

# Biomaterial centred chronic osteomyelitis\*

## (An experimental model at the cortical bones of rats)

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*Staphylococcus aureus 209 p was inoculated into the cortical bones of 43 Sprague-Dawley rats, together with a stainless steel implant (s) to establish and evaluate biomaterial centred chronic osteomyelitis. On the third week of bacterial inoculation osteomyelitis developed in 42 out of 43 of the animals (97.7%). The established osteomyelitis was evaluated chronologically by its clinical, gross pathological, histopathological, radiological, and bacteriological findings. This model approximates the conditions of human biomaterial centred chronic osteomyelitis, and can be used for the evaluation of the pathomechanism of the disease, and the effectiveness of advanced therapy materials and methods.*  
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Key Words: Biocompatible materials, Osteomyelitis

Post operative osteomyelitis following arthroplasties or internal fixation of fractures remains as a serious complication, yet a few experimental models have been established. When the increasing opportunity of replacement and internal fixation, and the development of a biomaterial centred chronic osteomyelitis (BCCO) model seemed to be essential.

Early attempts to device experimental osteomyelitis by injecting sclerosing agents into the bone marrow of animals prior bacterial inoculation have been used to evaluate haematogenous chronic osteomyelitis (1,2). Humblen realised an increase in the number of infected animals when he inoculated the bacteria at the presence of surgical fibrin foam (3). Since then, different materials as cotton pellets (4), or biomaterials as acrylic bone cement (5-7), and stainless steel implants (8-11) have been used as foreign bodies to facilitate development of osteomyelitis (3-13). Although an increase in the number of infected animals have been recorded, a detailed evaluation of BCCO have not been presented in any of these studies. Furthermore,

the implants were mainly used to immobilise the established fracture in order to develop a BCCO model (8-11).

On the basis of this concept we can summarise the aim of our study as; (a) to establish an experimental model in the cortical bones of rats to evaluate the pathomechanism of BCCO, (b) to present the detailed chronological changes of BCCO by a clinical, gross pathological, histopathological, radiological, and bacteriological study, and (c) in addition to elucidation of BCCO we have aimed at an adequate new therapy for the condition.

### MATERIALS AND METHODS

43 albino Sprague-Dawley rats weighing 300 to 600 g were inoculated by a stock strain of staphylococcus aureus 209p. A stainless steel implant (SSI) was implanted into the medullary cavity of the tibia after bacterial inoculation, and the aperture at the bone was sealed by bone wax.

The animals used for this study were referred as the control group of a later study, where a new therapy method was investigated for the treatment of BCCO.

The animals were evaluated in two groups. The first group consisted of 30 animals. They were sacrificed weekly starting on the fourth week of bacterial inoculation to access the early stage of BCCO. The second group consisted of 13 animals which were followed up for three months, and the late stages of the disease were detected.

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**Bacterial Strains and Cultures:** Two strains of staphylococcus aureus were obtained by the courtesy of Kozo Inoue of the Department of Bacteriology, The Research Institute of Microbial Diseases of Osaka University. Staphylococcus aureus serotype 1 Cowan (Coagulase negative; Phage type 52/52A/80/95) was not used at this experiment as it is known to be the causative bacteria of acute haematogenous osteomyelitis.

Staphylococcus aureus 209p (Coagulase positive; Phage type 42d)\* was preferred for inoculation, as this strain has a slime producing capability, but could not devise osteomyelitis when inoculated together with a sclerosing agent (1). Bacterial sensitivity to antibiotics were determined by tri-disk (Eicom Co., Tokyo, Japan) method, and this strain was found to be susceptible to gentamicin (>2g), cefoperazon (>1g), and flomoxef (>ig).

The lyophilised bacteria was dissolved in 5 ml trypticase soy broth (TSB), and pure colony was obtained by several subcultures. Following incubation at 37°C in TSB by overnight culture, 0.1 ml of the bacterial suspension was transferred into 10 ml TSB, and further incubated at 37°C for 5-6 hours to get the bacteria in the exponential growth phase. The amount of death or inactive bacteria were decreased by this manner. The tubes containing the bacterial suspension were transferred into dry ice, and kept there during the operative procedure to prevent the increase of the amount of bacteria. 2.5 to 7.0 x 10<sup>7</sup> CFU/ml of bacteria were obtained by this method (Table 1).

