Official Journal of TESMA
Regenerative Research
www.regres.tesma.org.my
E-ISSN 2232-0822
Tissue Engineering and Regenerative Medicine Society of Malaysia

Regenerative Research 7(1) 2018 66-68

FABRICATION AND OPTIMIZATION OF POROUS 3D BIOCERAMIC SCAFFOLD USING GEL CASTING METHOD

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ARTICLE INFO

Published: 26th August 2018
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KEYWORDS

Hydroxyapatite;
Beta-Tricalcium Phosphate;
Bioceramic;
Porous Scaffold

SUMMARY

Hydroxyapatite and Beta-Tricalcium Phosphate has been clinical used as bone substitute. Porous substitute suitable to act as scaffold as pores allow cells attachment. Interconnected pores are needed for infiltration of osteoprogenitor and host cells all over the whole construct. This study is to fabricate and evaluate the physical characteristics of porous bone substitute fabricate from HA and β-TCP by gel casting method. HA and β-TCP powders were mixed homogeneously to fabricate into 3D porous bone substitute. XRD and FTIR analysis showed the sintered bioceramic retain same structural formulae as raw materials. MicroCT analysis showed fabricated bioceramic contain interconnected pores. Based on the obtained results, we have successfully fabricated porous scaffolds that can perform as bone substitute.

1.0 Introduction

Bone loss could be caused by various incidences such as bone disease, trauma, surgical processes, bone inflammation etc. Bone has the ability to regenerate on its own. However, there are certain bone loss such as non-union defect which are too large to be regenerate on its own. Non-union defect requires bone graft to aid in the regeneration. There are various types of bone grafts are been currently clinical practiced to treat bone loss. All these bone loss treatments can be grouped into few categories such as autograft, allograft, xenograft, metal implants, synthetic materials and tissue engineered bone graft. Autografts transplanting require no chemical processing as autograft is osteoconductive in nature make it always the gold standard as bone substitute for orthopedic patients. Although autograft provide the best recoveries for bone defect treatment, it is quite limited and it caused inevitable injury to the donor site. Therefore, allograft and xenograft are being used in clinical practice to extend the graft volume. Allografts poss the risk of caring bacterial, viral disease transmission or immunogenic response which also happen to xenografting which origin from animal. Metal implants which provide strongest mechanical strength does not promote bone formation and have the risk of obstruction of blood flow on the injury site. All the well-known limitation of the bone grafts on clinical uses are driving the development of new alternatives to overcome the shortcoming of the bone grafts. 3D synthetic bone grafts hence been proposed to perform as bone grafts on bone repair and restoration of damaged bone.

Ideally, 3D synthetic bone grafts should pose natural bone's properties. Synthetic biomaterial should possess certain properties to be functionally used in body, including good biocompatibility, sufficient mechanical strength, good osteoconductivity, and osteoinductivity, ability to be fabricated into functional shapes, no immunologic potentiality, and controlled bioresorbability. Like bone, 3D synthetic bone grafts should contain interconnective pores for vascularization and blood flow. But we have always take into consideration that the bone synthetic grafts are not mechanical strong when it is porous, therefore, we need to always to balance the porosity and mechanical strength of the grafts. Interconnective pores also allow to host cells to house in and further develop into bone tissue. In the past, many materials such as alumina ceramics, bioglass, and calcium phosphate have been used to fabricate synthetic bone grafts. Calcium phosphates like Hydroxyapatite/HA ($Ca_{10}(OH)_2(PO_4)_6$) and Beta-Tricalcium Phosphate/β-TCP (β -$Ca_3(PO_4)_2$) has been clinical used in orthopedic surgery as bone substitute. Porous bone substitute suitable to act as scaffold for bone tissue engineering as the pores allow cells attachment. Interconnected pores are needed for infiltration of osteoprogenitor and host cells all over the whole construct.

This study is done to fabricate and evaluate the physical characteristics of porous bone substitute fabricate from HA and β-TCP by gel casting method so that further optimization on cell density can be performed.

2.0 Materials and Method

HA and β-TCP powders were mixed homogeneously and dissolve in monomer (14.17 wt% Dimethacrylamide and 2.83 wt% N,N-methylenebisacrylamide) solution in the weight ratio of HA:β-TCP = 7:3 to fabricate into 3D porous bone substitute. HA and β-TCP powders were fabricated using gel casting method by using polyurethane foam/PUF (Sigma Aldrich, USA) as the mold. The powder solution crosslinked by adding ammonium per sulphate and N,N,N`N` tetramethylenediamine (TEMED) and turn into gel together with the PUF with very a few seconds. Bioceramic gels were sintered with PUF at very high temperature to become 3D porous HA/β-TCP bioceramics as PUF was burned to ash in between of the sintering process. The space of PUF burned become the interconnected pores of the 3D bioceramic after sintering. After sintered, the product has been undergo physiochemical analysis which is Fourier Transform Infra-Red Spectocopy, X-Ray Diffraction and MicroCT.

3.0 Results

Figure 1 shows fabricated bioceramic scaffold and its cross section revealing the inner structure of the scaffold.

Fig 1. Gross image of a HA/TCP scaffold and its cross section after sintering

3.1 Fourier Transform Infra-Red Spectocopy/FTIR

FTIR spectrum shows that presence of ${PO_4}^{3-}$ and ${HPO_4}^{3-}$ in fabricated 3D HA/ β-TCP porous bioceramics. We compared it with the spectrums of HA and β-TCP. It appears that HA and β-TCP show similar peak of ${PO_4}^{3-}$ at (1021-1025 cm^{-1}), (599-601cm^{-1}), (545-561cm^{-1}) and ${HPO_4}^{2-}$(962-973cm^{-1}) (Fig. 2).

