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Use of a transparent rabbit ear chamber in the study of biomaterials

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Tests of biocompatibility are obligatory in the development of implants. Use of a polycarbonate transparent rabbit ear is a practical and relatively economical way of observing and documenting progressive interaction between potential prosthetic materials and granulation tissue. Fabrication of the chambers is technically straightforward and the surgical procedure involved in their insertion is simple and minimally invasive.

1. Introduction

The reactions of tissues to contact with artificial materials has been studied using a number of techniques, including cage implants [1] and direct subcutaneous [2, 3] and intramuscular implantation [4, 5] in various experimental animals. These methods have provided useful information on the various stages and aspects of tissue responses. A less well-known technique is the use of transparent ear chambers [6]. Historically, ear chambers of various designs and complexity have been used over many years to study tissue growth and microvascular responses [7–9]. Early types of ear chambers were handicapped by cumbersome, multicomponent designs and lack of suitable inert materials which did not themselves interfere significantly with the healing process. More recently the technique has been adopted for the study of interactions between healing tissue and potential prosthetic implant materials. For this purpose it is essential that the tissue responses which are observed truly represent only the effect of the test material on the host tissue without interference from any reaction to the chamber itself. A modification to a simple, polycarbonate ear chamber [10], which enhances its capability for testing putative prosthetic materials, is described below.

2. Materials and methods

2.1. Construction of the chamber

Stock, clear, 20 mm diameter polycarbonate rod (Ensinger GmbH & Co, Cham & Anröchte, Germany) is cut into 30 cm lengths, placed in a close-fitting pipe and annealed at 135 °C for about 2 h to relieve internal stresses that might cause cracking during machining. The main body of the chamber is turned on a lathe from the end of the rod, using a custom made cutter as shown in Fig. 1.

The new, important feature of the chamber is a single 'dimple', 2.0 mm diameter and 1.0 mm deep drilled in the central table of the "well". The other dimensions of the chamber, the anchoring "plug" and eight counter-sunk tissue-access holes are as described previously [10]. After machining and drilling the chamber is cleaned in an ultrasonic bath and solvent polished by dipping briefly into dichloromethane and drying in a divided stream of compressed air. The end of the plug usually needs polishing manually to remove machining marks. A circular 'skirt' of stiff surgical dacron or nylon net (0.5 mm mesh), with a central hole large enough to clear the tissue-access holes (Fig. 2) is glued to the outer part of the underside of the body of the chamber with polycarbonate cement (5% solution of polycarbonate dissolved in dichloromethane).

2.2. Assembly of the chamber

The chamber is assembled for use by first inserting a "spacer-washer" into the well. The thickness of this spacer determines the subsequent depth of the chamber and therefore the thickness of any tissue growing in it. It also limits the thickness of any material which can be placed in the chamber for biocompatibility testing. More than one spacer can be used if material samples cannot be made very thin. The 'roof' of the tissue chamber is formed from a thin, transparent polyester (Melinex ICI, Dumfries, Scotland) disc which in turn is protected by an external washer, similar to the internal spacer, and held in place by a truarc internal steel circlip. In the original design of the ear chamber, the annular washers were made of teflon. However, where relatively thick materials are being tested, necessitating the use of deeper tissue chambers, teflon spacers do not seal well. In the latest design, internal spacer-washers of dental rubber dam (Dentsply, Weybridge, UK) have been substituted for the teflon. Thus the completed tissue chamber is formed with a polycarbonate floor, rubber wall and a melinex roof.