Table 1. Preparation of bacteria

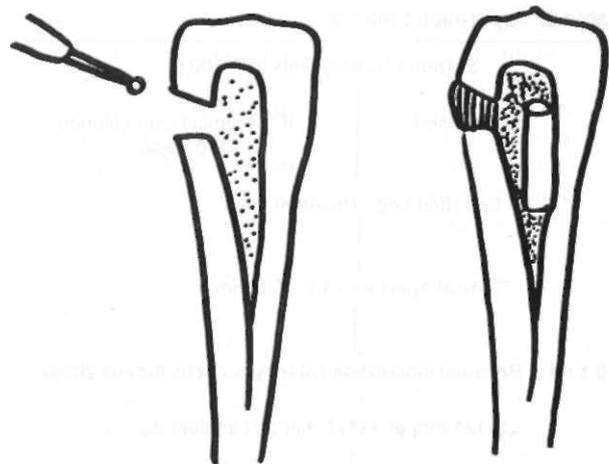
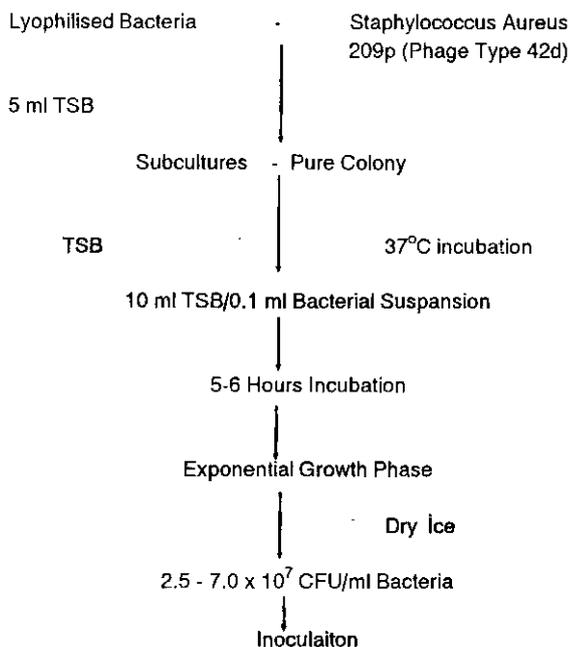


Figure 1. Operation protocol for the inoculation of the bacteria. The medullary cavity was exposed through a aperture performed by an electric burr. 0.1 ml of Staphylococcus aureus containing 2.5 to 7.0x10<sup>7</sup> CFU/ml of bacteria was inoculated through this aperture at the presence of stainless steel implant (s).

**Operative Procedure and Inoculation Protocol:** The animals were anaesthetised by intra peritoneal ketamine hydrochloride (10 mg/kg; Sankyo Co., Tokyo, Japan) injection, and the left hind leg was cleaned by 70% of ethyl alcohol. The proximal tibia was exposed from its anterior, and an aperture was drilled through the cortex down the medullary cavity by an electrical drill with a 1.2 mm burr. 0.1 ml of the bacterial suspension was injected through this aperture, and a stainless steel implant 4x1x1 mm in size, or 3 to 4 implants 1x1x1 mm size (AISI 316 L, Switzerland) were implanted into the medullary cavity. The aperture was covered by bone wax (Leukems, USA) to prevent the bacterial leakage into the soft tissues (Figure 1). A flow diagram of the operative procedure and inoculation protocol is given at table 2 (Table 2).

Sclerosing agents were not used as they cause aseptic necrosis at the bones, and may have an adverse effect on the bacterial viability. The skin was closed by staples, and the animals were returned to their cages, allowing free motion.

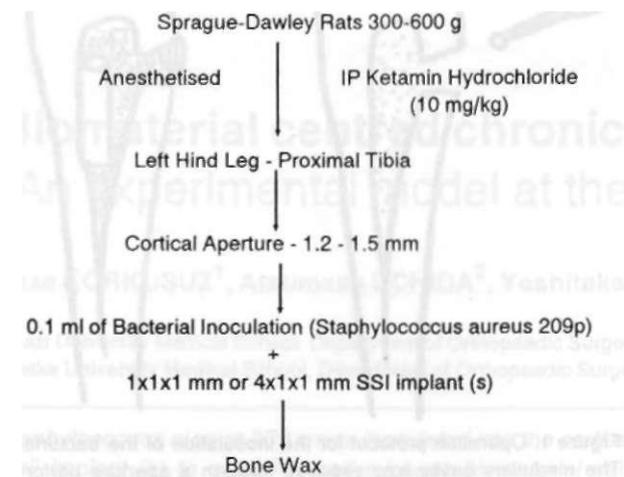
Evaluation of Infection

Clinical Appearance and Gross Pathology; Soft tissue swelling, the presence of fistula, purulent exudate, abscess formation, sclerotic and lytic changes, periosteal reaction and new bone formation, cortical thickening, and sequester were evaluated.

