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Acta Biomaterialia 5 (2009) 3132–3140
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Comparison of enhancement of bone ingrowth into hydroxyapatite ceramics with highly and poorly interconnected pores by electrical polarization

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Received 25 October 2008; received in revised form 10 April 2009; accepted 24 April 2009
Available online 6 May 2009

Abstract

The effects of electrical polarization of porous hydroxyapatite ceramics with different structures on bone ingrowth were compared. Two types of cylindrical porous hydroxyapatite ceramics with high and low interpore connection (hydroxyapatite-H and hydroxyapatite-L, respectively) were utilized in this study. Hydroxyapatite-H or hydroxyapatite-L with and without electrical polarization was implanted into the right or left femoral condyle of rabbits ($n = 10$ in each group) and histological examination was performed 3 and 6 weeks after operation. Each cross-section was divided into three regions, outer, middle and inner region, and the percentage of total newly formed bone area/total area of each region (% bone area) was calculated. Bone ingrowth throughout the region of implant was significantly larger in the hydroxyapatite-H group than in the hydroxyapatite-L group. Electrical polarization was effective in enhancing bone ingrowth through all the pores of hydroxyapatite-H implant, however, this advantage was not apparent in the hydroxyapatite-L implant. It is suggested that enhanced bone ingrowth into hydroxyapatite porous bodies due to electrical polarization may be a cooperative interaction between the osteoconductivity of hydroxyapatite porous bodies and enhanced osteogenic cell activity induced by large charges stored on the pore surfaces.

Keywords: Bone ingrowth; Interpore connection; Hydroxyapatite ceramics structures; Electrical polarization; Osteogenic cells

1. Introduction

Hydroxyapatite ceramics have been widely used as an artificial bone substitute because of their high biocompatibility and good bioaffinity, as well as osteoconductability. Induction of bone growth into hydroxyapatite blocks is unsatisfactory, however, because it is very slowly replaced by host bone after implantation. For this reason, porous bodies and granules of hydroxyapatite ceramics have been developed and have attained popularity in clinical applications. Due to the closed structure of conventional porous hydroxyapatite, which has a non-uniform pore geometry and low interpore connections, however, it is very difficult for the pores of implants to be totally filled with the newly formed host bone [1]. Meanwhile, porous hydroxyapatite ceramics with highly interconnecting structures have been developed, as osteoconduction can occur deep inside hydroxyapatite ceramics with an interconnected porous structure. The size and density of the interpore connections as well as those of the pores are important factors for osteoconduction into the central area of porous hydroxyapatite. It is reported that osteoconduction was greater with a pore size of 500 μm than a pore size of 200 μm [2]. In another report, interconnecting pore sizes of 30, 60, 100 and 130 μm were compared, and it was found that

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1742-7061/$ - see front matter
doi:10.1016/j.actbio.2009.04.036

osteoconduction was greatest for 130 μm pores [3]. However, although a larger pore size and interconnecting pore size lead to greater osteoconduction, the compressive strength of these porous ceramics is reduced, resulting in limited clinical applications.

Therefore, implantation of hydroxyapatite ceramics with interconnecting pores in conjunction with bone marrow stromal stem cells (MSCs) has been attempted to improve bone formation early after surgery [4]. However, the development of techniques that encourage bone growth into interconnected pores of hydroxyapatite without the use of MSCs is desired to eliminate the complications associated with MSCs implantation.

We previously reported that proton transport polarization induces a large surface charge on hydroxyapatite ceramics [5] and that a polarization-induced surface charge and hydroxyapatite ceramic surface bioreactivity synergistically promote osteoconduction [6]. Osteogenic cell activity and new bone ingrowth in regions bordering the charged surface and central region of hydroxyapatite ceramics with highly interconnected pores with and without electrical polarization have been compared [7]. It has been suggested that bone ingrowth is favored by cooperative interaction between the osteoconductivity of hydroxyapatite ceramics with interconnecting pores and the high charges on their pore surfaces following electrical polarization. In this study the effects of electrical polarization of porous hydroxyapatite ceramics with highly or poorly interconnected structures on bone ingrowth were compared.

