Q1

(a) Type I (or juvenile) diabetes occurs when there is autoimmune (i.e. self-inflicted) destruction of insulin-producing beta cells of the pancreas. The subsequent lack of insulin leads to increased blood glucose (hyperglycaemia). The classical symptoms are frequent urination, increased thirst, and increased hunger but simultaneous weight loss. It is fatal if not treated with exogenous insulin.

Two current technologies used in diabetes care: (i) glucose sensing devices, either external or implanted, to measure the current blood sugar, followed by insulin injection; (ii) insulin pumps for drug delivery.

Glucose sensing has dominated the biosensor literature and has delivered large commercial successes to the field. Oxidation of the glucose in a blood sample is catalyzed by a fungal enzyme (glucose oxidase) and the hydrogen peroxide H_2O_2 is the target of either electrical or calorimetric quantification.

The development of convenient hand-held glucose biosensors for one-shot measurements of glucose in a pin prick of blood have been a major help to diabetic patients, but further improvements are essential. Two principal avenues of potential improvement: (i) implantable subcutaneous glucose electrodes, that allow for continuous monitoring, and (ii) minimally invasive or non-invasive instruments for glucose measurement.

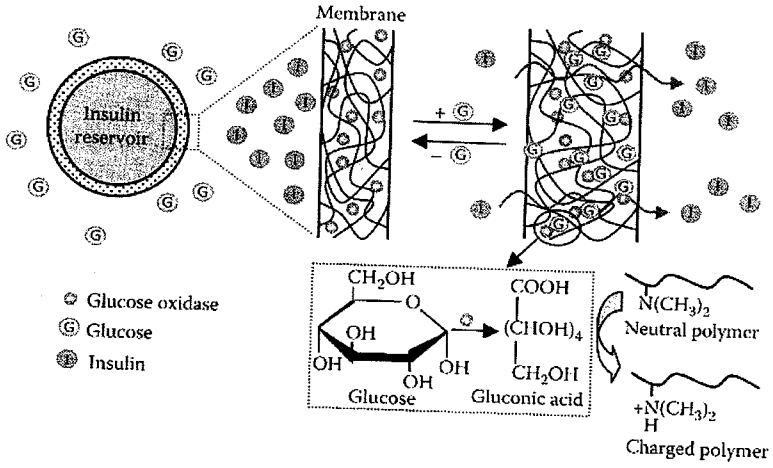
For implanted glucose sensors, microfabrication technology has aided in the design of enzyme electrodes that can be inserted under the skin, typically in the abdominal area. A monitor attached to the patient receives a measurement from the biosensor every ten seconds and stores an average glucose value in its memory once every five minutes. This implantable sensor has a lifetime of up to three days. An alternative non-invasive approach uses reverse iontophoresis to extract glucose from skin tissue and to measure amperometrically the hydrogen peroxide resulting from oxidation of the glucose in the presence of glucose oxidase. In this case, the instrument is in the form of a wrist watch providing automatic readings up to three times per hour for as long as twelve hours.

Insulin pumps have been introduced as an alternative to multiple daily injections of insulin and have been growing in use, especially in people who exhibit poor compliance with blood monitoring and injections, such as diabetic children. The modern pumps are the size of a pager, controlled by microprocessors, and pump different insulin concentrations for daytime, night-time and mealtime. Although one commercially available pump has a continuous glucose monitor on the same device, the two components are not currently allowed to communicate and thus current insulin pumps are open loop.

(b) The tip of the finger can become callous and sensitive following repeated pricking with a lancet for measurements using an external glucose-monitoring device, potentially requiring the use of a different device that can use blood sampled from another location. Subcutaneously implanted glucose sensors trigger a local inflammatory and wound healing response, which results in a fibrotic layer that covers the implant and impairs diffusion of glucose to the sensor after a few days, thus rendering the implant non-functional. A similar fibrotic response over a similar time-span impairs the subcutaneous delivery of insulin from an implanted insulin pump.

