performance is the key measure of success in the EU, rather than effectiveness.
F. A Notified Body needs to be selected to audit the manufacturing of the product. The MDD requires the Member States to notify the Commission and other Member States of the bodies they designate for carrying out the conformity assessment procedures. As discussed in class, these are private-sector bodies.

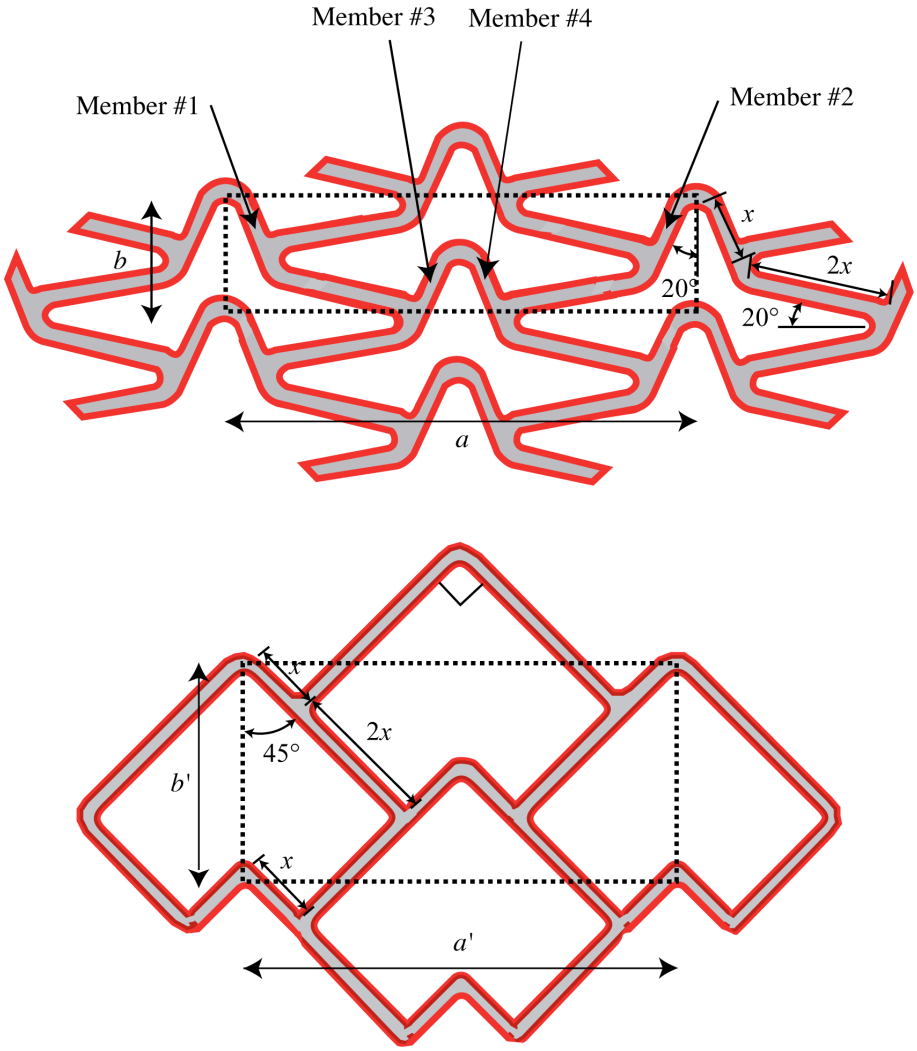
(ii) The students may think here about biocompatibility tests, which are covered in the lectures and examples paper. Cytotoxicity studies were described and especially identification of the different exposure routes and how to simulate them.
Direct contact with a material, diffusion of components from a material into a liquid, indirect exposure (exposure to a liquid that was in contact with the material).
Cells are watched in culture for a limited period of time, i.e. 3 days and thus the results are indicative only of short term implantation. Tests need to relate to end use also (short or long term). Diagram in lecture is also acceptable way to explain the different exposure simulations. Outstanding answers will give examples of when biocompatibility problems let to serious adverse events in medical devices.

Another test that has to be carried out is validation of sterilisation. The lectures discussed the process of using measurements to calculate the appropriate sterilisation of a product. Sterilisation measurements need to demonstrate broad spectrum antimicrobial efficacy and that the sterilization

process is in compliance to standards or guidelines. This usually means identifying the most resistant organism and using this to test the sterilisation.
Tests on the product properties or functionality after sterilisation may be noted also because sterilisation techniques may have a negative effect on the polymer.

4 (a) A stent is an expandable tube used to open and widen blocked or occluded vessels. These narrowed regions are made of fatty plagues that restrict the movement of the blood to the tissue, resulting in pain to tissue breakdown in several cases.
They can be classified into two main groups on the basis of the method of expansion: *balloon expandable* and *self-expanding stents*. Balloon expandable stent arrive premounted on a balloon anglioplasty catheter. While mounted, the stent is moved into place and the balloon is inflated to expand the stent to the desired diameter. Self-expanding stents come premounted or sheathed. Once deployed to the treatment area, the sheath is pulled back, allowing the stent to expand to its predetermined diameter.
Balloon expandable stents expand by *plastic deformation* by an angioplasty balloon while self-expanding ones use "*superelasticity*" or the "*shape memory*".
Balloon expandable stents are manufactured primarily from 316L austenitic stainless steel. Tantalum (Ta), cobalt-chromium (Co-Cr) and cobalt-platinum (Co-Pt) alloys have also been used.
Self-expanding stent are made from an equi-atomic alloy of Ni and Ti, known as Nitinol.

(b) (i) The procedure involves an incision in the patient's groin and then a guidewire is inserted through the aorta (largest artery leaving the heart) to the arteries surrounding the heart. A catheter is then inserted along the guidewire. Once the catheter is in place, a small amount of contrast material (dye) is injected through the catheter and is photographed with an X-ray as it moves through the heart's chambers, valves, and major vessels to find the blocked vessel. The doctor will move the catheter into the point of narrowing in the vessel. The catheter contains a balloon, known as a balloon angioplasty catheter, the balloon is then inflated to open and widen the blocked vessel, pushing the plaque against the wall.
The main criterion in deciding on the placement of a stent is the outcome of balloon angioplasty. If the vessel collapses after balloon angioplasty, then the surgeon will place a stent.
(ii),(iii)



$$a = 2(\cos 20 \cdot 2x) + 4(\sin 20 \cdot x) \sim 5.13x$$

$$b = \cos 20x + \sin 20 \cdot 2x \sim 1.62x$$

$$b' = \cos 45 \cdot 3x + \cos 45 \cdot x \sim 2.83x$$

$$\frac{b' - b}{b} \sim 0.74$$

(iv) The main advantages of this stent wall design is its low axial beam stiffness (high flexibility) before expansion and its low axial contraction ratio. In the unexpanded form, an enhanced flexibility (low axial beam stiffness) is obtained by the introduction of these high-θ members (members #1 & #2 and #3 & #4). The beam stiffness of these sections of the stent is low. However, as the stent expands, these flexible segments form part of the rigid expansible structure. The two oppositely-inclined high-θ members (vertical loops) rotate to smaller θ as the low-θ members (horizontal members) rotate to a greater θ, the whole forming together an interlocking rigid latticework structure. The combination of vertical loops and horizontal loops results in minimised foreshortening i.e. low axial contraction ratio.

From a surgical point of view, a low axial contraction is desirable as it allows precise positioning of the stent. A low axial beam stiffness (high flexibility) before expansion is highly desirable since this eases passage of the stent through tortuous vessels. 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.