Micro-CT evaluation on osteoconductivity of DCPD-coated β-TCP granule using experimental rats

Khairul Anuar Shariff¹, Kanji Tsuru², and Kunio Ishikawa²

¹ School of Materials and Mineral Resources Engineering, Engineering Campus, Universiti Sains Malaysia, 14300, Nibong Tebal, Penang, Malaysia
² Department of Biomaterials, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan

Email: biokhairul@usm.my

Abstract. β-tricalcium phosphate (β-TCP) bone substitutes have been widely used because of its excellent tissue response and good osteoconductivity. However, recent study reported that the osteoconductivity of β-TCP could be enhanced by treating β-TCP surfaces with saturated acidic calcium phosphate solution. In this study, the osteoconductivity of treated β-TCP granule were evaluated using micro-computed tomography (micro-CT) scan technique. The granule specimens were obtained by exposing β-TCP granules with saturated acidic calcium phosphate solution for 10, 30 and 60 mins, respectively. Then, critical bone defect size with dimension of 9 mm were made at rat calvarial, and the defect was reconstructed with 10, 30 and 60 mins treated β-TCP granules and β-TCP granules without treatment as a control. After 2 and 4 weeks implantation, the specimens with the surrounding bone tissue were taken out and examined using micro-CT machine. Based on the cross section of micro-CT images, new bone formations were observed from the edge of the defect towards the center of the bone defect area in all specimens. At 2 weeks, new bone formations were observed for 10 min and 30 min treated β-TCP granules when compared with other specimens. The 30 min treated β-TCP granules showed faster new bone formation rate after 4 weeks implantation. It can be concluded that 30 min treated β-TCP granules revealed the highest osteoconductivity in comparison to the other specimens.

Keywords: Osteoconductivity, Dicalcium Phosphate Dihydrate, β-Tricalcium Phosphate, Microtomography, Acidic Calcium Phosphate Solution.

1. Introduction

Numerous studies have been reported that the β-TCP bone substitute shows an osteoconductivity [1, 2–4]. For example, comprehensive studies by Ogose et al. have been reported that the β-TCP bone substitute shows greater osteoconductivity than hydroxyapatite (HAp) bone substitute after implanted in human femur [1, 3, 4]. Although these findings demonstrated that the β-TCP bone substitute has a potential to be used as an osteoconductive material, its osteoconductivity is still inferior to the natural bone [5, 6]. In fact, Shinomiya et al. reported that if bone defect size becomes larger, the amount of new bone formation becomes low [7]. Therefore, if the ability of new bone formation for β-TCP bone
substitute can be enhanced, its range of clinical application is expected to be wider. Based on the histological study by Khairul et al. [8] reported that the osteoconductivity of β-TCP granule were enhanced by treating β-TCP granule surfaces with saturated acidic calcium phosphate solution. However, till now no study has been done using micro-CT scan technique. Therefore, the objective of this study is to investigate the osteoconductivity of treated-β-TCP granules using micro-CT scan technique.

2. Experimental study

2.1 Preparation of granular specimens

The granular specimens used in this study were 10 mins, 30 mins, 60 mins-treated β-TCP granule and DCPD free β-TCP granule. These granular specimens were prepared in the same manner as described by Khairul et. al [8]. The granular size used in this experiment was regulated between 300-600 μm using a sieve. Before implantation, these granular specimens were sterilized using gamma ray (2.27 kGy/hour, 11 hours, total dosage 25 kGy at 25°C) [9].

