



A bone chamber for investigation of gas pressure Oxygen tension measured in rabbits

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Titanium implants containing a chamber that allowed ingrowth of bone were modified from a previous design to allow the use of oxygen microelectrodes. The modification consisted of the placement of ports in the superior surface of the implant for the insertion of oxygen electrodes into the tissue within the bone growth chamber. Forty titanium bone growth chambers were inserted into the proximal tibial metaphyses of 20 rabbits. From 2 to 20 days, oxygen tension was measured inside the chambers of 2 rabbits immediately prior to death. The oxygen tensions increased, declined, and increased again. Our results indicate that oxygen tensions fluctuate during the initial period of healing reflecting cellular changes in the callus.

Albrektsson (1982) described a chamber for study of endosteal bone formation; the chamber allows the harvesting of tissue for histologic and micro-radiographic analysis. We have modified the bone chamber for dynamic measurements of tissue oxygen.

Materials and methods

The bone chamber implant (Figure 1) was constructed of pure titanium, measuring 6.8 mm in diameter and 4 mm in height. The assembly was made in two parts that were joined with two small screws. The division of the implant was horizontal, the upper part having a self-tapping thread to achieve stability at installation. The cavity for bone ingrowth, which measured 1.0 mm in diameter by 6.8 mm in length, resulted from the apposition of two hemispheric grooves in each half of the implant. Three vertical ports passed

from the upper surface to the bone chamber to accommodate the oxygen microelectrodes.

Twenty-seven gauge platinum-iridium wire was soldered to heavy gauge copper wire. These platinum-iridium electrodes were then electropolished with a solution of sodium cyanide and sodium hydroxide to tip diameters varying from 125 to 200 μ (Cater and Silver 1961).

The electrodes were then insulated with Araldite[®], leaving the tip bare (Figure 2). The insulated part of the electrode and part of the adjoined copper wire were then coated with self-curing acrylic resin in such a way that short of the active tip a stop was formed that would contact the superior surface of the chamber implant when the electrode was introduced into the ports. The stop ensured that the bare electrode tip would extend to the center of the bone chamber (Figure 2). Anodes were constructed from tapered no. 50 endodontic silver points.

A Keithly model 600-B electrometer was used

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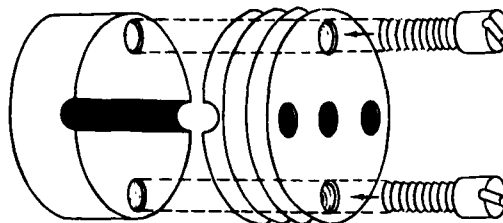


Figure 1. The bone growth chamber disassembled.

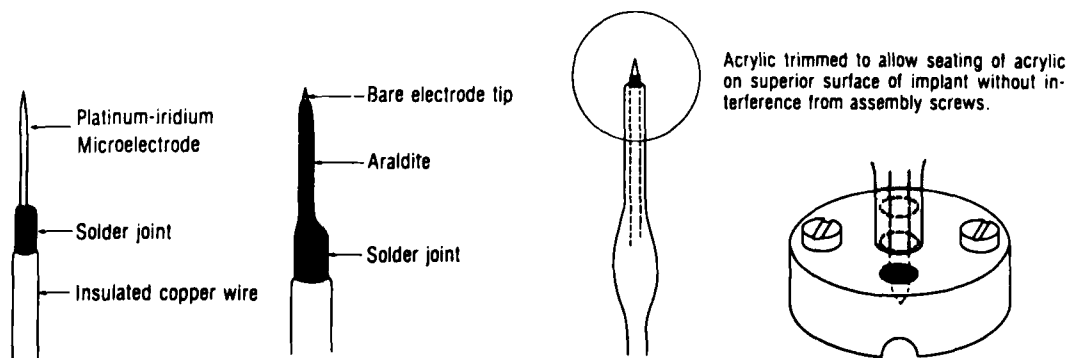


Figure 2. Insulation of the oxygen microelectrode with epoxy resin.

to measure current at the platinum-iridium electrode. Polarizing voltage, supplied by a 1.5 volt dry cell, passed through a potentiometer and voltmeter. A metal junction box was incorporated into the circuit to allow the use of different platinum-iridium electrodes and different silver-silver chloride anodes, and to act as a shield for the connections. The female component of a coaxial connector was mounted in each end of a metal box of approximately 10 cm \times 5 cm. Compatible male coaxial connectors were then soldered to the insulated copper wire that had been connected to the electrodes.

