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Significance of Three-Dimensional Printed Beta Tri Calcium Phosphate Scaffold as an Adjuvant to Bone Reconstruction of Oral and Maxillofacial Surgery Defects as a Future Facet in Oral and Maxillofacial Surgery Reconstruction.

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ABSTRACT

Maxillofacial trauma, chronic odontogenic infections and resection of benign or malignant tumors may leave defects in the bone. It's been an endeavor to majority of oral and maxillofacial surgeons to reconstruct these bony defects so as to improve esthetics, function and overall quality of life of the patients. Various materials have been tried in the form of allografts and alloplasts. Autogenous bone transplantation especially free vascularized tissue transfer is considered a "gold standard of care" for maxillofacial reconstruction in patients undergoing major ablative surgery, as it provides three essential elements: osteoconduction, osteoinduction, and osteogenic cells. But the major drawback is that it necessitates a second surgical site with a significant risk of morbidity. Moreover, despite the availability of various reconstructive methods by means of autogenous and allogenic tissue, perfect maxillofacial reconstruction, including restoration of continuity, sensation, dentition, soft tissue, function, and aesthetics is still not achievable. As a consequence, maxillofacial bone reconstruction still remains a surgical challenge in reconstructive surgery. Biocompatible 3D scaffolds designed to accommodate these mass transport requirements while offering a load-bearing matrix during the bone healing process.

Keywords: Beta Tcp scaffold, stem cell, biomaterials, tissue engineering, maxillofacial, 3D printing.

INTRODUCTION

The maxillofacial region, tissue regeneration is in high demand due to trauma, chronic odontogenic infections and resection of benign or malignant tumors may leave defects in the bone. Successful regeneration of affected tissues is necessary to reconstruct facial support, allow for functional and cosmetic makeover. One of the recent advances in the field of tissue engineering aims to restore tissue function by growing cells on a designed scaffold that creates a three-dimensional microenvironment for cell support. 3D scaffolds designed to accommodate these mass transport requirements while offering a load-bearing matrix during the bone healing process. A fundamental requirement for tissue-engineered bone grafts is the ability to integrate with the host tissues, while providing the capacity for load-bearing and remodeling. The size of scaffold-tissue constructs that can be cultured is limited due to high metabolic activity of bone cells. Thorough knowledge of the molecular biology of bone formation and remodeling is a stepping stone for making advances in craniofacial bone tissue engineering. Combining biomaterials, often with competing properties to fabricate optimized scaffolds for use in craniofacial skeletal regeneration is representative of current research trends and the most promising strategy for tissue engineers and craniofacial surgeons. New advances unlocking the osteogenic potential of several stem cell types, as well as the discovery of more readily available stem cell sources, are also providing exciting prospects for craniofacial bone regeneration. The combination of stem cells, growth factors, small molecules, and Nano printed scaffold materials used in reparative bone tissue engineering will largely be guided by these and other complicating factors.

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Beta Tcp scaffold synthesis and fabrication:

β-tri-Calcium phosphate, Hydroxyapatite Nano powder, were used for scaffold synthesis by bioplotting.

3D-Bioplotting of HA/TCP Scaffolds

First, the β-TCP powder was sieved through a mesh size of 500 to get uniform particle size of 25µm. Afterwards, Nano size (<200 nm) HA powder was added to TCP at a concentration of 20%. Binder solution was prepared by mixing 3 grams of polyvinyl alcohol (PVA) in 37 grams of water. The solution was stirred for 30 min to get the binder solution which was subsequently sieved to remove suspended particles. Finally, 10 grams of HA/TCP powder was further added to 7 grams of binder solution to get a uniform viscous ceramic paste.

The solid freeform process was carried out using 3D-Bioplotter, Developer Series, Envision TEC, Germany. The system consists of

- Air pressure system to control the flow of the ceramic paste;
- Paste dispensing unit having syringe and nozzle
- Control unit which is connected to a computer having software (Visual Machines) to regulate the fibre deposition path.