2.2 Animal surgical procedures

Animal experiment was performed according to Animal Care and Use Committee of Kyushu University (Reference number: A25-231-0). Twelve Wistar male twelve-weeks-old rats (Kyudo, Saga, Japan) with an average weight of 350 g were used. The rats were anesthetized with intraperitoneal injection of ketamine and 2% xylazine hydrochloride by using liquid to body weight ratio of 0.2 ml/100 g. The fur on the skin of rat calvarial was shaved and disinfected with 10% povidone iodine solution (Meiji Seika Pharma, Tokyo, Japan). An infiltration of localized anesthesia with 0.4 ml of 2% lidocaine-epinephrine solution (Dentsply Sankin, Tokyo, Japan) was applied over the surgery area in rat calvarial to arrest the bleeding and to control early post-operative pain. Then, the skin was incised sagittally with a surgical blade, and the calvarial bone area was carefully opened. A circular bone defect was created in each rat using 9 mm trephine burr under irrigation of saline solution (Otsuka, Osaka, Japan). The resected bone was gently removed to avoid injury the underlying tissue. Furthermore, the bone defect was irrigated with saline solution to remove debris that occurred during the drilling process. After wiping with gauge, the bone defect was reconstructed with 0.04-0.05 g of the granular specimens. Next, the periosteum layer and skin was sutured to close the wound. Then, the sutured area was disinfected with 10% povidone iodine solution (Meiji Seika Pharma). Figure 1 show the granular specimen condition reconstructed with the bone defect. After 2 and 4 weeks implantation, these rats were sacrificed by using overdosage of anesthesia solution. Then, the block section of rat calvarial bone containing granular specimens with surrounding tissue was taken out for micro-CT analysis.

Figure 1. Treated β-TCP granule specimens (300-600 μm) was reconstructed with 9 mm in diameter of bone defect size at rat calvarial.
2.3 Microcomputed tomography analysis

Morphology of bone contained implant specimens was analysed using microcomputed tomography (μ-CT: Skyscan 1075 KHS, Kontich, Belgium) at a source of 59 kV and source content of 169 μA using a 0.5 mm aluminum filter [10]. The samples were scanned using the high-resolution mode (9 μm voxel resolution) image.

3. Results and discussion

3.1 Appearance of granular specimens

Figure 2 shows the appearance of the β-TCP granules (a) before and after reacted with saturated acidic calcium phosphate solution for (b) 10 min, (c) 30 min and (d) 60 min at 25°C. The physical appearance of β-TCP granule reacted with saturated acidic calcium phosphate solution is similar with the unreacted β-TCP granule. The 10 mins, 30 mins and 60 mins treated β-TCP granules were successfully fabricated after exposing β-TCP granule to 50 mmol/L MCPM-25 mmol/L H₃PO₄ solution at 25°C for 10, 30 and 60 min, respectively. The precipitation of DCPD crystals on the surface of β-TCP granule occurs through dissolution-precipitation reaction. [8]. Therefore, prolong the exposure time of β-TCP granule to acidic calcium phosphate solution results in the larger amount of DCPD coated on β-TCP granular surface. The 1 mass%, 5 mass% and 15 mass% of DCPD were produced after exposing β-TCP granule to saturated acidic calcium phosphate solution for 10 mins, 30 mins and 60 mins, respectively.

Figure 2. Appearance of the β-TCP granules (a) before and after reacted with acidic calcium phosphate solution for (b) 10 min, (c) 30 min and (d) 60 min at 25°C.

3.2 Micro-computed tomography analysis

Figure 3 and 4 show the cross-sectional view of the bone defect filled with (a) β-TCP specimen, (b) 10 min-treated β-TCP, (c) 30 min-treated β-TCP and (d) 60 min-treated β-TCP specimen at 2 and 4 weeks after implantation. These granular specimens remain in the bone defect in all specimens. At 2 week, the new bone formation was observed at the edge of bone defect area for 10 mins and 30 mins-treated β-TCP specimens as indicated by black arrows. At 4 week, the new bone formation was observed underneath the implanted granules in all specimens. Also, the new bone was regenerated from the surface of the original bone in all specimens at opposite direction. The new bone formation results obtained in this study indicated that the osteoconductivity of β-TCP granule could be enhanced by coating its surface with appropriate amount of DCPD. Interestingly, effect of the amount of coated DCPD on bone formation was not simple. Based on micro-CT results, new bone formation rate for 10 mins-treated β-TCP was faster compared to DCPD free β-TCP granule after 2 weeks implantation, indicating even 10 min of treated time on β-TCP granular surface is effective to stimulate new bone formation. However, no significant difference was observed between 10 mins-treated β-TCP and DCPD free β-TCP granule at 4 weeks implantation. In the case of 30 mins-treated β-TCP, the new bone
formation rate was faster after 4 weeks implantation. Although, detailed mechanism of the improvement of osteoconductivity of β-TCP granules coated with DCPD has not been clarified in the present study, it seems that the amount of coated DCPD is useful to enhance the osteoconductivity of β-TCP granule. Based on the reported by Khairul et al [8] mentioned that the enhancement of the new bone formation of DCPD-coated β-TCP granule may be explained by the release of Ca\(^{2+}\) and PO\(_4^{3-}\) from the specimens. The concentration amount of Ca\(^{2+}\) and PO\(_4^{3-}\) release from the specimens was increased with increasing the treatment time on the β-TCP granule surfaces. The 30 mins-treated β-TCP granules may release appropriate amount of Ca\(^{2+}\) and PO\(_4^{3-}\) that promoted the proliferation and differentiation of osteoblasts [8]. In the case of 60 mins-treated β-TCP granules, the large amount of Ca\(^{2+}\) release might decreased the proliferation and differentiation of osteoblasts cell [8].