In vitro testing of oxygen electrodes. When the anode and cathode are placed in solution with a constant concentration of oxygen and stepwise increasing voltages are applied, the current developed at the cathode will rise, plateau, and rise again. The amperage at different voltages will form a curve characteristic of that electrode. Construction of polarograms was necessary to determine satisfactory characteristics of the electrode and to determine the correct polarizing voltage. A platinum-iridium oxygen electrode and a silver-silver chloride electrode were connected to the circuit through the junction box previously described. These electrodes were then placed in a 500 ml glass bottle into which approximately 200 ml of 0.9 percent sodium chloride solution had been placed, so that the tips were immersed in the fluid. A porous stone bubbling device was fitted to a polyethylene tube that was connected to an air compressor. The saline in the bottle was bubbled for 20 minutes with this apparatus. The turbulence in the saline was then allowed to settle. The potentiometer was used to deliver stepwise increases in voltage of 0.1 to 1.1 volts across the

electrodes. Electrometer readings were taken for each incremental increase in voltage to produce a polarogram for each of the electrodes used in this experiment, and thus verify the electrode's performance (Figure 3).

Apparatus identical to that used to make the polarograms was assembled. Instead of the air compressor, however, the bubbling device was connected to a Y-connector that joined delivery hoses from tanks containing medical grade pure oxygen and nitrogen. These tanks had been fitted with flow meters that would allow rates of flow to be accurately controlled. The saline in the glass bottle was bubbled with nitrogen for 20 min and the turbulence was allowed to settle. Then, a polarizing voltage was selected from the polarogram for that electrode, and an electrometer reading was taken. This procedure was repeated with mixtures of oxygen and nitrogen containing 25 to 100 percent oxygen bubbled into the saline.

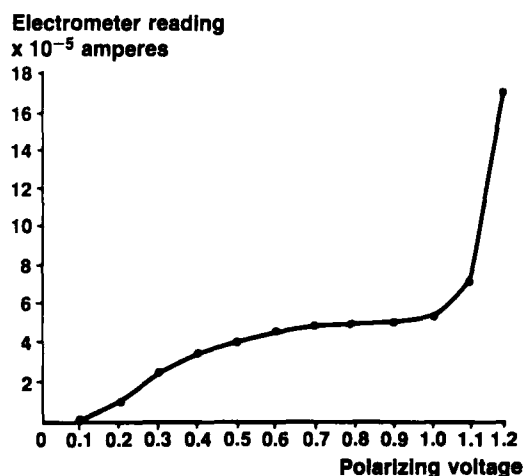


Figure 3. A polarogram drawn from measurements made with one of the electrodes used in this study.

This procedure verified the linear response of the electrode to changes in oxygen tension, and was used to calibrate the electrode prior to each use.

Chamber placement. Twenty adult New Zealand white rabbits weighing approximately 4 kg were anesthetized. Neuroleptic analgesia was achieved with a combination of fentanyl and droperidol (Innovar-Vet®) in a dose of 0.22 mg/kg i.m. supplemented with 2.5 mg of diazepam i.m. The inner aspect of the legs was depilated and prepared with Betadine and infiltrated with 0.9 ml of Xylocaine 2 percent with 1:100,000 parts epinephrine. A 1.5 cm curvilinear incision was made on the medial aspect of the region of the tibial metaphysis. Care was taken not to enter the joint capsule during the dissection. The site for placement of the implant was located just anterior to the tibial collateral ligament. A circular portion of periosteum was removed, approximately 7 mm in diameter. Then, with a trephine having an outside diameter of 6.5 mm, a circular piece of cortical bone was removed, irrigating with saline. The self-tapping implant was then inserted into the defect created in the cortex and rotated clockwise. The piece of cortex removed was used as a guide in determining the depth to which the implant was placed. The implant was placed so that the chamber was at the junction of cortical and cancellous bone, to ensure that the open ends of the chamber were in close apposition to bone and not to the marrow cavity. Blood was allowed

to fill the chamber and was observed flowing from the electrode ports. The fascia and skin were then closed in layers with absorbable suture.

Measurements. On alternate days after placement of the implants, an electrode was calibrated. Two rabbits were anesthetized with a combination of Innovar-Vet and diazepam. The skin was opened and the superior surface of the chamber implant exposed. A 2-mm incision was made on the medial aspect of the leg approximately mid-way along the tibiofibular length. The anode was inserted deep to the subcutaneous tissue. The platinum-iridium electrode was dipped in colloid and inserted into an electrode port of the superior surface of the chamber until the acrylic shoulder rested on the implant. Electrometer readings were recorded from each of the three electrode ports; the oxygen values taken from three positions in each chamber were calculated. The rabbits were then killed and tissue was removed from the chamber for histologic studies.

Results

There was wide variability in the values for several time points, causing us to question the shape of the curve produced (Figure 4). Analysis of variance, however, showed that changes in values shown from successive time points were significant (probability $> F = 0.01$).

Histologic sections of harvested tissue clearly showed extensive bone formation within the chamber (Figure 5).

