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High porous titanium scaffolds showed higher compatibility than lower porous beta-tricalcium phosphate scaffolds for regulating human osteoblast and osteoclast differentiation

Makoto Hirota [a,*], Tohru Hayakawa [b], Takaki Shima [c], Akihiro Ametani [c], Iwai Tohnai [a]

[a] Department of Oral and Maxillofacial Surgery, Yokohama City University Graduate School of Medicine, 3-9 Fuku-ura, Kanazawa-ku, Yokohama 236-004, Japan
[b] Department of Dental Engineering, Tsurumi University School of Dental Medicine, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama, 230-8501, Japan
[c] Medical Device Department, HI-LEX Corporation, Inc., 1-12-28 Sakae-cho, Takaraduka 665-0845, Japan

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ABSTRACT

We compared osteoblast and osteoclast differentiation when using beta-tricalcium phosphate (βTCP) and titanium scaffolds by investigating human mesenchymal stem cells (hMSCs) and osteoclast progenitor cell activities. hMSCs were cultured for 7, 14, and 21 days on titanium scaffolds with 60%, 73%, and 87% porosity and on βTCP scaffolds with 60% and 75% porosity. Human osteoclast progenitor cells were cultured with osteoblast for 14 and 21 days on 87% titanium and 75% βTCP scaffolds. Viable cell numbers with 60% and 73% titanium were higher than with 87% titanium and βTCP scaffolds ($P < 0.05$). An 87% titanium scaffold resulted in the highest osteocalcin production with calcification on day 14 ($P < 0.01$) in titanium scaffolds. All titanium scaffolds resulted in higher osteocalcin production on days 7 and 14 compared to βTCP scaffolds ($P < 0.01$). Osteoblasts cultured on 87% titanium scaffolds suppressed osteoclast differentiation on day 7 but enhanced osteoclast differentiation on day 14 compared to 75% βTCP scaffolds ($P < 0.01$). These findings concluded that high porosity titanium scaffolds could enhance progression of hMSC/osteoblast differentiation and regulated osteoclast differentiation cooperating with osteoblast differentiation for calcification as compared with lower porous βTCP.

1. Introduction

Titanium and ceramics have a high affinity for bone. Based on this affinity, various clinical biomaterials have been developed. Titanium bone fixation and reconstruction plates that exploit the mechanical strength of titanium are widely applied. Dental implants made of titanium are the most widely used artificial bone substitutes, and this technology is well developed. Then, many other bone substitutes using ceramics have been developed, and in recent years, absorbent porous beta-tricalcium phosphate (βTCP) has become widely used as a material for bone replacement [1–3].

The porous nature of bone substitutes is essential for their osteoconductivity [4]. Even when using titanium, the surface characteristics of implants are important considerations for osteoconductivity and osteointegration [5]. In recent years, using dental implants with rough surfaces has become the norm. Rough surfaces have similar nano- or micro-porous structures and improve the affinity for bone tissue. Numerous techniques are used to manufacture the porous coatings on titanium surfaces, with porosity and pore size ranging from 35%–86% and 10–200 μm, respectively [6]. Titanium fiber scaffolds are porous materials made from pure titanium fibers with a diameter of 50 μm. Research and development on bone tissue regeneration has resulted in the widespread use of titanium fiber scaffolds with a porosity of 86% and a pore size of 250 μm [7–9]. Osteoblasts proliferate and differentiate well on titanium fiber scaffolds with these porous structures [10].

The proliferation and differentiation of osteoblasts and their progenitors on porous materials are critical for bone tissue formation on a scaffold. Osteoblasts are osteogenic cells that are associated with bone formation through their production of osteoids and subsequent mineralization of the osteoid matrix. During osteoblast maturation, type I collagen is expressed as the first differentiation marker of osteoblasts, followed by alkaline phosphatase (ALP). Osteocalcin expression is induced during the final differentiation stage when calcification occurs [11]. Bone tissue is constantly replaced by osteoblasts and osteoclasts. Osteoprotegerin (OPG) inhibits osteoclast differentiation, whereas the receptor activator of NF-kappa B ligand (RANKL) enhances their differentiation [12]. High expression of tartrate-resistant acid phosphatase (TRAP) 5b and RANKL indicates the progression of osteoclast differentiation [12].

Very few studies have directly compared titanium and ceramic porosities. The differences between titanium and ceramic scaffolds could affect cell proliferation and the expression of osteoblast and osteoclast differentiation markers. Mesenchymal stem cells (MSCs) have been used in many studies of bone tissue regeneration on or around

* Corresponding author.
E-mail address: mhirota@med.yokohama-cu.ac.jp (M. Hirota).

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biomaterials, such as ceramics and titanium, because their multipotent differentiation capability provides them with osteogenic potential [13–15]. Previous study revealed that block shape scaffold with low porosity could not show sufficient bone formation compared to those with higher porosity, *in vivo* [14].

Thus, in this study, to investigate the effects of different block materials and their porosity on osteoblast and osteoclast differentiation, we used titanium fiber and βTCP scaffolds with different porosities and compared the effects of their porosity on progression of osteoblast differentiation using human MSCs (hMSCs). In addition, we also assessed osteoclast differentiation markers' expression during co-culture of osteoblasts and osteoclasts.