The depth of the tissue cavity has to be adequate for the material under investigation but it must be borne in mind that deeper chambers take longer to fill with granulation tissue. If the material being considered

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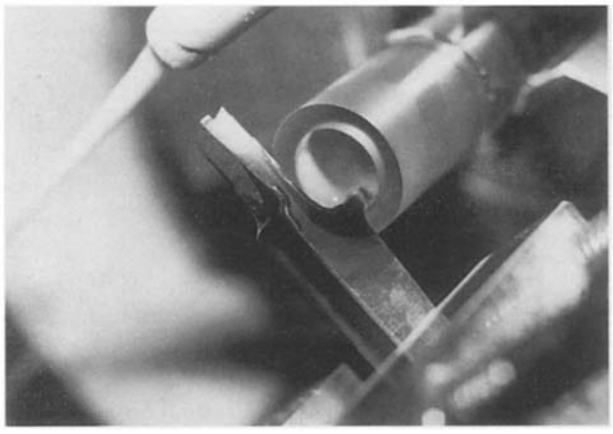

Figure 1 A piece of annealed polycarbonate rod being machined on a lathe. The special cutter is excavating the well. Also shown is the cutting edge used for turning the anchoring plug.

swells, then a trial pre-swelling must be done and the depth of chamber adjusted by using an appropriate number of spacers.

Before the chamber is inserted into the rabbit ear, it is either heat sterilized by boiling for 20 min or autoclaving at 120 °C, or gas sterilized with ethylene oxide. In the case of the latter method several days degassing are required to extract ethylene oxide that has dissolved in the plastic. Chambers may be sterilized before or after final assembly. Test materials may be added to the chamber at the time of assembly and sterilized *in situ* if they are capable of withstanding the sterilization procedure. More fragile materials are placed in pre-sterilized chambers or added when the chambers have already been inserted into the rabbit ears and are partly or completely filled with new tissue.

Immediately prior to insertion, the chamber is filled with sterile normal saline (0.9%) through a tissue hole (Fig. 2). This preliminary step tests the water-tightness of the assembly and also provides a medium for growth of granulation tissue. When the chamber is inserted as described (*vide infra*) the cartilage and outer skin fit snugly into the recess of the anchoring plug, effectively sealing the tissue holes and converting the whole arrangement into a watertight chamber.

2.3. Insertion of the ear chamber

Sandy half-lop rabbits (Belgian Hare × Full-lop rabbit) are most suitable for implantation of these chambers and animals should be selected with ears large enough to accommodate two to three chambers in each ear (Fig. 3). The main surgical instruments required for the procedure are a scalpel, a flat undermining instrument (spatula with bevelled edge) and a punch which exactly fits the outer diameter of the chamber "plug" [10].

The rabbit is anaesthetised with Hypnorm (Janssen Pharmaceutica, Beerse, Belgium) 0.5 ml/kg. The ears are plugged with cotton-wool to prevent liquid from running into the external ear canal during the procedure. The hair on the inner and outer aspects of the pinna is removed with a domestic proprietary depilatory cream and the skin washed free of the preparation and cleaned with hibitane.