![Figure 3](image_url)

**Figure 3.** Cross-sectional view of the bone defect filled with (a) β-TCP specimen, (b) 10 min-treated β-TCP, (c) 30 min-treated β-TCP and (d) 60 min-treated β-TCP specimen after 2 weeks implantation. Yellow arrow = defect margin; Black arrow: new bone formation
Figure 4. Cross-sectional view of the bone defect filled with (a) β-TCP specimen, (b) 10 min-treated β-TCP, (c) 30 min-treated β-TCP and (d) 60 min-treated β-TCP specimen after 4 weeks implantation. Yellow arrow = defect margin; Black arrow: new bone formation.

4. Conclusion

It is found that 30 mins-treated β-TCP granule showed faster new bone formation rate than DCPD-free β-TCP granule for both 2 and 4 weeks after implantation. However, 60 mins-treated β-TCP granules showed lower new bone formation rate than 30 mins-treated β-TCP granule with similar implantation period. This results proved that appropriate treatment time of β-TCP granule with acidic calcium phosphate solution could improve osteoconductivity of β-TCP granule.

Acknowledgement

This study was supported by Short-Term Research Grant Universiti Sains Malaysia (Grant no: 304/PBAHAN/60313046), in part, by the Strategic Promotion of Innovative Research and Development Program (15im0502004h0004) from Japan Agency for Medical Research and Development. The first author would like to thank Department of Biomaterials, Faculty of Dental Science, Kyushu University for micro-CT analytical service.
References
[1] Ogose A, Hotta T, Kawashima H, Kondo N, Gu W, Kamura T and Endo N 2005 Journal Biomedical Materials Research - Part B Applied Biomaterials 72 94
[2] Onodera J, Kondo E, Omizu N, Ueda D, Yagi T and Yasuda K 2014 Knee Surg, Sports Traumatology, Arthroscopy 22 2763
[3] Ogose A, Hotta T, Hatano H, Hatano H, Kawashima H, Tokunaga K, Endo N and Umezu H 2002 Journal Biomedical Materials Research - Part B Applied Biomaterials 63 601
[4] Ogose A, Kondo N, Umezu H, Hotta T, Kawashima H, Tokunaga K, Ito T, Kudo N, Hoshino M, Gu W and Endo N 2006 Biomaterials 27 1542
[5] Frota R, Silva-Junior VAD, Teixeira M, Veras Sobral AP, Oliveira e Silva ED, Fonseca Da Silveria MM and Aragao-Neto AC 2011 Med Oral Patol Oral Cir Bucal 16 190
[6] Khatiblou F 2011 Journal Oral Implantology 37 727
[7] Shinomiya K, Ishizuki M, Morioka H, Matasumoto S, Nakamura T, Abe S and Beppu Y 2012 Seikei Geka 63 921
[8] Khairul A S, Tsuru K and Ishikawa K 2017 Materials Science and Engineering C 75 1411
[9] De Moraes M A, Weska R F and Beppu M M 2013 Journal Biomedical Materials Research.-Part B Applied Biomaterials 102 869
[10] Shariff K A, Tsuru K and Ishikawa K 2016 Journal Biomaterials Applications 30 838