2. Materials and methods

2.1. Porous materials

We used titanium fiber scaffolds (Hi-lex Co, Kobe, Japan) and βTCP scaffolds (Olympus Terumo Biomaterials, Tokyo, Japan) with different porosities in the form of porous blocks that measured 10 × 10 × 3 mm (Fig. 1). We used porous titanium scaffolds with porosities of 60% (Ti60), 73% (Ti73), and 87% (Ti87). The diameter of a titanium fiber was 50 μm, and a sintered titanium fiber scaffold was processed to have an average internal pore size of 250 μm (range: Ti60; 100–150 μm, Ti73; 200–250 μm, Ti87; 300–350 μm). Porosity was determined by adjusting the titanium fiber scaffold weight per unit area for every desired porosity.

We also used porous βTCP scaffolds with porosities of 60% (TCP60) and 75% (TCP75). Fine βTCP powder was produced by wet milling. A slurry mixture of $CaCO_3$ and $CaHPO_4 \cdot 2H_2O$ (molar ratio of 1:2) in pure water was prepared in a pot mill for 24 h, and then dried at 80 °C. Crystals of calcium-deficient hydroxyapatite were converted to βTCP by calcination at 750 °C. This βTCP was then mixed to produce a foaming slurry, which was dried at room temperature for 1 day and at 40 °C for 1 additional day. Porous βTCP (purity of 99.9%) was obtained by sintering the material at 1050 °C. Porosity was determined by adjusting the dry weight of βTCP. The pore size distribution was 100–400 μm. A 90% porous body could not be made because the mechanical strength of βTCP is weak and its form cannot be maintained. The surface of each material was observed using a stereoscopic microscope (M80; Leica, Solms, Germany) for low magnification and a scanning electron microscopy (SEM) (VE9800; Keyence, Tokyo, Japan) for high magnification.

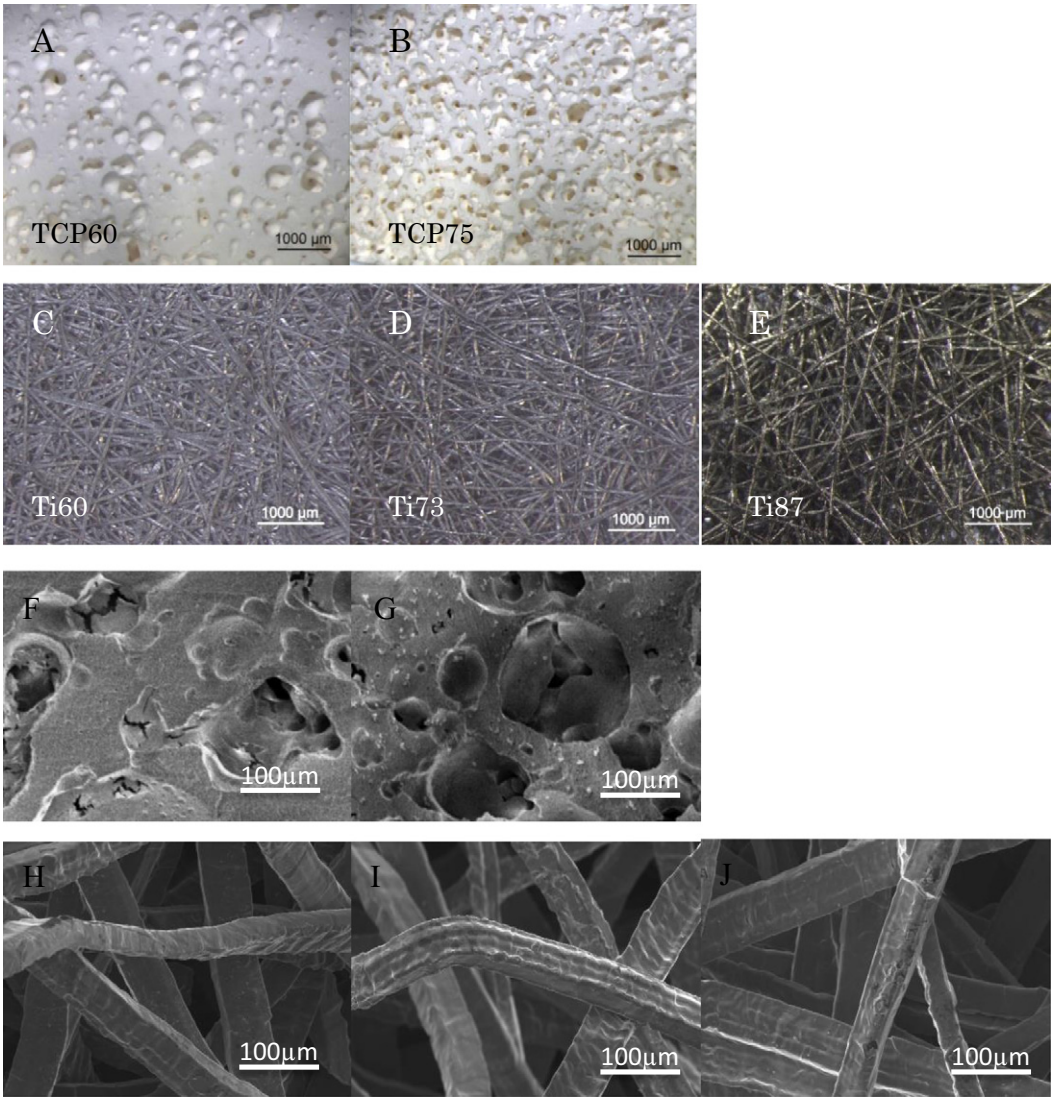

Fig. 1. Porous materials used in this study. (A, F) βTCP with 60% porosity, (B, G) βTCP with 75% porosity, (C, H) titanium fiber scaffolds with 60% porosity, (D, I) titanium fiber scaffolds with 73% porosity, and (E, J) titanium fiber scaffolds with 87% porosity.