

# Porosity and pore size of $\beta$ -tricalcium phosphate scaffold can influence protein production and osteogenic differentiation of human mesenchymal stem cells: An in vitro and in vivo study

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## Abstract

The interaction of stem cells and ceramics in bone regeneration is still poorly understood. The aim of this study was to examine the influence of the porosity (25%, 65% and 75%) of  $\beta$ -tricalcium phosphate (TCP) ceramics on osteogenic differentiation of mesenchymal stem cells (MSC) in vitro and in vivo. For the in vitro portion of the study, TCP scaffolds loaded with MSC were kept in osteogenic induction medium for 21 days. For the in vivo portion of the study, scaffolds loaded with undifferentiated MSC were implanted subcutaneously into SCID mice for 8 weeks and compared with similarly implanted controls that were not loaded with MSC. Measurements of total protein as well as specific alkaline phosphatase (ALP) activity were taken as indicators of growth/matrix production and osteogenic differentiation. An increase in the total protein concentration was noted from day 1 to day 21 on the in vitro TCP 65% and TCP 75% scaffolds ( $p < 0.05$ ) with no such increase noted in the TCP 25% specimens. However, the specific alkaline phosphatase activity increased from day 1 to day 21 in all three in vitro specimens ( $p < 0.02$ ) and reached similar levels in each specimen by day 21. In vivo, ALP activity of cell-loaded TCP 65% ceramics was higher when compared with both the TCP 25% and TCP 75% specimens ( $p < 0.046$ ), and higher in the TCP 75% than TCP 25% specimens ( $p = 0.008$ ). Histology revealed mineralization by human cells in the pores of the TCP ceramic scaffolds with a trend toward greater calcification in TCP 65% and 75%. In summary, a higher porosity of TCP scaffolds does not necessarily mean a higher ALP activity in vivo. The distribution and size of the pores, as well as the surface structure, might play an important role for osteogenic differentiation in vivo.

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**Keywords:** Porosity; Stem cell; Osteogenesis; Ceramic structure; Bone

## 1. Introduction

Regenerative medicine with the use of stem cells is a rapidly emerging field in the treatment of bone defects [1–5]. Mesenchymal stem cells (MSC) can easily be expanded to high cell numbers [6] and addition of MSC facilitates the healing of bone defects [7]. Cell suspensions are difficult to apply as they hardly remain in bony defects and do

not provide any biomechanical stability. Therefore, MSC are combined with biomaterials in a tissue engineering approach [8–11]. Calcium phosphate ceramics are used clinically because they combine good stability with porosity and interconnectivity, and they are non-toxic during the dissolution and degradation process [11–13]. Moreover, they allow the adhesion and growth of MSC and osteoblasts [14]. Among calcium phosphate ceramics,  $\beta$ -tricalcium phosphate (TCP), which dissolves in the presence of acids released by cells such as osteoclasts or macrophages, is distinguished from hydroxyapatite (HA), which is hardly

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degradable at all [15]. In general, it seems favourable to have cell-mediated degradation that proceeds at the same speed as new bone forms, as this allows the formation of bone with homogeneous elasticity and reduced fracture risk [16]. Critical factors that may determine the success of a MSC/biomaterial construct for osteogenesis include

- the initial adhesion of the MSC;
- survival of the MSC on the biomaterial;
- cell proliferation after loading; and
- the extent of osteogenic differentiation.

It is well described in previous studies that the porosity of the biomaterial plays a significant role in the success of an MSC/biomaterial construct [17–19]. Although several solid TCP ceramics are on the market, there is sparse data indicating what degree of porosity is most favourable for adhesion, proliferation and osteogenic differentiation of MSC in vitro and in vivo [20,21].