(c) *Active hydrogels for drug delivery*

Insulin reservoirs are contained within a membrane that has an affinity for glucose molecules. When excess glucose is in the local tissue fluid, it binds to the hydrogel membrane, which then swells. The increased permeability leads to insulin diffusion through the membrane and into the tissue. When the local glucose concentration decreases, the bound glucose diffuses back into the tissue environment and the corresponding membrane shrinkage prevents further insulin release. Thus the glucose sensing and insulin delivery functions of the islet cells are accomplished by a single device, instead of two separate devices. The membrane material must be biocompatible and reliable in performing this function. Since the insulin reservoir is limited in size, the capsules are unlikely to be present for long dwell-times in the body (new ones need to be delivered periodically) so the biocompatibility requirements are less than for that of a device implanted for longer time-frames. Some variability in reliability of each device can be tolerated since the overall effect is achieved by large numbers of microcapsules being deployed at any one time. This is a significant improvement over a single (and separate) device for each function.



Cell transplants and encapsulated cell transplants

Islet cell transplants may trigger adaptive immune responses that lead to the immune rejection of the transplanted cells unless successfully managed by immunosuppressive drugs, which can be toxic and leave a person susceptible to infection (not to mention at some risk of tumor development).

For this reason, islets for transplant are sometimes encapsulated with a hydrogel material such as alginate (a sugar found in some algae). Encapsulation of islets may impair the function of the islets themselves by lack of biocompatibility or impaired access to nutrients or growth factors; moreover, the encapsulating material itself may trigger inflammatory (innate immune) or allergic (adaptive immune) responses. An optimal encapsulation material remains to be identified, but would have to satisfy these constraints, as well as allowing release of secreted insulin and providing an effective barrier to immune cells and molecules.

Examiner's Comments:
Descriptions of device operating principles lacked detail (e.g. of chemicals involved). Part (c) specified discussion of the biological response, but many diverted into a standard commentary on ethical issues. Some discussed whole organ transplant, rather than implant technologies, and few mentioned hydrogels (possibly due to the overlap with Q2).

Q2

(a) Hydrogels are 3-dimensional networks of hydrophilic polymer chains that do not dissolve but that swell substantially in water. Examples include gelatine and PEG.

Swelling ratio: $R = (W_s - W_d)/W_d$, where W_s = swollen weight, W_d = dry weight

Determinants of the swelling behaviour:

- type and concentration of monomers
- cross-link density
- temperature
- pH
- ionic strength

Hydrogels have excellent biocompatibility because of the high water content and soft surface characteristics. They can also be environmentally-responsive, e.g. to changes in pH, temperature, light, or chemistry. Swelling behaviour largely determines the gel mechanical strength and permeability (related to diffusivity). Hydrogel materials can be 80% or more water, such that they have relatively poor mechanical properties and this is the key limitation for their use in biomedical applications.

(b) Hydrogels are typically used in the context of swelling-controlled systems for drug delivery. Swelling-controlled systems are particularly useful when the diffusivity of the drug in the polymer is very low. Water enters the pore spaces in the polymer, opening them up (causing swelling) and the swelling enhances drug diffusion.

This behaviour is controlled by two competing diffusivities:

(i) Diffusivity of drug in the polymer (as in diffusion controlled systems, above)
(ii) Diffusivity of water in the polymer

Semi-empirical expression for the drug release: $M_t/M_0 = kt^n$
where M_t is the amount of drug released at time t, M_0 is the total amount of drug, k and n are constants.
If there is only Fickian diffusion, n = 0.5 (the Higuchi limit): $M_t/M_0 \propto (Dt)^{1/2}$

In swelling-enhanced diffusion, the diffusion constant for drug in the polymer can be approximated to increase linearly with time, as $D = D_0 + kt$ such that

$$M_t/M_0 \propto ((D_0 + kt)\, t)^{1/2} \approx k' t \quad (\text{for } kt >> D_0)$$

where k' is a different constant. Thus the difference between diffusion-only drug delivery systems and swelling-enhanced systems is the shift from kinetics with square root of time to kinetics that are linear in time.

(c) (i) Nanoparticles are characterized according to:

- physicochemical properties (particle size and size distribution, surface area and surface area to volume ratio, surface electrical charge, shape, surface functionality, and aggregation state);
- toxicity and immune response in *in vitro* biocompatibility assays (e.g. cultures in an incubator);
- toxicity and immune response in *in vivo* biocompatibility assays (e.g. in the body, as in an animal).

