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Osteoblast-like cell proliferation on tape-cast and sintered tricalcium phosphate sheets

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Abstract

The influence of sintering temperature on the in vitro proliferation of osteoblast-like cells to sintered tricalcium phosphate (TCP) sheets prepared by tape-casting was investigated. Green sheets of tape-cast β-TCP were sintered for 2 h in a furnace at atmospheric pressure at five different sintering temperatures: 900, 1000, 1100, 1150 and 1200 °C. The number of osteoblast-like (MC3T3-E1) cells deposited onto TCP sheets was counted after cell cultivation for 1 week and was found to have increased with increasing sintering temperature. The TCP sheets sintered at 900 °C exhibited a significantly lower cell number than TCP sheets sintered at 1000, 1100, 1150 and 1200 °C. In the attenuated total reflection infrared spectra, the peaks around 900–1150 cm^{-1}, corresponding to the P–O vibration mode of the phosphate group, gradually decreased and shifted to lower wavenumbers with increasing sintering temperature. Meanwhile, the zeta potential of TCP sintered at 900 °C showed a highly negative charge when compared with the other groups. This would suggest that the higher solubility of the TCP sheets sintered at 900 °C exerted the higher negative charge obtained from zeta potential measurement. Within the limitations of this study, it was indicated that osteoblast-like cell proliferation increased with increasing sintering temperature. The biological stability of the sintered TCP sheet surface was considered to have affected cell proliferation.

Keywords: Calcium phosphate; Bone substitute; Cell cultivation; Zeta potential; Sintering temperature

1. Introduction

Tricalcium phosphate [$Ca_3(PO_4)_2$, TCP] is a calcium phosphate-based ceramic that is widely used as a bone substitute because of its good bioactivity and osteoconductivity. In our previous study, we prepared TCP sheets as a bone substitute by a tape casting technique [1]. The major advantage of tape-casting is that the thickness of the ceramic sheet can be precisely adjusted by varying the gap between the blade (the so-called doctor blade) and the glass surface. Thus, a TCP sheet with uniform thickness could be prepared by tape-casting. TCP sheets prepared by a tape casting technique have potential for clinical applications because complex shapes with three-dimensional structures, such as cylindrical and laminated shapes, can be produced through controlled wrapping or stacking of individual flexible sheets before sintering. Thus, sintered TCP sheets are expected to find applications as tailor-made bone substitutes for different types of bone defects.

By sintering, several forms of calcium phosphate can be controlled to various densities and porosities [2,3]. Meanwhile, TCP characterized with a calcium/phosphate (Ca/P) molar ratio of 1.5 has two main phases, β-TCP and α-TCP:β-TCP is stable below 1180 °C, and α-TCP is stable between 1180 and 1400 °C [4]. Thus, the physical and chemical properties of TCP ceramics are generally influenced by the sintering processes and additives [4–6]. Wang et al. [6] reported that the physical properties, such as hardness and Young's modulus, of TCP ceramics increased

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with increasing sintering temperature. Ryu et al. [4] investigated the sintering behavior of pure β-TCP and $Ca_2P_2O_7$-doped β-TCP, and revealed that $Ca_2P_2O_7$ was an effective sintering agent for β-TCP, as it controlled the phase transformation of TCP. With these points for reference, the sintering temperature is an especially important factor in TCP ceramics because it determines the phase transformation, sintering degree and porosity; all factors that can affect the physical and chemical properties of TCP ceramics.

There are very few reports with respect to biological responses on sintering temperature of TCP sheets prepared by the tape-casting technique. It is well-known that in vitro assays with primary cultured osteoblast-like cells are a useful method for evaluating the interactions between biomaterials and osteoblast-like cells. For example, Gough et al. [7] investigated the primary attachment of osteoblast-like cells to tape-cast bioactive glass discs sintered at temperatures ranging from 800 to 1000 °C. They reported that the greatest cell attachment was seen on the 900 and 1000 °C samples. However, the in vitro proliferation of osteoblast-like cells on TCP sheets sintered at a wide range of sintering temperatures have not been investigated in detail.

