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Original article

In vivo erosion of orthopedic screws prepared from nacre (mother of pearl)

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ABSTRACT

Background: Biodegradable biomaterials have been proposed to prepare orthopedic devices. Nacre is a natural aragonitic material made of calcium carbonate and is bioerodible.
Working hypothesis: We postulated that nacre is biodegradable without provoking bone erosion and favors bone apposition.
Material and methods: We prepared orthopedic screws from nacre of the giant oyster Pinctada maxima. Threaded screws (3.5 mm diameter) were implanted in 6 ewes in the upper tibial metaphysis (3 to 4 screws per animal). Their trajectory was transcortical and intramedullary to the opposite cortex. Animals were kept for 3 months ($n = 2$) and 6 months ($n = 4$). They did not develop local inflammation. Before euthanasia, they received a double calcein labeling. Bone samples were analyzed by X-ray nanotomography and histology after embedding in poly(methyl methacrylate). The fractal dimension of the screw profiles (measured by the box-counting method) was used to quantify surface erosion.
Results: 3D nanotomography showed a gradual erosion of the threads, which was confirmed by a decreased fractal dimension. Histologically, multinucleated cells (non-osteoclastic appearance) were visible at the surface of the screws. No ruffled border was seen in these cells but they had extensions creeping in the organic matter between the aragonite tablets. Bone apposition was noted in the transcortical path of the screws with limited osteoconduction at the endosteum. Mineralization rate was increased in these zones composed of woven bone in contact with the nacre.
Discussion and conclusion: Screws prepared from nacre have the advantage of an in vivo resorbability by macrophage-derived cells and an osteoconductive apposition in contact with the material without triggering a local inflammatory reaction.

1. Introduction

Biodegradable orthopedic devices such as screws, pins interference screws have been developed in the last decades. Their interest is that spontaneous degradation in the body does not necessitate a second surgery for removal. In contrast to metallic implants, they ensure a progressive transfer of loads to the healing bone [1]. A number of polymers such as polylactide, polyglycolide and copolymers has been proposed. Although they have proven to be useful, a number of reports have raised side effects (such as inflammation) during biodegradation of these materials [2,3].

Other substitutes are available to prepare bioerodible devices. Nacre (also known as mother of pearl), from the giant oyster Pinctada maxima is a natural material composed of calcium carbonate glued by an organic matter containing chitin. Its structure is made of flat polygonal tablets of aragonite cemented by the organic phase. This results in a 1000 folds mechanical strength compared with inorganic crystals [4]. The oyster's shell is machined to prepare orthopedic devices with suitable biomechanical properties. When nacre is implanted in bone, it performs a tight welding characterized by surface erosion due to a microionic environment. Bioerodibility can be improved by specific treatments and precedes bone apposition by osteoblasts [5–7]. However, the kinetics of bioerosion of nacre has not been accurately characterized after in vivo implantations.

The aims of the present study were to evaluate nacre bioerosion and bone apposition in vivo in a sheep model. Nacre screws were implanted in the tibial metaphysis during 3 and

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6 months. Biodegradability was studied on nanocomputed tomography images (nanoCT) of the screws and the thread resorption was assessed by fractal analysis. Bone apposition was evaluated after a double calcein bone labeling. We hypothesized that nacre bioerosion would not produce inflammatory effects and searched:

- if biodegradability was effective and quantifiable over a reasonable time, compatible with bone healing;
- which cell is responsible for bioerosion in vivo in association with bone apposition.

2. Material and methods

2.1. The nacre biomaterial

Orthopedic screws and plates were prepared from the inner shell of Pinctada maxima coming from an oyster park in Indonesia. Oysters were approximately 20 cm in diameter corresponding to a mean age of 7–12 years. Devices were obtained through a process combining physico-chemical treatments, machining and coating operations providing an hybrid semi-synthetic material. Devices were sterilized by γ radiation at 25 Gy and stored until use.