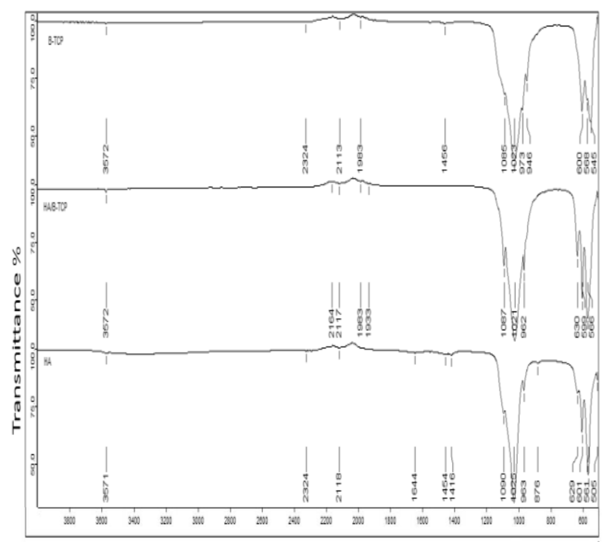

Fig 3. FTIR spectrum of HA/TCP scaffold. Spectrums of HA and TCP alone are used as the reference.

3.1 X-Ray Diffraction/XRD

XRD results shows similar pattern of peaks between fabricated HA/ β-TCP with the pure HA and pure β-TCP powders. The results show that the 3D sintered HA/ β-TCP still retain the same chemical structural formulae (Fig.2).

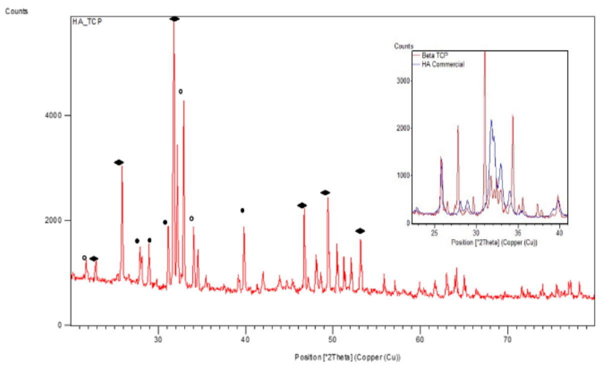

Fig 2. X-ray diffraction spectrum of HA/TCP scaffold. Insert: Spectrums of HA and TCP alone as the reference.

3.3 Micro-Computered Tomography/µCT

MicroCT scanning was carried out to investigate the porosity and pore properties of fabricated porous bioceramic (n=6). MicroCT analysis showed that the fabricated porous bioceramic scaffolds are porous and contained interconnected pores (Fig.3). Using ctAN software, we managed to quantify the distribution of pore sizes of each scaffold as shown in Table 1. The mean porosity (n=6) was 40.47%.±0.047% while the mean open porosity (n=6) was 38.84%.±0.046%. Thus, 95.97%.±0.001 % of the total pores are open pores.

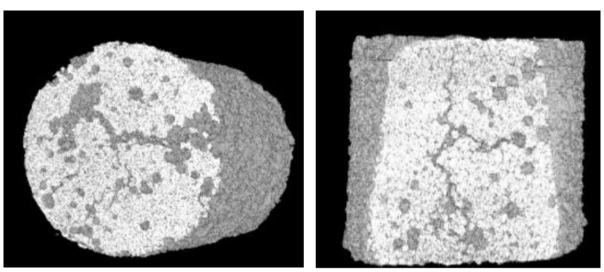

Fig 4. µCT images of longitudinal and transverse sections of a HA/TCP scaffold

Table 1 Distribution of pore size for six fabricated HA/ β-TCP scaffold

Pore Size	Percentage Distribution					
	1	2	3	4	5	6
<0.2mm	26.15%	22.87%	36.58%	26.61%	25.94%	20.10%
0.2mm-0.5mm	25.79%	22.78%	27.40%	25.57%	24.94%	19.60%
0.5mm-1mm	22.16%	35.14%	23.18%	21.49%	23.87%	17.24%
1mm-2mm	20.17%	17.08%	12.46%	7.51%	6.73%	8.20%
2mm-3mm	5.73%	2.13%	0.38%	6.27%	3.47%	9.10%
3mm-4mm	0.00%	0.00%	0.00%	7.44%	7.45%	14.51%
4mm-5mm	0.00%	0.00%	0.00%	5.11%	7.60%	11.25%

4.0 Discussion & Conclusion

X-ray powder diffraction/XRD and Fourier-transform infrared spectroscopy/FTIR analyses showed that the 3D bioceramic scaffolds were able to retain the same structural formulae and the functional groups as the raw materials (HA and β-TCP). This ensures the functional activity of these scaffolds and its biodegradability..

MicroCT images reveal that there were areas especially in the central region of certain scaffolds that contain large pores. This is probably due to the incomplete soaking of the PUF with the ceramic slurry. The insufficient ceramic in the scaffold could be overcome by repeated soaking of the PUF in the ceramic slurry during the formation of green body.

Relationship between porosity and mechanical strength had always been taken into consideration in the fabrication of tissue engineered scaffolds. Based on our results, the fabricated HA/TCP scaffolds achieved 40% porosity. Although this may be lower than the targeted > 50%, 98% of the pores were open and interconnected. This feature is ideal for scaffold meant to be incorporated with cells. In conclusion, the method employed successfully produced bioactive ceramic scaffolds with highly interconnected pores.

Acknowledgement

This work is supported by funding from Trans Disciplinary Research Grant Scheme TRGS/2/2014/UKM/02/4 and Fundamental Research Grant from the Faculty of Medicine, Universiti Kebangsaan Malaysia (FF2016-111).

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