Heparin-assisted BMP-2 Release from Beta-Tricalcium Phosphate Surface for Bone Tissue Engineering

Xiaoyan Liu [1], Yongzhi Qiu [1], Xiaowei Li [1], Yunzhi Yang [3], Xuejun Wen [1, 2]

[1]Department of Bioengineering, Clemson University, Clemson, SC, United States
[2]Department of Orthopedic Surgery, Cell Biology, Medical University of South Carolina, Charleston, SC, United States
[3]Department of Restorative Dentistry and Biomaterials, University Texas at Houston, Houston, TX, United States

Introduction: One decisive factor to the success of tissue engineering strategies for bone regeneration is osteoconductivity and osteoinductivity of the scaffolds. Biocermics are often used for bone tissue engineering owing to their biocompatibility, osteoconductivity [1-2]. Through osteoconduction mechanism, biocermics can form chemical bonding with living bone tissue. In order to further offer osteoinductivity to the bioceramics, growth factors, such as BMP-2 and TGF-β1, are physically absorbed on the biocermic scaffolds. However, the release of these growth factors is not well controlled and can only be released for a very short period of time. To obtain long term stable release of growth factors from bioceramics, we used β-tricalcium phosphate (β-TCP) substrate as a model bioceramic for the immobilization of model osteogenic growth factor BMP-2.

Methods: The modification of β-TCP was performed based on two steps. Firstly, chitosan and phthalic anhydride were heated with stirring at 130°C under a nitrogen atmosphere for 12 hours to form phthalized chitosan. Secondly, β-TCP discs with DMF, lysine diisocyanate and dibutytindilaurate were reacted at 60°C under a nitrogen atmosphere. After 4 hours, the solution was mixed with phthalized chitosan at 60°C under the protection of nitrogen overnight. The β-TCP discs were then reacted with hydrazine monohydrate and water with stirring for 15 h at 100°C under a nitrogen atmosphere to form de-phthalized β-TCP discs. The reaction scheme is shown in Figure 1. In order to graft heparin on the surface of modified β-TCP discs, heparin was activated with EDC and NHS for 10 min at 37°C. Then beta-TCP discs were immersed into this solution and reacted for 4 hours at 37°C. X-ray photoelectron spectroscopy (XPS) was used to measure the elemental change on the surface after modification. The immobilized heparin on the surface was qualitatively and quantitatively measured by toluidin blue staining. Heparin grafted β-TCP discs were immersed into the BMP-2 solution at 4°C for overnight. The controlled release of BMP-2 from modified surface was confirmed by Enzyme-Linked Immunosorbent assay (ELISA).

Table 1: Elemental components of four types of disc surface.

	Ca	O	P	C	N	S	C/O
I	19.56	67.56	12.87	--	--	--	--
II	3.21	31.01	2.42	60.13	3.23	--	1.94
III	7.21	50.07	3.65	35.28	3.79	--	0.70
IV	6.68	45.84	4.48	36.35	5.24	1.40	0.79

Results: The XPS was performed on untreated β-TCP discs (I), phthalized-chitosan grafted β-TCP discs (II), de-phthalized β-TCP discs (III) and heparinized β-TCP discs (IV). The chemical elemental components were listed in Table 1. Only calcium, oxygen and phosphor were detected on the surface of untreated β-TCP discs, which is in agreement with its chemical components. It was found that nitrogen appeared on the surface after being modified with phthalized chitosan due to the existence of amide and imide groups on phthalized chitosan. After heparinization, sulfur was detected on the surface of β-TCP discs, which can be assigned to the sulfate groups on heparin, thus indicating the success of heparin immobilization on the surface. C/O ratio decreased after de-phthlization, which is due to the higher carbon ratio in phthlic groups. Fig. 2 showed the staining of these β-TCP discs after being incubated in toluidine blue solution overnight. Since positively charged toluidine blue formed complex with negatively charged heparin, purple color from the complex was observed on heparinized discs other than other discs. By quantitatively analysis, 7.62 ± 0.30ug heparin was grafted on each disc (total surface area is about 150mm^2). The release character of BMP-2 from heparinized beta-TCP discs is shown in Fig. 3. The discs can sustain BMP-2 release over 3 weeks.

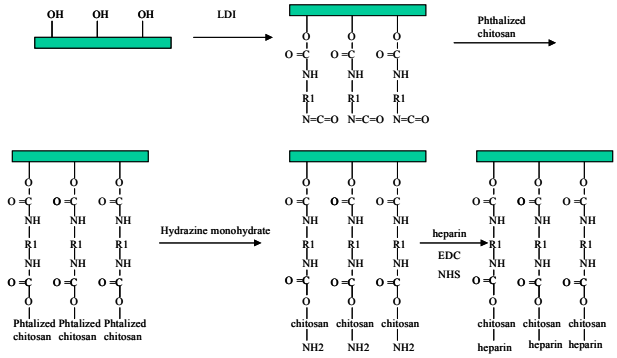

Fig. 1: The scheme of surface modification for β-TCP.

Fig. 2: Toluidine blue staining: I (A), II (B), III (C) and IV (D).

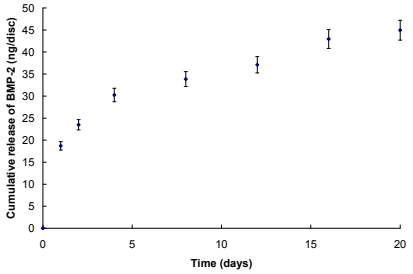

Fig. 3: BMP-2 released from heparinized beta-TCP discs.

Conclusions: Heparin can be conjugated on the surface of chemically modified β-TCP discs for immobilization and control release of BMP-2.

References:

1.Wang X, et al. Biomaterials 2002; 23(24):4787-91.
2. LeGeros RZ. etc. Chem Rev 2008; 108(11):4742-53.