

**3D Printing Highly Porous Bone Scaffolds.**  
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**Statement of Purpose:** The use of 3D printing in the medical industry in tissue regeneration is expected to grow prominently in the coming years. Our lab has previously developed nanofibrous implants for bone regeneration, but in response to developing 3D printing technologies we have designed and fabricated bone scaffolds using 3D printing. Specifically, we have adopted our methods for producing porous, osteoconductive and vasculature-inducing, nanofibrous bone scaffolds for 3D printing. The results of this study display the ability of this porous scaffold to develop new bone and vasculature while bearing physiological load. These osteoconductive scaffolds have high porosity and optimal micro pore size in the range of 150  $\mu\text{m}$  and macro pore size in the range of 400  $\mu\text{m}$ . The scaffold with mineralized trabecular sections and pre-vascularized cortical sections promote cell adhesion, proliferation and formation of vascular networks. As seen in our previous scaffolds, these 3D printed implants have cortical sections modeled based on the structure of osteons. These scaffolds have good mechanical properties and are further reinforced using structural posts. Using 3D printing technology ensures greater control over the structure, porosity and pore size, and also reduces time, effort and human error.

**Methods:** PLA scaffolds were printed using an Ultimaker 2+ and designed using Solidworks with emphasis on porosity and pore size. Additional porosity was added using either water soak-freeze technique or NaOH treatment. Pore size and structure was checked using SEM imaging. Before cell studies, PLA scaffolds were sterilized using glutaraldehyde and UV radiation, and scaffolds made of PLA and HAP were sterilized using 70% ethanol and UV radiation. Rat fibroblasts were seeded on the scaffolds to check cell adherence behavior on printed material. The media used for this study was  $\alpha$ -MEM with FBS and Penicillin-Streptomycin. Presto blue assay was conducted on days 3 and 7, and DAPI and Phalloidin stain imaging was also done for the same. Scaffolds were mineralized under static conditions using SBF and  $\text{NaHCO}_3$  and conditioned for vascularization. The degree of mineralization was checked using Alizarin red staining method. Vascularization was achieved using HMECs in a ten day cell study with presto blue assay conducted on days 3 and 7 to check cell metabolism. The media used for this study was MCDB131 with hydrocortisone, glutamine, Penicillin-Streptomycin and epidermal growth factor. Compression testing using Instron 5869 was done to check the scaffold's load bearing capacity. Bone tissue formation was induced by seeding scaffolds with HMSCs, and a ten day study involved measuring ELISA osteocalcin secretion levels on days 1, 3 and 7.

**Results:** Similar to our previous electrospun scaffold the 3D printed design scaffold sponsored vascularization due to the presence of decellularized vascular tissue and also lead to bone tissue formation due to the presence of calcium phosphate from mineralization confirmed by ELISA. Pore size and porosity is optimized using the soak-freeze technique and 3D designing as can be observed by SEM imaging. 'Soak-freeze' technique has higher effect on mineralization when compared to 'no-freeze' treatment as can be observed from the Alizarin Stain data. NaOH treatment does not seem to affect or better mineralization. Scaffolds that haven't been put through the 'soak-freeze' technique have greater mechanical properties than those which have been put through the 'soak-freeze' technique. This could be because the latter has a higher number of micro pores. However, when combined with NaOH treatment and mineralization, mechanical properties of 'soak-freeze' scaffolds greatly improved. The micro pores are created to serve as pockets for mineral deposits (as well as cell movement), and therefore only 'soak-freeze' technique is preferred over the other treatments leading to optimal porosity and mineralization. Mechanical properties are improved greatly by addition of structural columns. Biological response is confirmed using presto blue assay and DAPI and Phalloidin imaging which shows that 3D printed material can be used to generate bone scaffolds successfully.

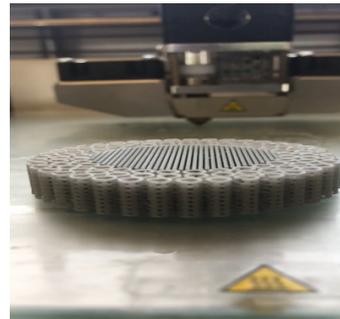


Figure 1. The printed porous bone scaffold.

**Conclusions:** Porous, osteoconductive, load bearing, vasculature inducing bone scaffolds can be successfully designed and 3D printed. Greater control over design structure, pore size and porosity, and time is ensured by using 3D printing technology.