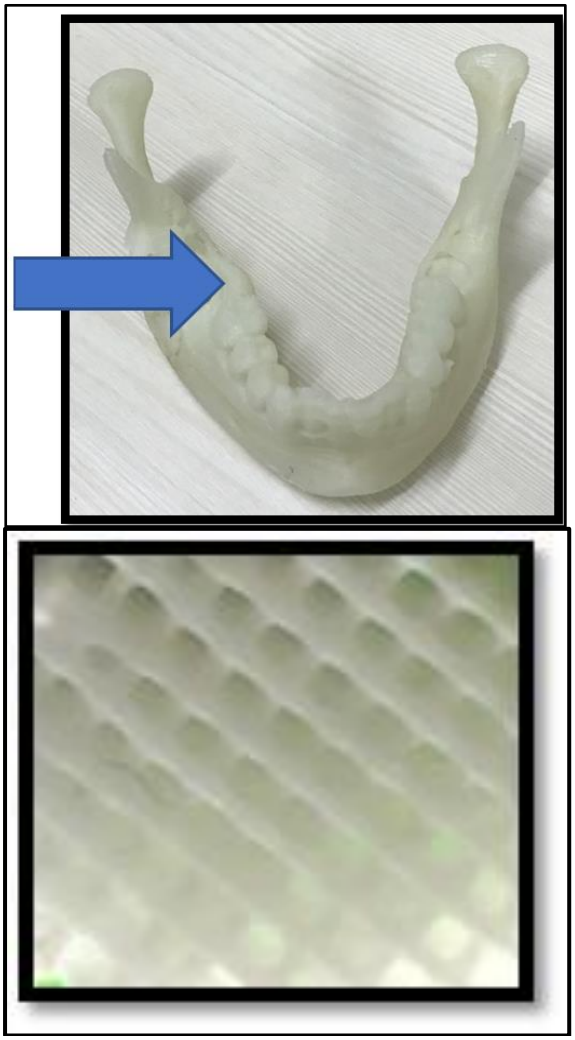

Figure 1a,b: 3-D printed beta TCP structure of mandible post bio plotting.

self-assembly and phase separation:

A 30ml PE (Polyethylene) syringe was filled with the ceramic paste and was placed inside the low-temperature head, which is fixed on the vertical axis of the machine. The paste was pushed outside the nozzle by applying air pressure to the plunger. Rectangular models of size 10mm x 10mm x 3mm were loaded on the software of the machine.

Scaffolds were printed using PE plastic nozzle having length 32mm and an inner diameter of 400µm. For all architecture types, the distance between strands was set to 1mm in order to generate pore size of around 500 to 600µm. The temperature of the printing head was set to 30C and the printing was done at a speed of 15mm/s with a pressure of 3.6 bars. The fabricated scaffolds were first allowed to dry at room temperature for 24 hours after which they were kept in the oven for another 24 hours to vaporize the water present in the samples. Afterward, the samples were subjected to sintering process in a silicon carbide furnace. Courtesy by Virchow biotech pvt. Ltd.

Bone tissue regeneration:

Autogenicity induction can be performed using tissue engineering process by laboratory process of admixture of stem cells, fibrin glue with growth factors inoculated into the biocompatible 3D printed scaffold printed and sterile in a sterile medium and inserted into the growth medium. Cell infiltration, migration, and proliferation of human osteogenic stem cells on the scaffolds can be carried out under both static and dynamic culture conditions. A computational simulation model and a series of immunofluorescent H&E staining implemented to understand the mechanism of cell behaviour and scaffold response. Immediate research methodology to be published in the next sequence of the present study.

Patient-specific defects:

Because of the highly specific and unique characteristics of the facial skeleton and widely varied craniofacial defects and complex shapes, there is a need for patient-specific tissue regeneration. To serve this need, our laboratory has developed technology to create patient-specific, anatomically shaped scaffolds. A scaffold unique to each patient can be helpful in regenerating the shape of the facial skeleton, for example, since it has complex geometry and can vary highly between individuals. This can be done by the creation of a wax mold from computed tomography (CT) scans of the patient's mandible (Fig. 1a). The mold can be used to form the phase-separated nanofibrous scaffold with macroporous structure and patient-specific maxillofacial regeneration. Such biocompatible scaffolds supported bone formation when seeded with MSCs and growth factors. Similar biotech advancements in scaffold

regeneration will be of great endeavour in maxillofacial skeletal reconstruction. This technique holds great potential for maxillofacial tissue replacements that are specific to each patient for best restoration of natural appearance and function.