Targeting is the process of delivering a drug locally instead of systemically. In *passive targeting*, nanoparticles accumulate near tumours due to their size: tumour tissue has greater permeability and these particles would be excluded from normal tissue. In *active targeting*, molecules are conjugated to the nanoparticles, such as antibodies seeking antigens that are only expressed in the tumour tissue.

(ii) PEG is polyethylene glycol, a non-ionic polymer with a repeating CH_2-O-CH_2 backbone, and it is highly hydrophilic. PEGylation is the process of attaching PEG molecules to something else, to take advantage of the unique properties of PEG. Because it is so hydrophilic, attaching PEG to an otherwise less-biocompatible entity such as a nanoparticle, can make the entire complex more biocompatible and more efficient in terms of drug delivery.

(iii) Prior to sale of any implant, there is a process of regulatory control that varies depending on the country, although the general conditions are similar. The implant is typically developed in a research environment, where studies are done in vitro and in animal models to assess the biocompatibility and efficacy of the implant. The implant is then brought into limited clinical trials, to test the performance of the implant in a human context. This is regulated closely, e.g. in the US, the FDA has to authorize an "investigational device exemption" allowing the device to be implanted into humans without it having been fully approved yet. There is an important review of ethical issues for any implant before it is used in the human body. Success in limited clinical trials leads to more extensive clinical trials, and eventually an application to the appropriate regulatory authorities prior to clearance for sale.

The process is different in the US and the UK in both philosophy and in details of execution. The duration and rigour of the examination process for the implant depends on the risk it presents to humans, and the existence of a comparable product in the approved implant market. In the US there are three categories in order of increasing risk: class I, class II and class III. In the EU, class II is subdivided into class 2a and class 2b. Long term implants, as would be expected for a tissue-engineered replacement, are high risk (class III); further, there is little precedent for the approval of such implants such that the approval process will be more rigorous than for an established class of medical device.

The philosophical difference between the US and EU is that in the US the implant must be proven to be efficacious (benificent) while in the EU the emphasis is on safety and process control (non-malificence). In the US, federal regulations require the governmental body (FDA) to give the approval for all implants for sale, while in the EU authority is not centralized but is delegated via voluntary standards to a "notified body" which is an independent and private organization with authority to grant the CE mark.

For a nanoparticle-based drug-delivery system, there is the added complexity that it is considered to be both a medical device AND a drug. Typically there are separate procedures for the consideration and approval of devices and drugs, but modern medical devices increasingly include elements of both. There is currently no single regulatory framework for such cases and they are handled on a case by case basis.

Examiner's Comments:
Descriptions of hydrogels and nanoparticles lacked depth, and were occasionally inter-changed (for which modest credit was given). Kinetic discussion was poor, with some writing down Fick's Law from the Databook and no more. Standard discussion of ethical issues was done well.

Q3

(a) Sterile (definition): the complete absence of all micro-organisms, including bacteria, yeasts, moulds, viruses (i.e. target of zero micro-organisms on every implant/piece).
In practice we are dealing with the probability of there being any micro-organisms on a group of pieces; typically we aim for no more than one in a million parts to be contaminated.

Sterilization techniques—most used:
Physical sterilization: heat
Autoclaving: steam at elevated temperatures (121-125C) and pressures.
Commonly used for metal implants and surgical tools (high T can't be used on polymers).

Radiation: Gamma rays (from $60Co \rightarrow 60Ni + \gamma$)
Most effective and most common alternative.
Disadvantage: large costs of the infrastructure to build the equipment to house radiation. Also can't be used on Teflon (PTFE), which is often used to make implants "slippery" to prevent adherent tissue

Chemical sterilization: Ethylene oxide (EtO) gas
Low T process, good for polymers, OK for Teflon, although it can be difficult to get it to permeate into "nooks and crannies" of the parts.
Disadvantage: toxic and carcinogenic, particularly problematic since it can be absorbed by plastic parts; there are also significant environmental concerns.

Sterilization techniques—new:
Radiation: X-rays
Same energy spectrum as γ-rays but made by an X-ray generator machine instead of a decay reaction.
Electron- beams are also machine-generated radiation with a different energy spectrum and lower penetration into the implant.

Other chemicals are being investigated, such as ozone and hydrogen peroxide (but with disadvantages from an environmental perspective).

(b) The immune system has two facets: innate immunity and adaptive immunity.

