Peer Review File

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Reviewer Comments

Comment 1: The phrase “In a previous study, we investigated the effects of applying surface modifications with bioactive glass (BG) and mesoporous bioactive glass (MBG) to selective laser melted (SLM) Ti-6Al-4V scaffolds and demonstrated that such treatments led to enhanced attachment, proliferation, and differentiation of human bone marrow stromal cells (hBMSC).” In abstract sounds, weird for me.

Reply 1: The opening sentence was meant to set the background of the research reported in the present article. We have modified this sentence to make it more eloquent:
“The results obtained from our previous study have indeed demonstrated that surface modification of selective laser melted (SLM) Ti-6Al-4V scaffolds by applying bioactive glass (BG) or mesoporous bioactive glass (MBG) coating can enhance the attachment, proliferation, and differentiation of human bone marrow stromal cells (hBMSCs).”

Comment 2: Pag 3 Line 3 - a major clinical challenge [0, - which reference is this?

Reply 2: It was indeed an error and should be [1-2]. It has been corrected.

Comment 3: Pag 3 Line 25 “bone substituting material” I am not English native, but sounds weird for me

Reply 3: The phase is better hyphenated: “bone-substituting material”. We have changed it into “bone substitute material”, which is more commonly used in the field of materials for orthopedics, e.g.,

http://www.biomet.co.uk/userfiles/files/Biomaterials/Bone%20substitute%20materials.pdf

https://www.zimmerbiomet.com/medical-professionals/biologics/product/n-force-blue-bone-substitute-material.html

Comment 4: Pag 4, line 6 extensive research has brought BG - sounds weird for me

Reply 4: We have changed the sentence into:
"Since then, BG has been extensively used in a variety of biomedical fields including the repair of (mostly non-load-bearing) bony defects."

Comment 5: Pag 4 line 8 by the virtue of its - sounds weird for me

Reply 5: We have corrected the phase and modified the sentence into:
"In recent years, BG has exhibited great protential for bone tissue regeneration by virtue of its excellent osteoconductive and osteostimulatory properties.

Comment 6: Pag 4 line 9 osteostimulatory properties - sounds weird for me

Reply 6: "osteostimulatory properties" or "osteostimulation properties" is a term commonly used by the researchers in the field, e.g.,

https://link.springer.com/article/10.1007/s00223-010-9374-z

http://www.lifesciencesite.com/lsj/life140417/14_32123lsj140417_111_116.pdf

Comment 7: Pag 4 line 25 with the help of a rabbit bone defect model – sounds not scientific

Reply 7: We have changed the whole sentence into:
"Here, we set the next step and utilize the rabbit bone defect model to evaluate the bone tissue regeneration performance of 3D-printed Ti-6Al-4V scaffolds with BG and MBG surface modifications *in vivo*."

Comment 8: Pag 5 line 13 – sampless – misspelling

Reply 8: We have made correction.

Comment 9: Fig 9 – methylene blue micrography's technique must be improved. Light microscope condenser distance must be adjusted.

Reply 9: Thanks for the comment. In this study, the sections for fluorescence microscopy and histological methylene blue acid fuchsin (BM-AF) staining wer hard tissue sections. The final thickness of the hard tissue section was about 50 μm. In order to observe the processes of new bone formation and neovascularization around the scaffolds at different time points, we chose a large field of view. However, due to the limitation in section thickness, it was difficult to adjust the distance of the substage condenser of the optical microscope in such a large field of view. At a higher magnification, we could clearly observe the hyperfine structure of neovascularization, Haversian system, and lamellar bone (Fig. 1). In the future study, we will improve the technique of grinding hard tissue sections to obtain thinner hard tissue sections and better images.