**Histopathology:** The bones were dissected free of soft tissues, and were fixed in 10% of formaldehyde after the removal of the SSI(s), and decalcified and longitudinal paraffin sections were stained with hematoxylin eosin.

\*Phage typing were performed at the General Testing Research Centre, Japan Oil Stuff Inspectors Corporation (Kobe, Japan).

Table 2. Operation protocol



**Radiography:** Xeroradiograms were obtained on a softex film (Type C-SM; Fuji, Japan) on the third week of bacterial inoculation in the first group, and on the seventh week in the second group from the living animals, and when the animals were sacrificed weekly. Periosteal reaction and new bone formation, sclerotic and lytic changes, cortical thickening, and a radiolucent zone between the implant (s) and bone were assessed by two of the authors independently. Bacterial contamination, in the animals undergoing bacteriological assessment was prevented by taking the X-Ray pictures before the tibia was dissected free of soft tissues.

**Bacteriology.** The soft tissues were removed from the bones under aseptic conditions. Each bone was chipped into separate Falcon tubes containing 20 ml phosphate-buffered saline (PBS) using a Rongeur, and then homogenised by a Polytron homogeniser (Kinematica, Switzerland) for 4-6 minutes. The suspension was vortexed for 1-2 minutes in a Vortex-Genie (Scientific Industries, USA), and 0.1 ml of this suspension was serially diluted in PBS. Each dilution was spread over trypticase soy agar (TSA) plates, incubated at 37°C for 48 hours for colony counting. The SSI (s) implants were not removed at any stage of the bacteriological study. Isolated bacteria were re-examined by routine tube dilution tests to confirm sensitivity towards gentamicin, and its Phage type was determined to identify contamination.

## RESULTS

42 out of 43 animals (97.7%) developed biomaterial centred chronic osteomyelitis according to the criteria evaluated below.

**Clinical Appearance and Gross Pathology:** Soft tissue swelling was recorded in 7 of 29 animals (24%) in the first group, and in 4 of 13 animals (30%) in the

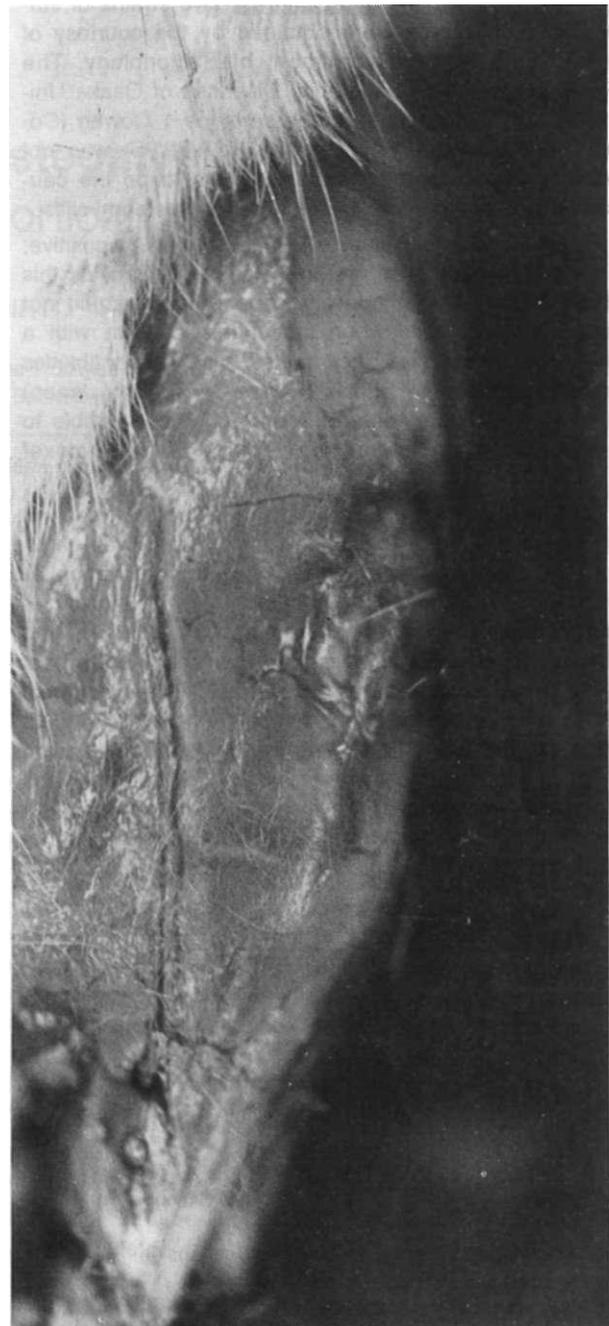


Figure 2. Macroscopic appearance of infection at the cancellous bone of a rat. Note the infection is also present at the surrounding soft tissues.