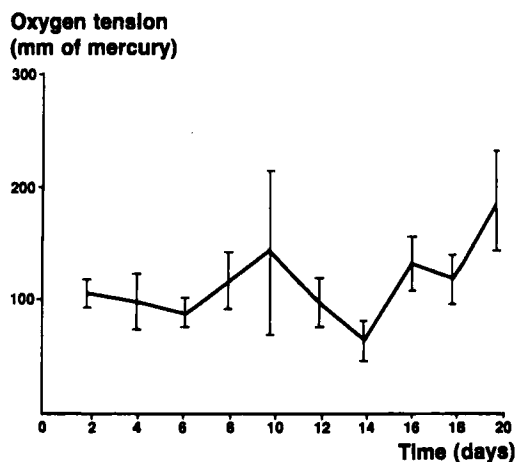


Figure 4. Means of oxygen measurements taken from tissue within the bone growth chambers over the initial 20 days of healing. Each point represents the mean of 12 measurements taken from four chambers in 2 rabbits. The bars represent standard deviations.

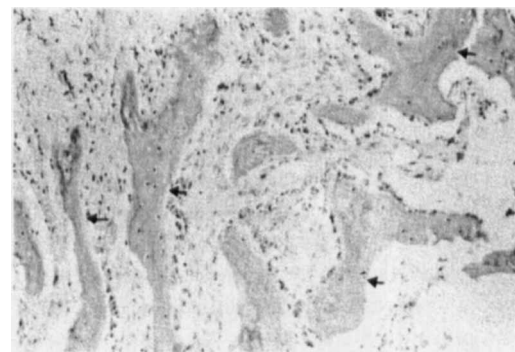


Figure 5. Tissue from within a chamber harvested at 16 days after chamber placement (X 80), showing woven bone in trabeculae (arrows).

Discussion

The neuroleptic agents used are potential respiratory depressants, and there was concern that this would alter the blood oxygen level. However, differences between preinjection and postinjection levels of hemoglobin saturation were observed in oximeter readings. Criticism could be leveled at the model system on the grounds that oxygen from the atmosphere could enter the tissue sample when the chamber is exposed; however, the maximum distance that oxygen will diffuse at a level that will maintain cell viability is 150 μ (Gray et al. 1953). Through skin, this distance is 48 μ (Fitzgerald 1957). Because the electrode ports were occluded by tissue and the surface of the port was 1,500 μ from the point of measurement, it was thought that diffusion of oxygen from the environment was not a factor.

The oxygen electrodes are very sensitive to environmental influences and also vary in their response at different times. Particular care, therefore, was taken to eliminate these effects. Static electricity, 60-cycle mains interference and capacitance effects are known to affect the current measurement (Cater et al. 1956-1957). The system was protected from electrical interference by the use of coaxial cables and short leads, and by the use of a grounded cage to enclose the apparatus.

Platinum-iridium electrodes are also shown to suffer a decrease in sensitivity with time, a condition known as poisoning (Cater and Silver 1961). This is thought to be due to deposition of protein on the electrode surface. Poisoning was not a factor in these experiments, for the measurements were made within a few seconds of introduction of the electrode into the chamber. Additionally, the electrodes were carefully washed after each use. The addition of collodion to the surface of the electrode served as a semipermeable membrane that prevented protein deposition on the active surface of the microelectrode. The responsiveness of the electrodes was checked each time they were used by calibrating them in normal saline, bubbled with known mixtures of oxygen and nitrogen. While Symington (1973) attempted to maintain the calibration solution at 37°C using a water bath, he ignored the cooling effect on caused by bubbling the gases through the solution. At higher oxygen concentration, there is insuffi-

cient time to allow the calibration solution to regain body temperature without the loss of dissolved oxygen. Because the quantitative effect of temperature is well established (Cater and Silver (1961), we decided to calibrate the solution at room temperature and apply the correction factor for the temperature difference between the calibration solution and the presumed temperature of the tissue sample.

The variability of the values for oxygen tension, particularly noticeable on the 10th day, presented a problem. This variability may have been due to inconsistency in the measuring apparatus or differences in the oxygen tension of the tissue measured. It is likely that there are variations in the tissue within one chamber to another, and there were probably differences in healing rates between different animals.

The analysis of variance for the values for oxygen for each day point would indicate that despite the broad range of values taken for each day, the shape of the curve is essentially correct. The wide variation, however, does not permit one to make accurate quantitative predictions of the oxygen levels for any given time point.

The curve presented of the tissue oxygen tensions shows a biphasic distribution that is similar in shape to that presented by Ninikoski (1977) for tissue oxygen tensions in healing soft tissue wounds, and that presented by Brighton (1972) for oxygen tensions in fracture callus.

The initial oxygen concentration measured was not, of course, zero because oxygen would be maintained within the clot partly by diffusion and partly from oxygen dissolved in the original blood. Macrophages are known to be stimulated by low tissue oxygen tension and migrate toward these areas (Hunt et al. 1983, Knighton et al. 1983). The oxygen concentration then rose. This occurred at a time when the ingrowth of new capillaries would be expected, stimulated to proliferate by the release of an angiogenesis factor produced by macrophages (Knighton et al. 1983). The oxygen then declined from 10 days to a trough at 14 days. It is possible that this reduction was due to a change in vascularity of the tissue, either through a true reduction in the number of blood vessels or as the result of shunting mechanisms. Finally, the curve rose. This period from 14 to 20 days was associated with increasing amounts of bone in the samples (Figure 5).

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