Original article

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

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A B S T R A C T

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 matrix 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.

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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 thiopental. 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 cortex. 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). Sheepes 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).

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 method.

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.

method [8]. Two unimplanted screws were processed and analyzed similarly to obtain the fractal dimension of the raw material. The fractal dimension of the screw profiles was compared at 3 mo and 6 mo with the control screws.

2.4. Histological analysis

Bone samples were embedded undecalcified in poly(methylmethacrylate) as previously reported [9]. Blocks were sectioned with a contact-point saw (Exakt 310, Norderstedt, Germany) providing ~ 30 μm thick sections. They were surfacestained with 1% toluidine blue allowing a clear identification of the bone matrix and cells. Additional sections were left unstained to study the calcein labeling under UV light. The mineral apposition rate (MAR, in μm/D) was determined in areas in close contact and at distance from the screws (> 3 mm) [10].

2.5. Statistical analysis

Statistical analysis was performed using the Systat statistical software release 13.0 (Systat Software Inc., San José, CA). Because this study was preliminary and mainly concerned the proof of concept, no attempt was done to compute the size of the sample. All data were expressed as mean ± standard deviation. Differences were compared using the Mann and Whitney’s U test. Differences were considered significant when P<0.05.

3. Results

3.1. Animals and nanoCT

No animal developed infection or side effects after implantation. Trajectory of the screws was well evidenced on nanoCT images either in 3D or after sectioning with a cutting plane in 2D (Fig. 3).

Erosion was evidenced at 3 months on all screws and some threads were completely resorbed 6 months (Fig. 4). Erosion occurred similarly at the threads present in the marrow cavity (Fig. 4C) and in the transcortical passage (Fig. 3B). The boundaries of the different types of screws is illustrated on Fig. 5. The magnitude of erosion was not similar on the different screws (Fig. 4A). Fig. 4C clearly illustrates that erosion heterogeneity is also observed along the same screw. The fractal dimension was significantly reduced at the two periods of analysis: at 3 months (v.s. control screws) (P=0.02) and between 3 and 6 months (P=0.0001) (Fig. 6). The high standard deviation observed at 6 months reflected the heterogeneity of the erosion foci. Apposition of woven bone was clearly evidenced by nanoCT (Fig. 4).

3.2. Histological analysis

Stained sections evidenced that giant cells were responsible of nacre resorption. The shape of these cells differed from a true osteoclast: at high magnification (~ 1000), they did not present a ruffled border but cytoplasmic processes were present, creeping between the stacks of aragonite tablets (Fig. 7E). An accelerated bone apposition was seen in the Haversian canals of the cortices near the screws and on the endosteal bone at the screw emergence (Fig. 7). A faint label was observed at the surface of the screws where eroding cells exposed free calcium ions. MAR was significantly increased in the cortices (around the screws) when compared to values measured at distance from the grafted site (P=0.01) (Fig. 6B). MARs were not significantly different at 3 months and 6 months in these two locations. In the bone marrow cavity, apposition of bone was

observed constantly at the surface of the threads that were closest to the endosteum. Foci of polymorphonuclear or lymphoid cells were never observed on the sections.

4. Discussion

In the present study, no inflammatory reaction was observed in contact with the biomaterial. A surface erosion of the threads occurred all along the screws as evidenced by a reduction in the fractal dimension of their profiles. Erosion was due to giant multinucleated cells that differed from osteoclasts. Bone apposition in contact with nacre was increased in direct contact with the biomaterial.

The small number of animals used may constitute a limitation of the study. However, the erosion of nacre was clearly identified in this pilot experiment and progressed during the time-course of the study. Another study is planned with longer implantation times to define more accurately the resorption rate of screws prepared with nacre.

NanoCT was used at a low resolution to obtain sharp details on the 3D images. Measurement of the surface roughness of an object can be appreciated by different methods such as atomic force microscopy (AFM) or profilometry but they do not work on large or curved surfaces (usually 100 × 100 μm for AFM). Analysis of boundary erosion was possible after extraction from nanoCT images and computation of the fractal dimension. In orthopedics, fractal analysis was found interesting in the analysis of bone [11,12] and to characterize the complex shape of wear particles [13].

In the present study, the unimplanted screws presented the highest fractal dimension due to the regularly disposed protruding threads. The surface erosion due to giant cells caused a progressive smoothing of the boundaries on the 2D sections. The surface erosion was significant when measured by the fractal dimension as early as 3 mo. At 6 mo, erosion was significantly more pronounced. On a given screw, erosion was not uniform and some threads were more eroded than others; this caused an increased standard deviation of the fractal dimension at 6 mo. There was no difference in the intensity of surface erosion in the transcortical path and the intramedullary trajectory. This is due to the nature of the eroding cells that are not true osteoclasts [5,14,15]. They do not have the cytological characteristics of osteoclasts (these cells had lacked a ruffled border, had more nuclei with a different chromatin pattern). Resorption of ceramics by multinucleated cells formed by fusion of macrophages has been described on calcium-phosphate ceramics, pyrolyzed bone and coral (calcium carbonate exoskeleton) [16–18]. The histological technique used here preserves cytological details, allowing a precise analysis of the interface of cells with extra-cellular matrix [19]. The organic phase of nacre contains water-soluble factors that cause differentiation of osteoblasts and inactivation of osteoclasts [20,21]. In vitro studies have confirmed that true osteoclasts (generated from progenitor cells) were inhibited by nacre [22,23].

Bone formation was stimulated in the cortical bone at the site of implantation and rapidly forming bone (woven bone) was evidenced. The double calcine label showed that the osteoblastic activity was significantly increased at 3 mo when compared to values measured at distance from the implantation area. Similar values were obtained at 6 mo, indicating that the osteoblastic activity was not reduced after a prolonged implantation period. Stimulation of the osteoblastic activity may be the reflect of numerous interacting factors; the Regional Acceleratory Phenomenon due to the surgical stimulation [24], the modification of strains around a biomaterial stiffer than bone [25], the release of bioactive molecules by nacre and a direct effect of the shape of the aragonite tablets on the osteoblasts [26]. Such an effect has been described for octacalcium phosphates but has never been explored with nacre [27].

Fig. 5. Profiles of the different types of screws processed according to the method illustrated on Fig. 2. A. Control, unimplanted screw. B. After 3 mo; note the beginning of erosion of the threads. C. After 6 mo; erosion interests a large number of threads.

Fig. 6. A. Fractal dimension of the screw boundaries in the different screws. The fractal dimension decreased upon time. B. Histodynamic determination of MAR at the vicinity and at distance from the screws.
5. Conclusion

Surface erosion was described on large screws prepared from the nacre of *P. maxima*. Erosion was confirmed by a reduction in the fractal dimension of the screw boundaries eroded by giant cells. Continuous bone apposition was noted around the biomaterial in the cortices. Resorption of nacre could be in the same order of time than polylactide screws (between 2–5 years) [28]. Devices prepared from physico-chemically treated nacre can represent interesting solutions when resorption of the material is expected in a prolonged period of time (e.g. fusion cages, mini-screws for dental surgery...) and when MRI is necessary for the follow up of the bone healing.

Disclosure of interest

Georges and Serge Camprasse are Scientific cooperators at MEGA BIO PHARMA.

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