second group (Figure 2). Two of the animals in the second group developed fistula (15%), but other clinical findings, such as joint effusion or limitation of motion were not observed. The infection did not extend through all the bone, but remained localised at the surrounding of the SSI (s). The cortex was soft and thin. Although cortical thickening or sequester could be detected in some of the animals, the main lesion was



Figure 3. Gross pathological and radiological appearance of BCCO. (a) Intramedullary abscess formation and a purulent exudate at the operation site is a common finding, (b) Periosteal reaction, new bone formation, and a radiolucent zone between the SSI (s) and the bones reveal that BCCO is present.

cortical erosion and lytic lesions between the implant and bone. Another common finding was the intramedullary abscess formation and a purulent exudate at the operation site (Figure 3a). The presence of a haematoma was usually mis-diagnosed as abscess formation or soft tissue swelling both clinically and radiologically.

**Histology:** Osteomyelitis progressed from subacute form to chronic form when the histological sections of the first and second group were compared. Photomicrographs revealed the abscess mass consisting of cellular debris, and the infiltration of inflammatory cells into the bone marrow and bone necrosis were replaced by fibrotic reaction in the early stages of the disease (Figure 4). Bone destruction, and the replacement of haematopoietic marrow by connective tissue that contains lymphocytes were observed during the progression, and the endosteum was covered by large active osteoblasts in the later stages of the disease.

**Radiology:** 30 radiograms obtained on the third week from the first group, and 13 radiograms obtained on the seventh week from the second group animals were assessed, and the progression of the disease was evaluated by comparing these X-Ray pictures to

the ones obtained from the sacrificed animals (Table 3). The discrimination of infection and trauma at the operation site was radiologically difficult. Periosteal reaction and new bone formation was related to infection when it was seen on the opposite cortex, or at the distal end of the implant. Although the infection expanded slightly during the chronological evaluation, the main difference between the radiograms of the third week and third month was the increase of the radiolucent zone between the SSI (s) and the bones (Figure 3b).

**Bacteriology:** All the animals in the first group, except one, developed osteomyelitis (97.7%) according to the bacteriological findings. The amount of isolated bacteria showed a slight decrease in the second group in the later stages of the disease, but always remained similar to the inoculated amount between the fourth and 13 weeks of bacterial inoculation (Figure 5).

