11 Standards of biocompatibility tests and sterilization methods

11.1 What does make a material biocompatible?

ISO system is for Europe and FDA is for USA and every material that exist in the list is biocomaptible.

11.2 How biocompatibility is measured?

Biocompatibility is measured by physical and chemical properties: particle release, oxidation, mechanical properties; in vitro cell testing: cell adhesion, cell growth; in vivo animal testing; clinical studies.

11.3 What information does the current biocompatibility test standards deliver (and not)?

The results of the tests can only declare a material as more biocompatible or less toxic (in general, but not for a specific host tissue). Biocompatibility remains the central theme for biomaterials applications in medicine. It is generally accepted that this term means not only absence of a cytotoxic effect but also positive effects in the sense of biofunctionality, i.e. promotion of biological processes which further the intended aim of the application of a biomaterial. The national and international standards for testing regimes represent a lowest common denominator for such applications and do not necessarily ensure that optimal function will be achieved.

11.4 What is the difference of in vivo and in vitro materials evaluation?

In the in vitro materials evaluation:

- Simpler, faster (shorter), cheaper, easier control of environment
- Only test for really specific question/field of interest
- Toxic potential of biomaterials
- Direct interaction of biomaterials with cells
- Quality control, including sterilisation, under well defined conditions

- Analyse the cytokines (at molecular level)
- Easily with different human cells

In the in vivo materials evaluation:

- The real environment of mixed culture of interaction cells
- The possible detoxification and excretion in the complex real situation
- Reasonable test period with duration from months to years

11.5 What are the advantages and disadvantages of the different sterilization methods for the different classes of materials?

- **Sterilization by Moist Heat**
  Boiling water: This procedure is unsuitable for sterilization since the destruction of bacterial spores is not certain and cutting instruments may be damaged by turbulence and handling. Also, the steam released into the theatre sterile supply unit (TSSU) would have been unacceptable in a positive pressure air-conditioning system.

- **Steam under pressure**
  Steam under increased pressure is biocidal, the efficiency of the steam as a sterilizing agent being due to its condensation which is accompanied by liberation of latent heat, reduction in volume and deposition of moisture. This is, from the bacteriological viewpoint, a most satisfactory method of sterilization and it was hoped that it would have been possible to sterilize the bulk of equipment and materials in a high vacuum autoclave. Unfortunately, it was found that sufficient moisture was retained to cause corrosion of carbon steel when wrapped, sterile instruments were stored for any length of time and, since it is essential in our system to store instruments after sterilization, it was evident that autoclaving was 2 unacceptable as a method for sterilizing metal instruments. It is, however, the routine method for sterilizing trolley tops, glove and gown packs, and sutures and dressings which are supplied from the central sterile supply depart ment (CSSD). It was obvious that under certain circumstances it would be necessary to sterilize an instrument quickly and without delay (e.g. the dropped instrument) and it was considered that a high speed downward displacement autoclave installed in the TSSU would most readily fulfil

this need. It should be emphasized, however, that in using this method instruments cannot be placed in a sealed wrapping and are wet when removed from the sterilizing chamber.

- **Sterilization by Dry Heat**
  Dry heat kills micro-organisms by causing oxi dative destruction of their protoplasm. High temperatures, above 150C, are required as des truction of bacterial spores does not occur below 140C. It is the usual and recommended practice to sterilize in dry heat at 160C for one hour, but it has been our experience that under these con ditions the cutting edges of many of the fine metal instruments are damaged, and consequently there has been increasing dissatisfaction with the method. The manufacturers of the surgical instru ments are well aware of this problem, and it is of interest to note that Grieshaber recommend that their instruments be sterilized at 120C for half an hour; this is, of course, quite unacceptable to a bacteriologist. It was considered by us that if the oven temperature were reduced and the time of exposure lengthened then a state of compromise could be reached whereby sterility would be guaranteed and most of the fine instruments would not be damaged, and it is now our practice to use the hot air ovens at 150C for 1 hours. At this time and temperature it is found that the large bulk of metal instruments suffer no significant damage. It is essential, however, to use an oven with a circulating fan to avoid serious temperature differences in the chamber during sterilization, and in our hospital, as an added pre- caution against over-heating, an automatic temperature-operated time switch has been added. The racks containing the larger sets of instruments are placed in closed stainless steel boxes which in turn are wrapped in Kraft sterilizing paper bags; smaller sets are packed in foil trays and a plastic wrap- 3 ping while individual instruments are double-wrapped in plastic material. After sterilization the wrapped instruments are stored until required for use. There remained the problem of sterilizing not only the very delicate surgical instruments such as fine knives and needles, corneal trephines and micro-surgical instruments, but also heat-destructible materials such as plastics and lenses.

- **Chemical Sterilization**
  ith a few exceptions chemical agents do not kill bacterial spores in the

times and concentrations at which they are expected to act and therefore can not be classified as sterilizing agents. Glutaraldehyde does, however, have a remarkably wide range of biocidal activity and is probably one of the most satisfactory of the chemical agents, but with this compound as with other chemicals there is always the risk of introducing a dangerous and highly irritant substance into the eye if it used for the sterilization of instruments. The biocidal action of formaldehyde is critically dependent on the relative humidity, which must be at least 75 Percent, and in the traditional method of using paraform tablets which slowly liberate formaldehyde it is important that the temperature is at least 60C, the humidity is high and the time of exposure is not less than twenty hours; such a method is rather cumbersome besides being unreliable. The biocidal activity of ethylene oxide is related to its power of alkylating proteins and nucleic acids. The vapour will destroy all microorganisms, including spores, when present in an atmos phere of moderate concentration, the time required depending on the temperature, which if raised will accelerate the process; humidity is also an important factor. Ethylene oxide is inflammable and explosive and to achieve ideal and safe conditions sophisticated and costly equipment is necessary. This method, however, does at the moment provide an answer to the problem of sterilization of the very delicate instruments and heatdestructible materials, and an ethylene oxide sterilizer which has been installed in the CSSD fulfils the purpose. It is essential that all instruments and materials be thoroughly clean and free from contamination with saline before sterilization. The gas must obviously be allowed to penetrate to the articles to be sterilized and correct wrapping is therefore most important. some equipment and instruments are placed in a plastic box with holes in the wall and this is then either wrapped in CSS paper or placed in a Kraft sterilizing paper bag while other articles are doubly wrapped in a similar manner. It is the current practice at the hospital to allow instruments and materials which will only touch the body tissues to be returned ready for use following removal from the sterilizing chamber, but materials which are to be embedded in the tissues and also tubing which may be used for irrigation are given a shelflife of two days to allow for the vaporization of ethylene oxide and its toxic products. Frequent bacteriological testing of the sterilizer using the method developed by Kelsey is essential since in contrast to heat sterilization no physical methods to judge the

efficacy of ethylene oxide sterilization are as yet available.

- **Advantages**
  Most commonly used, effective method of sterilization.
  Sterilization cycle time is shorter than with dry heat or chemical sterilants.

- **Disadvantages**
  Requires a continuous source of heat.
  Requires equipment (steam sterilizer), which must be expertly maintained to keep it in working condition.
  Requires strict adherence to time, temperature and pressure settings.
  Difficult to produce dry packs because breaks in procedure are common (e.g., not allowing items to dry before removing, especially in hot, humid climates).
  Repeated sterilization cycles can cause pitting and dulling of cutting edges of instruments (i.e., scissors).
  Plastic items cannot withstand high temperatures.