DISCUSSION

Hard tissue structure comprises of cortical and cancellous bone. Cortical bone is closely packed sharing 80% of the whole bone mass while remaining 20% is shared by cancellous part. Remodelling and maintenance of bone is carried out by different cells. Osteoblast cell is responsible for bone generation, whereas the function of Osteoclast cell is bone resorption. The communication among different cells (Osteocyte, Osteoblast and Osteoclast) together is responsible for maintaining healthy bone.[1] Such intricate structures can be successfully fabricated by additive manufacturing (AM) techniques. Some major techniques involve, Three-Dimensional Printing (3DP), Stereo lithography (SLA), Fused Deposition Modeling (FDM), Selective Laser Sintering (SLS), 3D Plotted, β-TCP is considered to be an important bone graft material in clinical use due to its solubility, excellent biocompatibility, osteoconductivity and ability to provide a temporary framework for bone remodelling.[2,3] However, the use of β-TCP is limited by its inherent lack of osteoinductivity, the ability to induce new bone formation. One of the most commonly adopted procedures to integrate osteoinductivity in calcium phosphate scaffolds is by incorporation of bone derived growth factors like BMP-2, IGF-1 & 2, VGEF etc.[4,5] However, it is necessary to ensure a sustained and controlled release of these growth factors from the scaffold and also, long term viability of these protein poses as a major challenge in scaffold incorporation.[5] Scaffold exhibited a very dense physical appearance with bright white shade having rough surfaces. The burning of ABS did not induce any deformation in the overall structure since the heating was achieved very gradually. However, high-temperature sintering caused volumetric shrinkage in the scaffold. ABS molds of size 7 mm × 7 mm × 7 mm each with ceramic slurry when subjected to sintering yielded scaffolds admeasuring 5 mm × 5 mm × 5 mm after sintering. This indicates the reduction in the volume by the ratio of 2.744. The shrinkage is caused because of, first, evaporation of binder from the samples and second due to diffusion of beta-TCP and ZrO2 particles. This leads to compaction in the sample, making it harder. Compression test results indicated an increase in compressive strength values for samples with an increase in ZrO2 content. Compressive strength and density of samples increased from 3.674 Mpa for the sample with 10% zirconia content to as much as 15.954 Mpa for the sample with 50% zirconia content. Although the overall porosity decreased from 82.5 to 68.5% with an increase in ZrO2. The above results indicated that the strength and porosity values were well within the required range for human trabecular bone. Compressive strength value depends on the shape and porosity of samples. Cubic samples with (5 mm × 5 mm × 5 mm) each were used in the present study for evaluating their mechanical properties. The achievement of cell proliferation on scaffold depends on various parameters like its porosity, surface area to volume ratio, strut/wall thickness, anisotropy, cross sectional area, permeability and interconnected porosity. A perfect scaffold should have interconnected macro pores in the range of 100 to 350 μm for good release of oxygen and nutrients and micro pores on the scaffold surface in the range of 5 to 10 μm for initial cell adhesion.[6,7] Development of a channelled porous scaffold, which can facilitate nutrient diffusion to the maximum extent without sacrificing its mechanical strength, and promote cell infiltration and proliferation, as demonstrated in this study, is significantly promising in enhancing angiogenesis for bone regeneration. However, limitations in the current study are as follows, first, the scaffold's function on promoting osteogenesis in vitro was to be studied yet. Second, the signalling pathway that regulates cell migration and angiogenesis was not deeply investigated, though we considered an integrin and CD318. The regulation mechanism could be investigated in the future. More cell motility related-markers need to be identified for fully discovering the mechanisms of the promoted cell infiltration by multiple channels. Third, the potential ability of the multiple-channelled porous β-TCP scaffolds without any growth factors or exogenous implanted cells to promote vascularization and bone regeneration in vivo needs to be verified in the next step. Future works will focus on the function of multiple-channelled scaffolds in promoting angiogenesis and osteogenesis in vivo.

CONCLUSION

The fields of maxillofacial surgery and tissue regeneration have advanced to create a suitable microenvironment for cell development for the regeneration of various tissues. Beta Tcp scaffolds are one such biomaterial approach shown to enhance patient specific bone regeneration and reconstruction. Growth factors imbibition on the scaffolds with osteogenic potential induction by mesenchymal stem cells will improve specific tissue formation through sustaining the bioactivity of the biological factors. However, more studies are required to understand the mechanisms of the

biocompatible effects. There remain significant technical challenges for the synergistic integration of structural cues with biological cues for cell-based reconstructive aspects to achieve functional maxillofacial tissue regeneration.

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