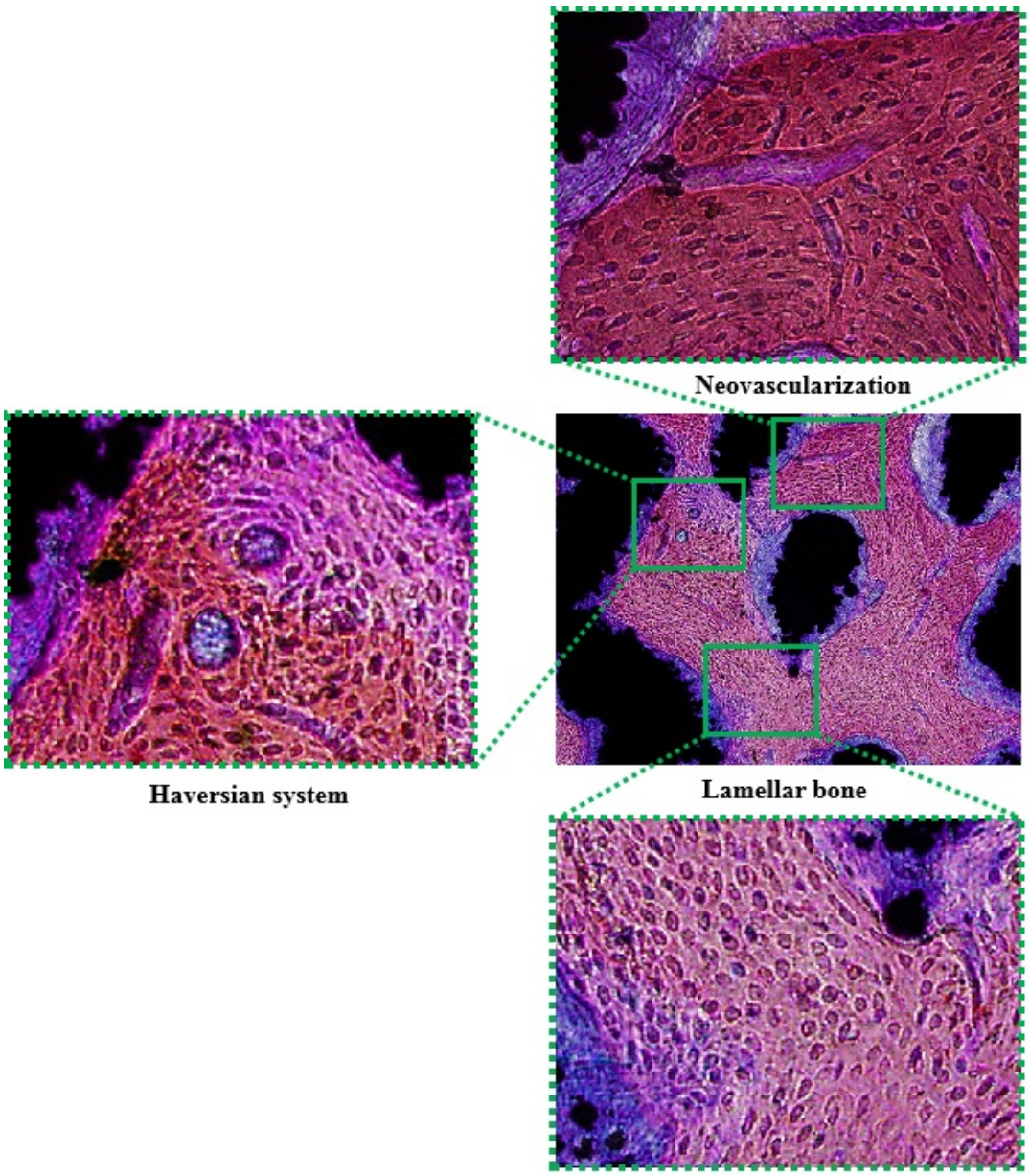

Fig. 1 High magnification images of hard tissue sections.

Comment 10: Pg 19 line 21 - They could, therefore, be used for the treatment of femoral condyle defects in rabbits. Is this condition a veterinary problem?

Reply 10: We have removed the phase “in rabbits” and changed the sentence into: “Therefore, they can be used for the treatment of femoral condyle defects.”

Comment 11: I wonder to know mechanical properties of the samples, for example elastic modulus.

Reply 11: Thanks for the comment. In this research, we placed the focus on the biological properties of the materials and therefore we did not perform mechanical

tests. In the literature, there is a wealth of information on the mechanical properties of the SLM Ti-6Al-4V scaffolds with diamond unit cells in relation to their topological features. In general, with increasing porosity, the elastic modulus of SLM Ti-6Al-4V scaffolds reduces. The elastic modulus of SLM Ti-6Al-4V scaffolds with a design porosity value of 70% was reported to 15.3 GPa, while that with a design porosity value of 60% was 24.4 GPa (*J Alloy Compd*, 2017, vol. 713, pp. 248-254. https://www.sciencedirect.com/science/article/pii/S0925838817313890). Our scaffolds had a design porosity value of 68% and their elastic modulus was estimated to fall between 15.3 and 24.4 GPa, likely being close to 15.3 GPa. Such scaffolds are suitable for cancellous bone repair. The elastic modulus of human bone is between 4-30 GPA (*J Biomech*, 1999, vol. 32, pp. 1005-1012. https://www.sciencedirect.com/science/article/pii/S0021929099001116?via%3Dihub). We still need to find a reliable method to determine the elastic modulus of our own scaffolds accurately.

Comment 12: Did authors think about to use reverse torque test to access bone-sample osseointegration force?

Reply 12: Thanks for the suggestion. The reverse torque test appears to be a safe, reliable method for verifying osseointegration with screw-shaped implants (*Int J Implant Dent.* **2016**, vol. 2, article number 26. https://europepmc.org/backend/ptpmcrender.fcgi?accid=PMC5143334&blobtype=pdf) With this test, it is however difficult to evaluate the osseointegration of porous scaffolds. After implantation, most of available pore space was occupied by *de novo* bone. We made an effort to evaluate the bone integration of porous scaffolds by using the reverse torque test, but the results were not satisfying. In the future, we will try to find a reliable method to measure the bone-implant osseointegration force in the case of highly porous bioactive material.

Comment 13: Discussion is quite repetitive and long

Reply 13: Thanks for the comment. It is indeed quite extensive and hopefully enlightening. In this section, we try to interprete our findings and observations in the context of the state-of-the-art scaffold materials for bone tissue repair, their deficiencies and the role of BG and MBG in resolving these deficiencies. We also try to explain the implicatioons of the results and make suggestions for future research.

Comment 14: Pg 22, line 3 - treatment of large bony defects, - give more specific application of the material

Reply 14: Thanks for the suggestion. We have added a few specific applications of the materials for the repair of large bone defects to the revised manuscript: “such as human maxilla, mandible and non-weight-bearing parts of limbs.”