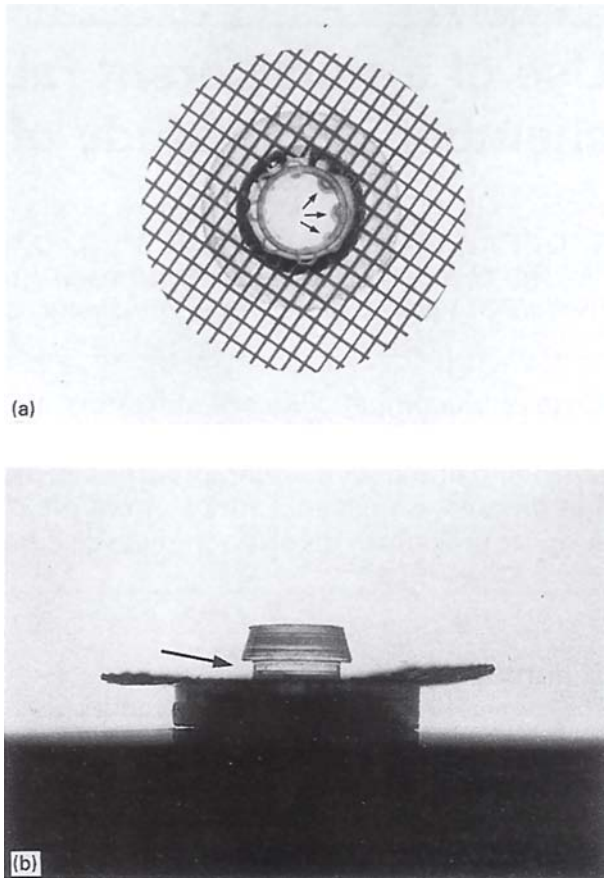

Figure 2 (a) The assembled chamber showing the skirt, internal circlip and the tissue holes (arrowed) partly covered by the rubber dam washers. (b) Profile of the chamber showing the plug, skirt and main body of the chamber, the anchoring plug and its recess (arrowed).

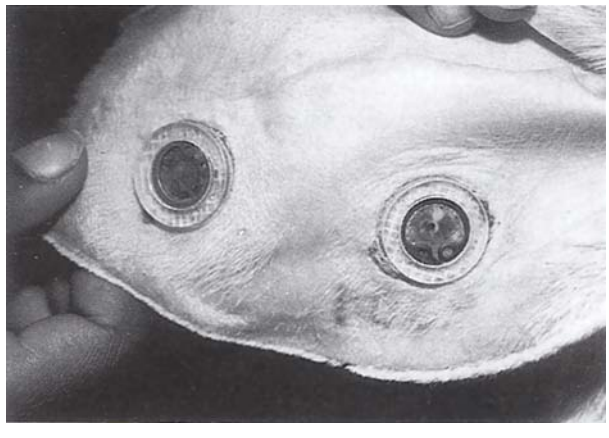

Figure 3 Two chambers inserted into the ear.

The surgical operation essentially involves incising the inner skin of the ear in cruciate fashion, at a site where there is minimal vascularization, undermining the skin to create a circular cavity for the skirt and punching a hole through the cartilage and external skin to admit the "plug". This plug is pushed firmly through the hole so that the cartilage of the pinna locks into the recess and holds the stub firmly. The skirt is then insinuated under the internal skin flap to provide additional anchorage.

Following surgery, the rabbit is provided with appropriate antibiotic cover by injection for 5 days.

3. Results and end-point

After insertion the ear chamber is observed periodically using a stereo microscope with adjustable magnification. The initial response is haemorrhage into the chamber of varying severity. This is minimal if due care has been exercised in selecting a suitable site, avoiding blood vessels and ensuring reasonable haemostasis (Fig. 4). The chamber is checked regularly to ensure that any initial haemorrhage progressively clears. If the test material was not already in the chamber at the time of insertion, it may be introduced after a few days when the haemorrhage has cleared and provided there is no evidence of infection. This is most conveniently done under general anaesthesia by removal of the retaining circlip, external washer and melinex cover, introducing the test material and resealing the chamber with a new coverslip, washer and circlip.

In chambers which are progressing satisfactorily, there is no leakage of fluid, no air bubbles and healthy granulation tissue grows through the tissue holes centripetally. This is manifest as arterioles and capillaries observed under magnification (× 50). Eventually the tissue incorporates the test material if it is non-toxic (Fig. 5).

After an arbitrary period (about 6 weeks) depending on the progress of the granulation tissue, the rabbit is sacrificed and the ear chamber excised and fixed for histological study.

4. Summary

This method allows continuous visual assessment of interaction of healing tissue and materials. Progressive changes can be observed and recorded directly without the need to sacrifice the experimental animal and study the reaction at arbitrary time points histologically. Fewer animals are therefore needed making it a very economical method. Where relevant, physical changes in the materials can also be assessed, e.g. when biodegradability is being studied.