The electrokinetic properties of biomaterials are of particular importance, especially the measurement of the zeta potential, as this provides a direct means of assessing the adsorption of biomolecules and the subsequent behavior of biological cells. Zeta potential measurement has been employed to quantify actual states in situ at the interface between materials and solution, depending on the polarity of the absorbed ions, the material surface charge and the ionic concentration in the fluid. Also, Smith et al. [8] suggested that zeta potential analysis is an effective predictor of biomaterial attraction to osteoblasts and bone, providing a useful in vitro method for predicting such interactions.

The purpose of the present study was to evaluate the influence of sintering temperature on the in vitro response of osteoblast-like cells onto TCP sheets produced by the tape-casting technique. Cell culture tests were carried out using MC3T3-E1 cells. We also used thin-film X-ray diffraction (TF-XRD), attenuated total reflection infrared (ATR-IR) spectroscopy and zeta potential measurement to examine the characterization of the TCP sheets at different sintering temperatures. The phase transformation, crystal reconstruction and surface charge of the TCP sheets at different sintering temperatures, and the accompanying discussion, are presented below.

2. Materials and methods

2.1. Materials

Fig. 1 shows a flowchart of the fabrication process for a β-TCP sheet. In this study, a β-TCP sheet was fabricated by a tape-casting technique of several steps, including slurry preparation, vacuum defoaming, casting and drying [1].

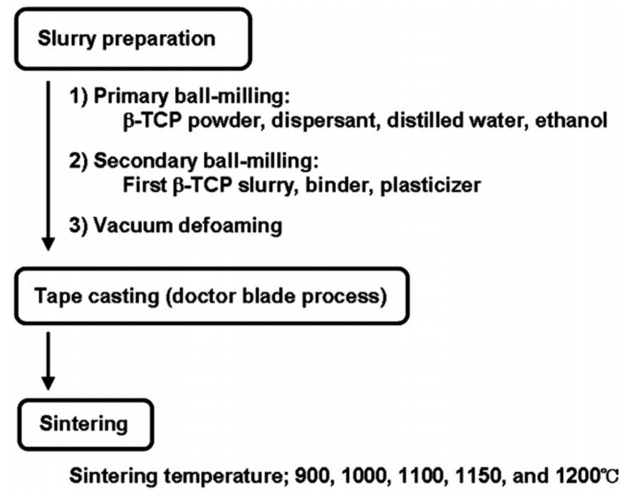

Fig. 1. Procedure for preparing sintered TCP sheets.

First, a β-TCP aqueous slurry was prepared by mixing 20 g of β-TCP powder (β-TCP-100, Taihei Chem. Ind. Co. Ltd., Osaka, Japan), 0.2 g of dispersant (AQ-2559, Lion Co., Tokyo, Japan), 4 g of double-distilled water (Wako Pure Chem. Ind. Ltd., Osaka, Japan) and 6 g of ethanol (Wako Pure Chem. Ind. Ltd., Osaka, Japan) in an Al_2O_3 container with one of two different sized Al_2O_3 balls ($\varphi = 3$ or 5 mm) for 18 h by means of a planetary ball mill (P5/2, Fritsch Japan Co., Ltd., Kanagawa, Japan). Next, a binder (3.0 g, HB-500, Lion Co., Tokyo, Japan) and plasticizer (0.4 g, PEG#600, Lion Co., Tokyo, Japan) were added to the initially prepared β-TCP slurry, and the resultant slurry was further mixed by ball milling for 2 h. Finally, the β-TCP slurry was degassed by a rotary pump for 10 min.

2.2. Fabrication of sintered TCP sheets by the tape-casting technique

A doctor blade system (DP-150, Sayama Riken Corp., Saitama, Japan) was filled with the obtained β-TCP slurry. Tape-casting was performed on a batch-process type caster, where the casting head and doctor blade traversed over a stationary floating glass slab, thereby discharging slurry onto the surface. The height of the blade was adjusted to 0.5 mm and the carrier speed was adjusted to 60 cm min^{-1}. The width of the β-TCP sheet was 150 mm. The β-TCP sheet was then dried at room temperature to remove the solvent and cut to the specimen geometry (10 mm long and 10 mm wide). Additionally, the thickness of the β-TCP sheet, as measured with a digital micrometer (CD-20CP, Mitutoyo, Kanagawa, Japan), was found to be approximately 0.18 mm, and substantially uniform.

β-TCP sheet specimens were sintered at maximum temperatures of 900, 1000, 1100, 1150 or 1200 °C, under atmospheric pressure in a furnace (MSFT-1520-P, Nikkato