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Original Research Article

Evaluation of the biocompatibility of a hydroxyapatite-$CaTiO_3$ coating in vivo

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ABSTRACT

Objective: This study was designed to evaluate the biocompatibility and osteointegrative activity of hydroxyapatite (HA)-$CaTiO_3$, titanium substrate, traditional HA coating and $CaTiO_3$ coating via an animal experiment.

Method: Four types of screws (type 1: coated with HA; type 2: coated with $CaTiO_3$; type 3: coated with HA-$CaTiO_3$; type 4: untreated titanium screws) were implanted into femur bone of 48 New Zealand rabbits. Histological and mechanical investigations were employed at the end of 2, 4, 8 and 12 weeks to evaluate the material osteointegration.

Results: (1) All of the experimental rabbits were healthy during the experiment process. (2) Histological investigation showed fully regenerated and well integrated bone tissue surrounding the screws coated with HA, HA-$CaTiO_3$ and $CaTiO_3$. (3) Mechanical investigation showed that the bonding strength of HA-$CaTiO_3$ coating was significantly higher than that of $CaTiO_3$ coating or titanium materials without coating, but was lower than those coated with HA.

Conclusion: HA-$CaTiO_3$ coating possesses similar admirable biocompatibility and osteointegration activity with HA coating, indicating a promising coating material for implants in orthopedics.

1. Introduction

Titanium and its alloys have been widely used in the medical field because of their excellent biocompatibility and good mechanical properties [1–3]. However, one of the disadvantages is the long period for these materials to obtain good fixation between the material and bone [4]. To shorten the bone fixation term, various surface modification techniques and implant-driven treatment considerations have been attempted [5–7]. A number of studies have attempted to use various bioactive coatings onto the surface of titanium implants in order to improve the implant osseointegration [8–10].

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Hydroxyapatite (HA) has long been used in medical field because of its compatibility with various tissues and can bond directly to bones [11–14]. However, one disadvantage of HA coating is the lacking of mechanical strength [15]. Therefore, major attempts have been conducted to achieve successful physical and chemical coating processes with HA on titanium to acquire good mechanical properties and excellent bone fixation.

Increasing attention has been paid on the calcium titanate ($CaTiO_3$) for medical application. Various studies have shown that $CaTiO_3$ enhances biocompatibility [16] and promotes osteoblastic adhesion [2]. Despite its excellent properties as a biomaterial, our previous study has found that the adherence of $CaTiO_3$ is weaker compared with HA (Supplemental Fig. 1). To solve this problem, we used a combination of bioactive HA and $CaTiO_3$ to develop cytocompatible composites with enhanced adhesion and fixation properties. Thus the objective of this study was to evaluate the potential of the HA-$CaTiO_3$ coating on being a novel substitution of implant coating material.

2. Materials and methods

2.1. Materials fabrication

All of the screw-shaped titanium implants (Zhejiang Guangci Medical Appliance Co., Ltd., Ningbo, China) whose external diameter and length are 4.1 mm and 8 mm, respectively, were roughened as previously described [17]. In brief, implants were polished, sandblasted, and washed with acetone, 75% alcohol, and distilled water in an ultrasonic cleaner. Subsequently, implants were treated with a solution containing HF and HNO_3 at room temperature, and then a solution containing HCl and H_2SO_4 at 80 °C. Implants were dried in an oven at 50 °C for 24 h. The high purity powders of HA and crystalline $CaTiO_3$ were synthesized using wet precipitation route and mechanical activation of a mixture of CaO and TiO_2 (anatase), respectively. Electrochemically deposited HA and $CaTiO_3$ coatings were applied as previously described [18]. Implants were used as the working electrode (cathode) for the deposition of HA or $CaTiO_3$-HA, with a platinum (Pt) plate as the counter electrode. The deposition process was performed with a DC power source operated at 3.0 V at 85 °C for 1 h.

2.2. Animals

This in vivo study was conducted after having received the approval of the Animal Care and Use Committee of the Central South University. All handling of animals were carried out in full compliance with the Chinese guidelines for animal welfare. New Zealand rabbits ($n = 48$) with an average age of 7.5 months (ranging from 7 to 8 months) and average weight of 2.4 kg (min 2.2–max 2.5 kg) were used. The rabbits were kept at 22 °C in a large cage and well fed during in vivo experiment. The animals were separated into four groups: 2w (after implantation), 4w (after implantation), 8w (after implantation) and 12w (after implantation), with each group have 12 rabbits. Six rabbits in each group were implanted with a HA-$CaTiO_3$ coated screw in the left femoral condyle and $CaTiO_3$ coated titanium screw in the right femoral condyle, with uncoated titanium screws and HA coated titanium screws as control implanted into the left and right femoral condyle of the other six rabbits.

2.3. Surgical technique

These implants were polished, sandblasted, and washed with acetone, 100% alcohol, and distilled water in an ultrasonic cleaner, individually packaged and labeled then autoclaved in Xiangya Third Hospital, Central South University. Ten minutes before surgery, 80,000 units gentamicin were injected intramuscularly. A dose of 30 mg/kg 3% pentobarbital sodium was intramuscularly injected to anaesthetize the rabbits. The operation area was arranged by shaving and disinfecting the outer surface of the lateral femoral condyle. The skin antiseptics benzalconium chloride 10% (Zefiran®, Vilsan) and povidone iodine 10% (Biokadin®, Adeka) were used for disinfection. Using an electric drill on the outer surface of the femoral condyle, drill horizontally from outside to inside. After the drilling is completed, remove bone fragments with saline flush, tighten the screw in, and then record the screwing torque.

2.4. Radiological and histological analysis

After titanium screws implantation at each time point, the rabbits were anesthetized intraperitoneally with pentobarbital sodium, and then were killed by air embolism. After radiologic X-ray and clinical examinations, all the femoral condyle were kept in the 10% formaldehyde solution for two weeks, and later decalcified in the Bouin's solution for two days (in 10% acetic acid, 80% NaCl and 10% formalin solution). For decalcified bone biopsy light microscopic observation, the bone tissues were fixed with 10% formalin in PBS at 4 °C, dehydrated in a graded series of ethanol, and then embedded in paraffin wax. The tissues were sectioned at 8 μm thick and mounted on slides. They were dewaxed, hydrated, and then stained with hematoxylin–eosin. For the tissues with titanium screw, the bone tissues were processed and embedded into paraffin, sectioned at 50 μm, and stained with toluidine blue according to a routine protocol [19].

2.5. Biomechanical test

It has been reported that HA coating of screws enhances fixation in normal and osteoporotic bone [20]. Insertion and extraction torque values allow us to determine screw fixation in bone. Briefly, the maximum torque value during the last turn of insertion and the first turn of extraction were measured using a torque wrench with a dial gauge and drag indicator, deviation 0.4% (Rahsol Company, Solingen, Germany). Then define the quotient maximum extraction torque over maximum insertion torque as fixation index, which appreciates the specific screw fixation strength and eliminates a great number of variables which are relevant for fixation strength of a screw in bone (such as coating, roughness diameter, contact area between screw and bone, e.g. cortical vs. trabecular contact zone) [21]. The fixation index of each group was measured. The fixation index greater than 1 is considered as there is osseointegration.