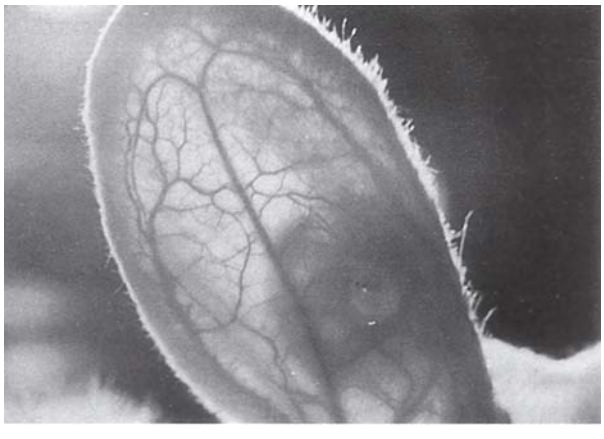

Figure 4 Optimal site for chamber insertion avoiding major vessels.

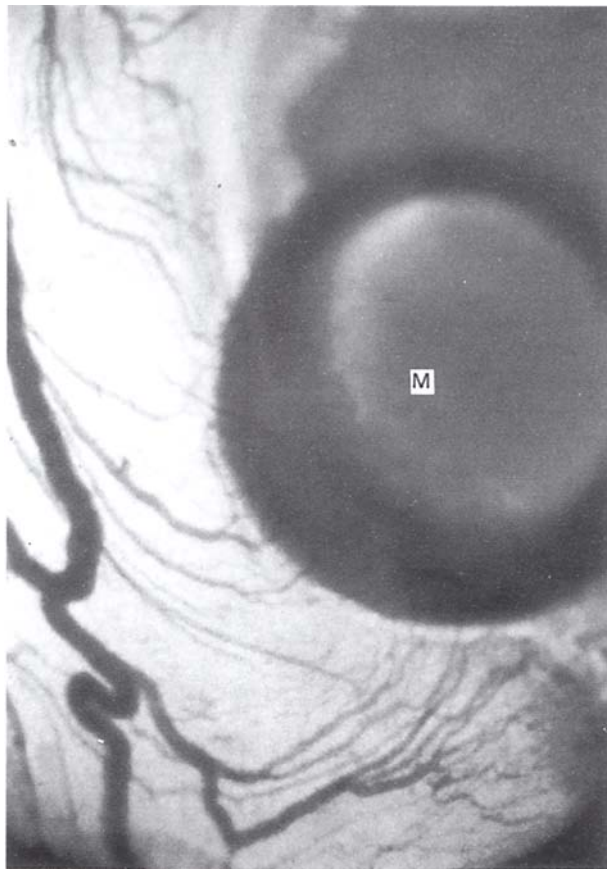

Figure 5 Microscopic view showing main vessels and capillaries closely related to the material (M) (magnification × 50).

Acknowledgement

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References

1. R MARCHANT, A HILTNER, C. HAMLIN, A RABINOVITCH, R SLOBODKIN and J M ANDERSON, *J. Biomed. Mater. Res.* **17** (1983) 301.
2. J AUTIAN, *Int Dent J.* **20** (1970) 481.
3. E. P BOULGER, *J. Amer. Dent Assoc.* **20** (1933) 1473
4. P. G LAING, A B. FERGUSON and E S. HODGE, *J. Biomed. Mater. Res.* **1** (1967) 135.
5. J. H. BREWER and H H BRYANT, *J Amer. Pharm. Assoc.* **49** (1960) 652
6 J. C SANDISON, *Amer. J. Anatomy* **41** (1928) 447.
7. *Idem., ibid.* **41** (1928) 475.
8. S WOOD, R LEWIS, J H MULHOLLAND and J KNAACK, *Bull. Johns Hopkins Hospital* **119** (1966) 1.
9. R H EBERT, H W FLOREY and B. D PULLINGER, *J. Path Bact* **48** (1939) 79.
10 G. F HOWDEN and I. A SILVER, *Int. Endodontic J.* **13** (1980) 3.

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