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PERFUSION CULTURE FOR CORTICAL BONE

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INTRODUCTION: Cell monolayers or organ cultures are still the most widely used tools in studying tissues in vitro. However, since both of these methods have limitations, the technique of perfusion chambers has widely spread and is in use by more than 100 research groups for a broad spectrum of studies. The advantages of ex vivo perfusion systems are obvious, with respect to reducing the amount of test animals used for biocompatibility studies and to overcome the drawbacks of in vitro cell and organ cultures. However, to study cortical bone in a set up that is suitable for investigations of material, bone and bacteria interactions we had to develop a new technique [1].

METHODS: Tibial bones from Swiss Mountain sheep's were acquired from the local slaughterhouse. With a sterile preparation process the bone was exposed stripped from periosteum and discs with a diameter of 10.6 mm were prepared with a saline cooled, diamond edged trephine (STRATEC). Bone cores were washed in medium containing antibiotics for 30 minutes before placed into the chambers.

We used custom-made bone chambers that consist of polycarbonate, which could be steam sterilized and reused several times. With an inlet at the lower and an outlet at the upper side of the chamber the in-between lying bone-disc is perfused in a perpendicular flow pattern with fresh media with the aid of a minimal dispersing (1 ml/ hr) computer controlled roller pump.

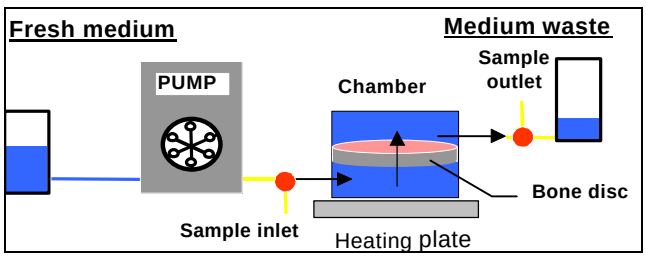

Fig. 1: Diagram of experimental setup

The system is a closed semi circuit system with cooled sterile media supply, a roller pump, valves for sampling before and after the chamber a warming plate at 37°C with isolation hood and disposal bottles for the used culture media. To use the system outside an incubator we also adapted the commercially available culture media BGjB [2] with HEPES buffer so that a carbon dioxide independent constant pH was maintained. To proof the viability and stability of the culture media we analyzed the in- and outflow daily for sodium, potassium, calcium, magnesium, glucose, lactate and pH. Cultures were run up to 21 days.

Fig. 2: Cart with warming plate, roller pump, laptop PC, refrigerator and waste bottles (left), close up of warming plate with 8 chambers (right).

Daily drawn 1 ml samples were analyzed in the laboratory of the local hospital. At the endpoint of every experiment the samples were perfused for 30 minutes with procion red and subsequently fixed in 4% formalin and embedded in resin for histological analysis.

RESULTS: To maintain a stable pH of 7,4 a flow of 2 ml per hour and a supplementation of 15 mM HEPES proved to be sufficient. However, a drop in pH during the first two days corresponded with the increase in lactate of up to 1 mmol/l. Potassium levels were higher in the beginning, the other electrolytes remained stable during the experiments. The culture medium was oxygenated by diffusion through the gas permeable silicon tubing. Portion red staining showed that there is a distinctive flow pattern across the perpendicular perfused bone discs. Histological evaluation revealed viable tissue in the center of the discs, but some destruction at the edges.

DISCUSSION & CONCLUSIONS:

In the recent years new models were developed to study bone ex vivo with rather sophisticated tools as in vivo loading [3]. However, to study

osteomyelitis only animal models and bacterial in vitro models so far are available. The bone disc in this model serves as a barrier between two compartments, thus resembling the inner and outer part of cortical bone. The system demonstrated to maintain ovine bone cells (osteoblasts, osteoclasts, osteocytes) viable up to 21 days. In pilot experiments we already used this two-compartment model in conjunction with different implant metals and bacteria. Since the interactions of osteosynthesis materials, bone and bacteria are essential in the course of an infection; our model seems capable of filling this gap in-between the above-mentioned methods, thus possibly reducing further the need of using living animals as test objects.

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