Innate immune responses employ soluble factors (such as the complement components) and phagocytes as a rapid line of first response against bacteria. Important mechanisms of action involve the direct lysis of bacteria by complement and their clearance by phagocytosis: the process by which phagocytes (macrophages or neutrophils) engulf and destroy bacteria.

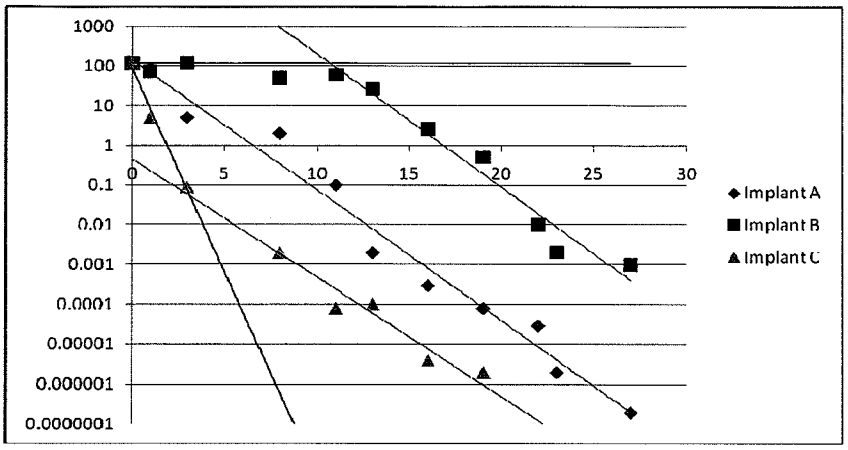
Adaptive immune responses enable delayed but highly specific defences against invading pathogens. These are based on the recognition of unique structures on the pathogen—antigens—by lymphocytes (T and B cells) bearing a clonally variable antigen receptor that binds the antigen. The antigen-specific B cells produce antibodies, soluble factors that bind to the bacterium itself or to bacterial toxins that can remain on an implant even after "sterilization" and killing of the bacteria. Activation of T cells and binding of antibodies allows innate immune responses to be amplified and focussed on the destruction of the bacterial pathogen.

(c) The sterility assurance limit (SAL) is the number of allowably contaminated parts following a sterilisation process. The target is normally 10^{-6} typically for a "one in a million" scenario. There is never a guarantee that every single part will be sterile, there is always some finite limit. The "decimal reduction time" D_T is the amount of sterilization time required to reduce the number of micro-organisms on the implants by a factor of 10.

A plot of the data (below) with a log-linear scale suggests that the number of micro-organisms decays exponentially with time on implant A. However, the relationship is more complex for implant B (NB implant C was not considered in the question). It appears that the micro-organisms on implant B decay exponentially only after an initial heating time of 10 minutes.

Lines of best fit can be approximated on the log plot and decimal reduction times can be estimated. The process times can then either be calculated using the decimal reduction times or by extrapolating the lines of best fit.

For a SAL of 10^{-6} the number of organisms needs to decay by 8 decades in total ($\log 120 = 2.08$). Following the ten minutes with no decay for implant B, the decimal reduction time for both implants A and B is approximately 3 minutes (from best-fit lines).



Process times assuming no error or variation in measurements:

Implant A: approx. 24 minutes (8 decades × 3 minutes).

Implant B: zero reduction for the first 10 minutes, then approx. 24 minutes of decay (similar to implant A: 8 decades × 3 minutes). Total: 34 minutes.

Process times with a factor of safety

The plots do not take into account population variations or measurement errors. Factors of safety of around 10 minutes (just over 3 times the final decimal reduction times of each of the implants) should be added to each time. Therefore, appropriate process times are: Implant A: 34 minutes; Implant B: 44 minutes.

Reasons for different trends

The organisms on implant A decay exponentially, suggesting that all of the organisms on the implant have equal heat sensitivity. The organisms on implant B do not initially decay. This suggests that they have a protective coating which much first be degraded by the heat and pressure.

Examiner's Comments:

Popular question with high average mark. In part (b) some candidates trotted out the full immune response to healing of a wound, which was not the question. Many commented that the difference in response of the two implants was simply that the organisms were different, without saying in what way they might differ.

