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The influence of foreign body surface area on the outcome of chronic osteomyelitis

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ABSTRACT

Reproducible animal models of osteomyelitis close to the clinical scenario are difficult to obtain as the animals either die shortly after inoculation of bacteria or the bone cures itself of infection. Additional materials used as foreign bodies offer increased chances for localized infection due to bacterial attachment and are closer to clinical pathology.

Through *in vivo* experimentation we investigated here the influence of surface area of a series of foreign bodies on the final outcome of the animal model, in terms of reproducibility, survival rate and time necessary for onset of chronic disease. Stainless steel Kirschner wire segments, stainless steel balls and cotton meshes were employed for this purpose.

The clinical, microbiological, radiological and histological results obtained were compared with the simple case where no foreign body was used. The follow-up period was 57 days. The cotton meshes, which had the highest surface area, were observed to provide the best outcome, with the lowest disease onset time interval (of 1 week earlier than the others), the highest survival (of 90%) and disease reproduction rate (90%). The only clinical pattern of the mesh group rabbits was short lived inflammation while the other rabbits presented also some other clinical signs such as rhinorrheas, abscesses, rush and/or dyspnea. Moreover, this model is the most suitable for further treatment studies, as the cotton meshes could be easily removed after disease onset, without any intervention on the bone. This is important, as the treatment would address the bacteria present within the bone parts (marrow, cortex, periosteum etc.) not those forming the biofilm.

1. Introduction

Osteomyelitis is a bone disease caused by bacterial or fungal infection involving parts of the bone. Due to the poor vascular system specific to the bone, the defensive system is not able to cure the disease and antibiotics have limited access, also. In the acute form, the infection is suppurative and the blood supplies of the medulla and the periost are compromised. An avalanche of bone destruction mechanisms leading to bone death follow and the chronic form is thus installed [1].

Treatment of staphylococcal infection is difficult because bacteria develop resistance to antibiotics [2]. Current treatment of osteomyelitis includes high dose antibiotics (like Nafcillin, Ceftriaxone, Cefazolin, Ciprofloxacin, Ceftazidime, Clindamycin, Vancomycin etc.) for 6 weeks to 6 months, pain medications and surgery [3–6]. Therefore, research on finding better therapies is still in progress.

The need to obtain improved therapies has triggered the necessity of finding reproducible experimental models of this disease.

It is known that chronic osteomyelitis is difficult to induce in animals. This is due either to the fact that the animals die shortly after inoculation of the pathogen, or the bone does not develop the disease.

Several animal models were tried and reviews were made by different authors [2,7]. A large range of species of animals, from mice and rats to chicken, rabbits, dogs, pigs and goats were involved until now. A trade-off between the size of bone/animal and

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the costs associated with the use of larger animals is considered when choosing the animal species. Different bones were used for induction of osteomyelitis: tibia, femur, mandible, radius. As observed by the above mentioned authors, the most used model is the rabbit tibial model. While being a relatively small animal, the rabbit has sufficiently large bones for complex surgical intervention involving insertion of foreign bodies and also for analysis.

Among the bacterial species usually involved for inducing osteomyelitis experimentally, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* are the most frequently used species [1–3,7].

Regarding infection promoter and route of inoculation, some models use fracture or bone hole creation together with insertion of a foreign body. Some authors injected sclerosing agents such as sodium morrhuate in the medullary cavity to better mimic the clinical scenario [8–10]. The benefit is the fast progression of infection by induction of aseptic bone necrosis, vascular congestion, and thrombosis, but there are drawbacks such as non-natural evolutional pattern of infection and possible interferences with treatment.

The main rationale for involving foreign bodies in animal models of osteomyelitis is to make surfaces available for bacterial adherence and proliferation which localize and increase infection [7].

It is well known that bacteria attach to surfaces and develop biofilms provided that the chemical and physical environment is suitable. By surface attachment, they protect themselves from hostile environments (such as antibiotics). The composition and porosity of surfaces are very important in biofilm formation. Therefore, the foreign bodies employed in animal models of osteomyelitis need to be biocompatible. Common such materials are: PMMA rods, PVA sponges, bone cement, metal rods/wires, plates or bone wax [2,11–14].