2.2. Surgical procedure

Six ewes (Île-de-France strain) were obtained from a breeding center in Bourges (France). Animals (~8 mo. old) were transferred to the Biomedical Research Centre in the National Veterinary School of Alfort and acclimated for 5 days. The experiment was conducted with ethical principles for animal studies and good clinical standards (agreement Cometh/ENVA/UPEC #12-013). Before surgery, animals were premedicated by sodium thiopenthal. General anesthesia was induced with an IV perfusion of Ketamine (1 mg/kg) and after endotracheal intubation, maintained with isoflurane. Cardiac monitoring, pulse and inspired gases was recorded during the operating time. The upper tibial extremity was exposed with a lateral approach. Holes adapted to the nacre screws were created with an electric rotary instrument. Nacre plates (which can receive four screws) were placed on the periosteal surface after the removal of fibrous tissues (Fig. 1). The screws were implanted in the drilled holes until they anchored in the opposite cortice. The incision was closed with resorbable sutures. Benzyl penicillin-dihydrostreptomycin was given IM as a prophylactic antibiotic. Pain was avoided by morphine injection (0.1 mg/kg) every two hours during surgery and at 20 mg/SC during 5 days post-surgery. A double label was done with IM injections of calcein (Aldrich-Sigma) (12 and 2 days before euthanasia, 10 mg/kg). Sheeps were sacrificed after 3 months (n = 2) and 6 months (n = 4) with an IV injection of natrium pentobarbital. Bones were harvested and fixed in formalin. A total of 18 screws was placed in this series (5 in the 3 mo group and 13 in the 6 mo group).

Fig. 1. A plate made of nacre with a T-shape is affined on the periosteal surface The plate can receive 4 screws also prepared with nacre. The biomaterial is easily identified by its optical iridescent properties.

2.3. Nanocomputed tomography

Bones samples were scanned while in the fixative in a nanoCT (Nanotom, Phoenix, Germany) at 100 kV, 150 μA, rotation angle 0.2° and 20 μm pixel size. Image reconstruction provided a stack of 2D sections for each specimen. 3D models were obtained with a volume rendering software. Erosion of nacre was identified on the 3D images by a reduction of the thread sharpness. Reslicing was used to expose the screws and to obtain sections through their center, parallel to the long axis of the screw. For each screw, two orthogonal images were obtained and nacre was threshold with ImageJ (NIH). Due to the higher calcium content of nacre vs bone, it appeared white on the re-sliced sections. Binarized images were obtained with nacre in black (surrounding tissues eliminated) and the boundary of the screw was extracted (Fig. 2). The fractal dimension of the boundary was then measured on the images with the Fractalyse software (ThéMA laboratory, http://www.fractalyse.org/fr/home.php) using the box-counting

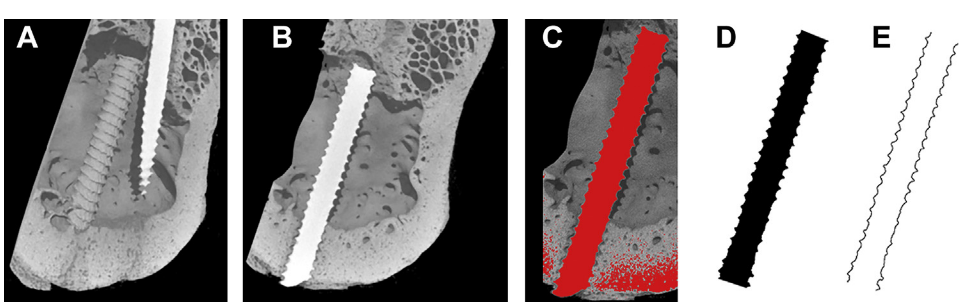

Fig. 2. Principles of identification of a screw boundary on nanoCT sections. A. NanoCT image of a sheep tibia of the 3 mo group; the deeper screw is positioned parallel to the image plane. B. A sectioning plane has been placed at the center of the screw, parallel to its long axis to eliminate irrelevant structures (e.g. cortical bone and the second screw). Due to the increased calcium content of nacre vs. bone, the screw appears in white. C. The image is threshold: nacre and irrelevant pixels are detected, these last ones are eliminated. D. Profile of the screw obtained on a binarized image. E. The screw boundaries are automatically extracted and used for the determination of the fractal dimension.