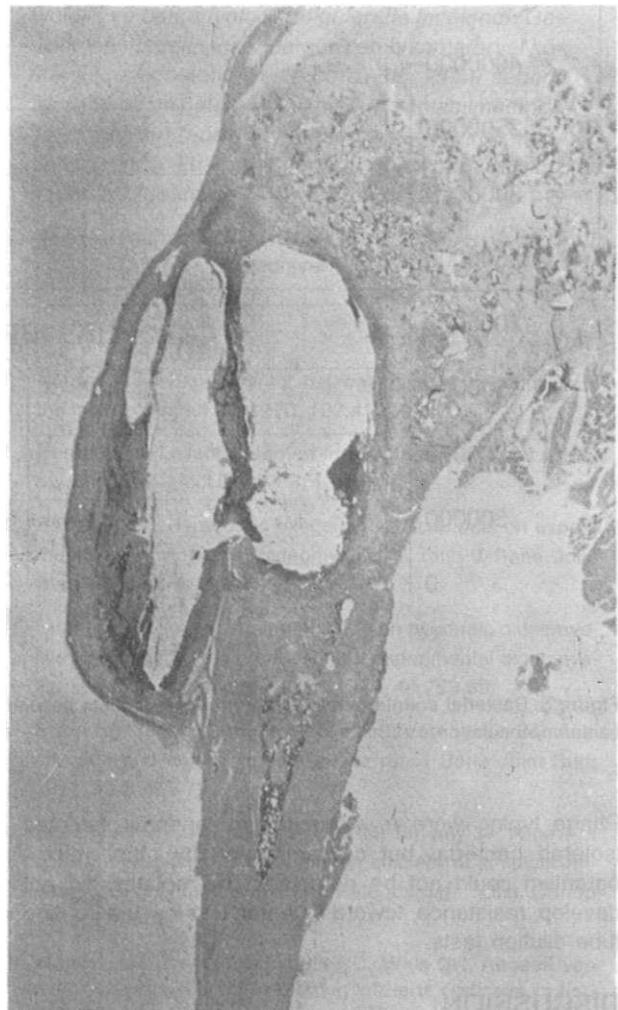


Figure 4. Abscess mass consisting of cellular debris, and the infiltration of inflammatory cells into the bone marrow and bone necrosis were replaced by fibrotic tissue (x40).

Table 3. Radiological evaluation of biomaterial centred chronic osteomyelitis

Weeks	Group I					Group II						
	3	4	5	6	7	7	8	9	10	11	12	13
Number of Animals	(30)*	(7)	(7)	(8)	(*8	(13)*	(2)	(2)	(3)	(3)	(-)	(3)
Periosteal Reaction and New Bone Formation	15(52%)	4(57%)	3(43%)	5(63%)	4(50%)	8 (62%)	2	1	3	3	-	2
Lytic and Sclerotic Changes	24(82%)	4(57%)	5(71%)	7(88%)	8(100%)	11 (85%)	2	2	3	2	-	3
Cortical Thickening	—	—	—	1 (13%)	1 (13%)	7 (53%)	1	1	2	2	-	0
Radiolucent Zone Between the Implant and Bone	6(21%)	1(14%)	1(14%)	3(38%)	3(38%)	12 (492%)	2	2	2	3	-	3

Bacterial Counts Between the 4th and 13th Weeks

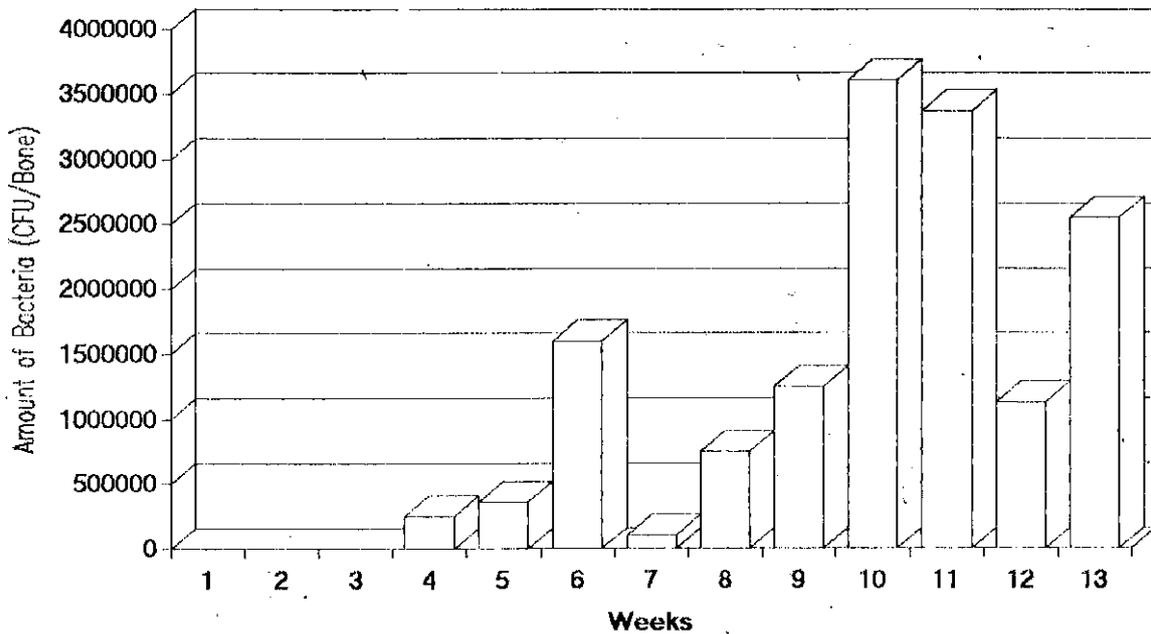


Figure 5. Bacterial counts obtained from the specimens between 13th week revealed a gradual increase following the 8th week of bacterial inoculation.

Phage typing were re examined in randomly selected isoletad bacteria, but contamination by other microorganism could not be detected. The isolates did not develop resistance towards gentamicin by the routine tube dilution tests.