Q4

(a) Nanoscale: bone is a composite of 30% (dry weight) mineralised collagen fibrils surrounded by 70% calcium phosphate.
Micron scale: collagen fibres arranged either as a block of randomly oriented woven fibrils or wrapped into densely packed, concentric lamellar structures known as osteons (cylindrical structures aligned with the long axis of the bone).
Macro scale: two types: *cortical bone*, dense (5-10% porous), tightly packed lamellar or woven structure; *cancellous* (or *trabecular) bone*, highly porous solid (50 to 90% porosity), 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).

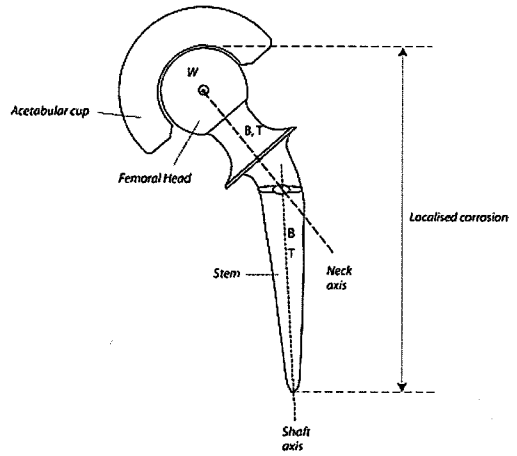
(b) According to Wolff's law, healthy bone will adapt to the loading regime to which it is subjected. If loading increases, the bone will model itself over time to become stronger to resist the loading and bring the strains back to the adapted range, i.e. at which bone-formation (modelling) and bone-removal (remodelling) are in equilibrium. If the loading decreases, the bone will remodel (i.e. bone density falls), e.g. occurs in bed-ridden patients, astronauts in microgravity.

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

Ti-6Al-4V and Al_2O_3 have Young's moduli of ~115 and ~350 GPa respectively i.e. stress shielding is stronger in alumina. In terms of mechanical performance, Ti alloys are tougher and more resistant to fatigue crack propagation but less wear resistant compared to Al_2O_3. However, Al_2O_3 is too brittle for any tensile loading.

In terms of biological performance, Al_2O_3 will provide a more appropriate environment for attachment, proliferation and differentiation of bone because of its chemical inertness. One possibility to improve the biological response of Ti alloys 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.

(c) The most common cause for hip replacement is *osteoarthritis*: 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*: cellular death of the femoral head due to interruption of the blood supply, leading to collapse of the femoral head and degeneration of the joint.



Implants are subjected to static and dynamic bending and torsional moments (B,T) around the intersection of shaft and neck axes of the implant – see schematic. There is also wear (W) resulting from articulation of the femoral head against the acetabular cup. The femoral component (stem + head) is also subjected to localised corrosion, i.e. only specific regions of the metal corrode, while the bulk remains passive. Main forms of localised corrosion expected are crevice and pitting corrosion. Other forms of localised corrosion such as stress corrosion cracking, corrosion fatigue and fretting corrosion may also be observed.

(d) Two types of implant fixation: cemented and cementless.
Cemented prostheses involve the use of bone cement, typically PMMA.
Cementless prostheses use surface engineering to give rough/porous surfaces to secure the implant via bone in-growth. Coatings include thermally-sprayed hydroxyapatite (HA) and Ti, bead-sintered coatings (Co-Cr, Ti) and wire/fibre meshes (Ti and stainless steel)

Cemented prostheses:

Advantages	Disadvantages
Patient can be mobile after 24 hours Prevents bedsores	Poor cement mixing can lead to poor mechanical properties Implant repositioning while 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) Two interfaces to contend with (bone-cement and cement-implant)

Cementless prostheses:

Advantages	Disadvantages
Better bone fixation to implant (direct fixation) Better long-term lifetime performance	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)

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.
Cementless implants are normally used in younger patients, since bone requires time to bond to the coated implants.

Examiner's Comments:
Popular question but without a correspondingly high average mark – in part due to omissions of sections of the question from some candidates (due to time pressure). Part (a) was not answered well, with many apparently unaware of the dense outer shell structure of bone, with more porous bone within. An extraordinary number of candidates thought Al_2O_3 was aluminium or an Al alloy, and quoted the wrong properties from the Databook.

H.R Shercliff
May 2011