To this end, we examined three different TCP block materials that are often used clinically: (i) Cerasorb<sup>®</sup>, (ii) Cerasorb M<sup>®</sup> (both Curasan, Kleinostheim, Germany) and (iii) a TCP produced by the Dr. Robert Mathys Foundation (RMS, Bettlach, Switzerland) that is similar to chronOS<sup>™</sup> (Synthes). Cerasorb<sup>®</sup> granules served as the positive in vivo control because of their well-known osteoconductive properties. The porosity between the tested biomaterials differed from 25% to 75% and pore size ranged from <10 to 600 µm. TCP Cerasorb<sup>®</sup> block material with a low porosity of 25% was included into the study as a negative control since it is known that small pore size is not favourable for bone formation in vivo [22]. Nevertheless, orthopaedic surgeons like to use this material because of its strong mechanical properties (e.g. in open wedge osteotomy of the acetabulum or the tibia). We examined adhesion, protein production and osteogenic differentiation of MSC on these ceramics for 3 weeks in vitro and osteogenic differentiation of freshly loaded undifferentiated MSC composites in vivo for 8 weeks.

## 2. Material and methods

### 2.1. TCP scaffolds

Three different porous TCP block forms were used: Cerasorb<sup>®</sup> (TCP 25), Cerasorb M<sup>®</sup> (TCP 65) and the TCP by RMS (TCP 75). In comparing these constructs, there are significant differences regarding micro- and macroporosity, while the specific surface area (SSA, in m<sup>2</sup> g<sup>-1</sup>) is equally low in all of them (Tables 1A and 1B). In the in vivo assay, Cerasorb<sup>®</sup> granules with particles ranging from 1000 to 2000 µm and micropores of <5 µm were used as a positive control. To illustrate the surface that is accessible to the cells, we calculated the surface per apparent volume (m<sup>2</sup> cc<sup>-1</sup> = SSA × density × (1-porosity)) and the total surface per scaffold (m<sup>2</sup> =

Table 1A  
Physical parameters of the biomaterials

Material	Total porosity (%)	Pore diameter			
		600–200 µm (%)	200–50 µm (%)	50–5 µm (%)	<5 µm (%)
TCP 25 block forms (Cerasorb <sup>®</sup> , Curasan) <sup>a</sup>	~25 ± 5	0	2	5	18
TCP 25 granules, 1000–2000 µm (Cerasorb <sup>®</sup> , Curasan) <sup>b</sup>	~59.3 ± 2.5	20	10	5	20
TCP 65 block forms (Cerasorb M <sup>®</sup> , Curasan)	~65 ± 5	5	15	20	25
TCP 75 block forms (RMS) <sup>c</sup>	~75 ± 0.4	54	0	0	21

These data were provided by the manufacturers.

<sup>a</sup> The block forms are usually provided with macroporosity by drilling interconnecting macropores with a diameter of 0.5–2.0 mm to reach an overall porosity of 65–80%.

<sup>b</sup> The overall porosity of granulates is calculated by the intergranular porosity (measured by Hg porosimetry) and intergranular voids.

<sup>c</sup> Structure similar to that of chronOS<sup>™</sup>.

Table 1B  
Physical parameters of the biomaterials

Material	Specific surface area (m <sup>2</sup> g <sup>-1</sup> )	Surface/ apparent volume (m <sup>2</sup> cc <sup>-1</sup> )	Surface/ scaffold (m <sup>2</sup> )
TCP 25 block forms (Cerasorb <sup>®</sup> , Curasan) <sup>a</sup>	0.12	0.28	0.0076
TCP 65 block forms (Cerasorb M <sup>®</sup> , Curasan)	0.18	0.2	0.0052
TCP 75 block forms (RMS) <sup>c</sup>	0.3	0.23	0.0068

weight of scaffold × SSA). Phase purity was high, at more than 99% in all scaffolds.

The TCP for the Cerasorb<sup>®</sup> products was produced by a solid-state reaction from calcium carbonate and calcium hydrogen phosphate. Microporous Cerasorb<sup>®</sup> block forms were produced by milling after cold isostatic pressing and a final sintering step. For producing Cerasorb M<sup>®</sup> block forms with additional meso- and macropores, an organic porogen substance was added for sintering before pressing. The porogen substance burns away, leaving pores behind. The TCP by RMS was synthesized in an emulsion process and subsequent sintering [15]. All ceramic bodies were machined in cuboids (5 × 3 × 2 mm) with a volume of 30 mm<sup>3</sup>. The Cerasorb<sup>®</sup> granule samples had the same weight as the block forms.

### 2.2. Structural and chemical analysis of the ceramics

The distribution of the pore sizes was analysed by mercury intrusion porosimetry (Pascal 140-240/440, Porotec,

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