2. Materials and methods

2.1. Preparation of the electrically polarized porous hydroxyapatite ceramics

Two types of cylindrical porous hydroxyapatite ceramics, 6 mm in diameter and 5 mm in length, were used for the following studies. One was a hydroxyapatite ceramic with highly interconnected pores (hydroxyapatite-H) having a porosity of 75%, while the other was a hydroxyapatite ceramic with poorly interconnected pores (hydroxyapatite-L) having a porosity of 50%. Specimens of these hydroxyapatite ceramics were ion sputter coated with platinum and examined by scanning electron microscopy (SEM) (Hitachi Model S-4500). The pore sizes and interpore connections were measured on SEM images of these ceramics using Image Pro Plus 6.0 software (Media Cybernetics, Carlsbad, CA) for Windows ($n = 3$ in each group). The density of pores and interpore connections were also calculated using the formula ($n = 3$ in each group) number of pores or interpore connections/area of each region.

A pair of platinum electrodes was used to electrically polarize these test pieces, with the negative electrode placed on the bottom surface and the positive electrode on the side [inducing positive (P) and negative (N) surfaces on the sample, respectively). A direct current field of 2.0 kV cm^{-1} was applied in air at 400 °C for 1 h [8] (Fig. 1). We confirmed the polarization of three randomly chosen samples in each group by the thermally stimulated current (TSC) method using a handmade measurement cell that was shielded against stray fields with a stainless steel pipe [9–12]. The measurements were carried out in air at from room temperature to over 700 °C at a heating rate of 5.0 °C min^{-1}. The stimulating current was measured with a Hewlett–Packard 4140B pA meter (Palo Alto, CA) and analyzed with appropriate computer software to calculate the stored electric charge.

Statistical comparisons of the size of pores and interpore connections, the density of pores and interpore connections and the stored electric charge between the hydroxyapatite-L and hydroxyapatite-H implants were performed. Differences between each group were determined and evaluated for statistical significance by the Mann–Whitney test (level of significance $P < 0.05$).

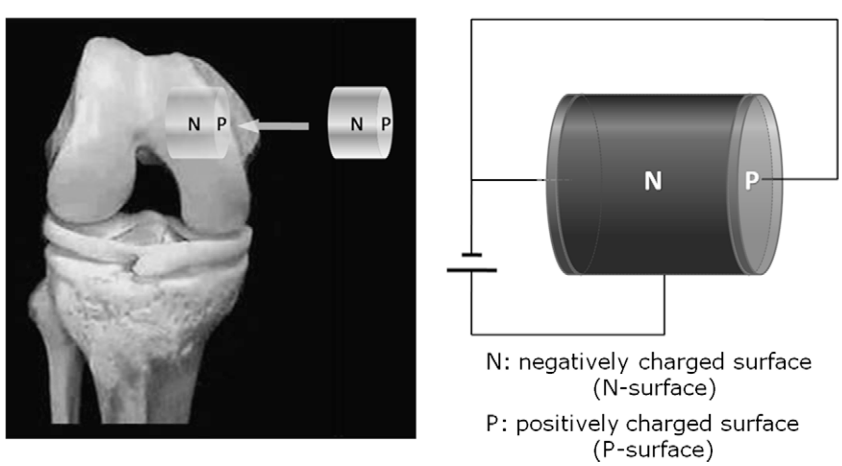

Fig. 1. Method of electrical polarization of hydroxyapatite ceramics and implantation. A pair of platinum electrodes is used to electrically polarize these test pieces, with the negative electrode placed on the bottom surface and the positive electrode on the side surface, applying a direct current field of 2.0 kV cm^{-1} at 400 °C for 1 h in air. A hole 6 mm in diameter and 5 mm in depth is drilled into the rabbit femoral condyle. A matching sized cylindrical hydroxyapatite porous body is implanted into the hole from the basal aspect. The base end surface of the polarized hydroxyapatite implant has a positive charge (P surface) and the lateral surface is negatively charged (N surface).