# 3D Interconnected Calcium Phosphate Scaffold for Bone Tissue Engineering: Enhancement of Mechanical and Biological Properties

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**Introduction:** The increasing need for skeletal reconstruction due to bone tumors, trauma, disease, birth defects, and/or war injury demands improved mechanical and biological properties of the existing scaffold materials. Along with biological and mechanical properties, architectural features are also important for scaffolds used in tissue engineering application. Three dimensional interconnected macropores are essential for scaffolds for better cell adhesion, mechanical interlocking between host tissue and scaffold, and flow transport of nutrients and metabolic waste [1]. Calcium phosphates (CP's) are widely used in bone-tissue engineering due to their excellent bioactivity and compositional similarities to bone**.** They offer the advantage of being custom manufactured with respect to the patient and target application, in addition to possible alternatives to autograft and allograft bone. **Objective** of this research is to understand the microwave heating effect on mechanical strength enhancement, and the effect of interconnected macroporores and pore size on *in vitro* and *in vivo* osteogenesis. Our **hypothesis** is that *improved mechanical strength will allow these 3D interconnected macroporous scaffold to withstand in vivo stresses at the site of application and interconnected macropores will help to reduce the healing time by enhancing bone ingrowth inside the 3D macroporous network.*

**Methods:** β-tricalcium phosphate with an average particle size of 550 nm was used in this study. Scaffolds architecture was designed by computer aided design (CAD) software having a dimension of 7 mm (h) and 10.5 mm (ϕ). Scaffolds with three different 3D interconnected macropore sizes, 500 µm, 750 µm and 1000 µm, were designed for mechanical strength comparison. 3D printing was performed by R-1 R&D 3D printer (ProMetal®, ExOne LLC). A conventional furnace, 1250 °C for 2 h, and a microwave furnace, 1250 °C for 1 h, were used to sinter green scaffolds. Phase analysis was carried out by XRD. *In vitro* bone cell interactions were investigated by culturing human fetal osteoblast cells (hFOB) for 3, 7, and 11 days of incubation period. Implants for the *in vivo* study were fabricated with a smaller dimension considering rat femur size. Scaffolds having 230 µm, 350 µm, and 470 µm interconnected macropores with a dimension of 5.2 mm (h) and 3.4 mm (ϕ) were fabricated for *in vivo* study in the cortical defect of rat femur.

**Results:** Calculated porosity of the scaffolds by Archimedes method was between 29% and 43%, while designed porosity was between 27% and 41%. **Fig. 1** XRD pattern of the scaffolds sintered at 1250 °C by conventional and microwave sintering. The characteristics peaks of β-TCP and α-TCP match well with JCPDS # 09-0169 (β-TCP) and 09-0348 (α-TCP). Presence of some additional α-TCP peaks after sintering is due to the high temperature phase transformation from β-TCP to α-TCP. β-TCP is not stable above 1125 °C. SEM images revealed intrinsic micropores (<5 µm) in addition to designed

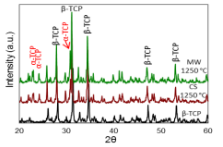


**Figure 1 .** XRD patterns of the scaffolds sintered after conventional (CS) and microwave (MW) sintering sintering.

**Table 1. .** Compressive strength comparison of the 3D printed scaffolds sintered at 1250 °C.

| Pore Size | Compressive Strength (MPa) CS | Compressive Strength (MPa) MW |
|---|---|---|
| 1000 µm | 2.71±0.67 | 4.61±0.33 |
| 750 µm | 4.37±0.40 | 6.42±1.14 |
| 500 µm | 6.62±0.67 | 10.95±1.28 |

macropores. Significant increase in compressive strength, 46-69%, was achieved by microwave sintering (MW) compared to conventional sintering (CS) as shown in **Table 1**. A compressive strength of 10.95±1.28 MPa and 6.62 ±0.67 MPa for scaffolds with 500 µm macropores was achieved by microwave and conventional sintering, respectively. **Fig. 2** shows the scaffolds after sintering. Cell morphology by SEM images and *in vitro* MTT assay by human osteoblast cells showed an increased cell

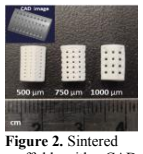


**Figure 2.** Sintered scaffolds with a CAD image (inset).

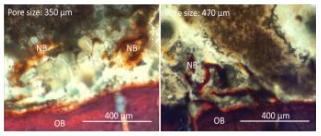


**Figure 3.** Photomicrograph images showing osteoid like new bone (NB) formation inside the macropores after 2W implantation in rat femur.

adhesion and proliferation with the decrease in macropore size. Histomorphological analysis after 2 and 4 weeks revealed improved tissue material integration by osteoid like new bone formation in the fibrous interzone and within the pores of these 3D bioresorbable β-TCP scaffolds, as shown in **Fig. 3**.

**Conclusions:** For the first time, we have applied microwave sintering to enhance the mechanical strength of 3D printed biodegradable β-TCP scaffolds with 3D interconnected macropores. Volumetric heating of microwave sintering [2] resulted in improved sinterability, which resulted in better mechanical strength of the 3D macroporous scaffolds. 3D printing of ceramic scaffolds coupled with microwave sintering could be very promising for tissue engineering applications. Mechanical strength obtained by microwave sintering of these 3D macroporous ceramic scaffolds makes them suitable for non-load bearing hard tissue replacement or repairmen. Good biocompatibility and osteogenesis was shown by these 3D printed, microwave sintered 3D macroporous ceramic scaffolds both *in vitro* and *in vivo*.

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**References:** 1. Hutmacher DW. Biomaterials 2000; 21:2529-2543.
2. Bose S. Acta Biomat. 2010; 6:3782-3790.