In terms of surface porosity, the higher the better for biofilm formation. Bacteria synthesize and deposit polysaccharide substances on surfaces and create a protective environment (matrix) for further colonization [15]. Direct correlations of surface area and amount of biofilm formed was observed since a long time ago and was reported by many authors [16–18].

Apart from the high surface area needed for increased bacterial adherence, the foreign bodies to be used in animal models of osteomyelitis should ideally be easily removable, a requirement necessary for further treatment studies where the material has to be removed before treatment administration. They should also be small for an increased chance of localization of infection, and also induce minimal invasion and less suffering for the animal.

The current work is aimed at finding a simple and reliable animal model of orthopedic chronic osteomyelitis based on foreign body materials which would comply with most requirements and could, hopefully, be used in therapy studies.

2. Experimental design

2.1. Foreign bodies

Three kinds of foreign bodies were used: 1 mm diameter and 10 mm length stainless steel Kirschner wire, 0.8 mm diameter spherical stainless steel particles and 1 mm diameter cotton fiber balls. The metallic materials were chemically corroded for 15 min in 3:1 HCl:HNO_3 solution to increase surface area.

The cotton balls were made using two 10 cm cotton threads (detached from standard medicinal cotton mesh) rolled together into a small ball.

2.2. Inoculum preparation

Being a common microorganism in human osteomyelitis, *Staphylococcus aureus* has been used in many animal models of this disease [1]. In this work, the microbial strain used for induction of bone infection was ATCC 6538.

In order to revitalize the bacterial culture preserved in our collection, after rehydration of the strain in nutritive broth (Biokar Diagnosis), three passages were made in the same media at 18–24 h interval. The bacterial culture was then incubated at 35–37 °C and a concentration of 5×10^6 CFU/ml was obtained from the last passage.

Table 1
Animal grouping and infection promoter.

Group no.	Infection promoter	Number of foreign bodies per drilled hole in the tibia
I	–	–
II	Kirschner wire	1 wire
III	Stainless steel spheres	4 spheres
IV	Cotton meshes	1 mesh

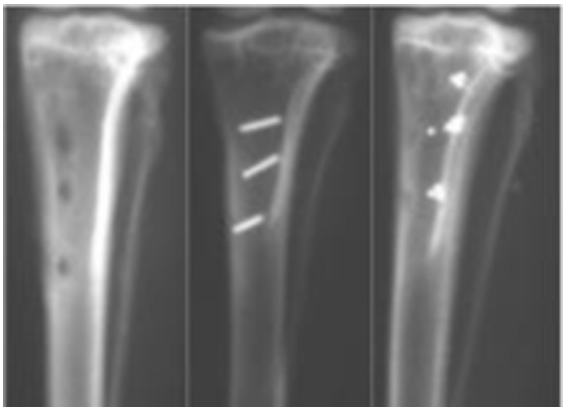

Fig. 1. Radiological images of rabbit tibia showing positions of foreign bodies, just after inoculation.

2.3. Animal groups and surgical procedure

Four groups of ten male and female white New Zealand rabbits each with different foreign bodies (Table 1) were contained in individual cages with computer controlled humidity (45–65%) and temperature (16–21 °C).

The animals were 6 months old with an average weight of 2.7 kg. Water and food were available *ad libitum*. The bacterial culture was inoculated in the tibia of the left leg, while the right leg was used as reference, in all animal groups.

The *in vivo* experiments were undertaken under national and international regulations concerning animal testing, using a protocol approved by the ethics committee of our institute.

The surgical procedure was undertaken as follows. Under total anesthesia and aseptic conditions, the skin on the antero-medial shaft of the tibia was incised directly down to the bone, exposing 3–4 cm. Three bone defects were then drilled perpendicularly at 3–5 mm distance one from the other, starting at 25 mm distance from the femoral-tibial-patellar joint using a 1.1 mm drilling pin. This procedure was applied to all rabbits in all groups. A quantity of 0.2 ml pathogen culture was evenly distributed among the three holes and inoculated using a 27 G needle. Insertion of foreign bodies followed. Relative positions of drilled holes and foreign bodies can be observed in the images presented in Fig. 1. Before insertion, the foreign bodies were sterilized under UV radiation.

The meshes were introduced into the holes leaving out of the hole one end of the thread. This setup is of great importance for further treatment studies, as it allows easy removal of the mesh by pulling out the end of the thread.