**DISCUSSION**

Biomaterial centred chronic osteomyelitis is an important entity, and its incidence is not rare even prophylactic antibiotics are used (14,15).

The interactions between the bacteria and biomaterials have been evaluated with an increasing interest (16-20). Biomaterial centred infections differ from haematogenous osteomyelitis as normally opportunistic bacteria can be the causative agent, if it has the adhesion capability to the implant (21,22). Although the glycocalyx formation of the bacteria (23,24), its properties (25,26), and the interactions of different bacteria with biomaterials (20), mainly plastic catheters (27,28) have been studied in vivo and in vitro, bone

infections were only evaluated in vitro, or on the implants removed from human beings.

Andriole et al. inoculated bacteria into the tibia of rabbits following fracture and rodding, or into the rodded tibia without fracture (8). The foreign body was suggested to provide a surface for the bacterial attachment, and enhance a delay in host defences. Elson et al. inoculated different kinds of bacteria into the tibia of rats at the presence of an acrylic bone cement plug, containing antibiotics or plain (5). His study concerns about the antibiotic release from acrylic bone cement for the prophylaxis of infection, Lida et al. produced an open fracture in mice tibia, and inoculated staphylococcus at the fracture site (9). Osteomyelitis developed in 77.8% of the animals when inoculated with  $10^2$  bacteria, and 100% with  $10^3$  bacteria when he stabilised the fractures by intramedullary rodding. Zimmerli et al. established experimental foreign body infection by implanting polymethylmethacrylate cages into the subcutaneous tissues of guinea pigs together with staphylococcus aureus Wood 46 strain (29). He was able to develop infection by  $10^2$  CFU, whereas in the absence of the foreign material even  $10^8$  CFU could not establish infection.

Our present study differs from the other studies by the establishment of a BCCO model, instead of using a biomaterial as a foreign body in order to increase the number of infected animals. A fracture was avoided and trauma was minimised during the drilling procedure to obtain a pure BCCO model, hence it was difficult to discriminate the trauma and infection radiologically. Although the clinical findings were not so remarkable, the gross pathological and histopathological findings revealed subacute osteomyelitis in the first group, and chronic osteomyelitis in the second group. The bacteriological study was regarded as the best criteria for the assessment of infection.

Although there were slight differences among the X-Ray findings, and the bacterial counts were almost similar between the two groups, histopathological sections confirmed the subacute to chronic change of the disease. The establishment of osteomyelitis was detectable starting from the third week of bacterial inoculation, and did not cure by itself till the end of three months.

Staphylococcus aureus 209p was preferred for inoculation in this study as staphylococci will remain one of the major pathogen agent in human BCCO as it has the adhesion capability to biomaterials. However, this strain did not establish osteomyelitis when it was inoculated with Ethicon sture material (Johnson and Johnson, USA), or when the aperture at the bone was not sealed with bone wax although SSI (s) were implanted into the medullary cavity after bacterial inoculation (unpublished data). These findings may erase the following hypothesis. The pathomechanism of BCCO may vary with biomaterials, and these infections may

be time dependent, where the bacterial contact with the biomaterial should be long enough for its attachment

The established model simulates the conditions of human biomaterial centred osteomyelitis and can be used for the assessment of the effectiveness of advanced treatment materials and methods.

Biyomateryal kökenli kronik osteomyelit (Sıçan kortikal kemiklerinde geliştirilen deneysel bir model)

*Biyomateryal kökenli kronik osteomyelit modeli oluşturmak ve incelemek üzere, stafilokok aureus 209 p tipi bakteriler paslanmaz çelik implant(lar) varlığında 43 Sprague-Dawley sıçanının kansellöz kemiğine inoküle edildi. Üçüncü haftanın sonunda 43 hayvandan 42'sinde (%97.7) osteomyelit geliştiği görüldü. Gelişen osteomyelit, kronolojik gelişim sürecinde klinik, patolojik, histopatolojik, radyolojik ve bakteriyolojik bulguları ile incelendi. Geliştirilen modelin insanda görülen biyomateryal kökenli kronik osteomyelite benzediği ve bu sebepten hastalığın patomekanizmasının anlaşılmasında veya gelişen tedavi yöntemlerinin etkilerinin incelenmesinde kullanılabileceği görüşüne varıldı. [Türk Tıp Araştırma 1992; 10(6):286-292]*

Anahtar Kelimeler: Biyokompatibl materyel, Osteomyelit

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