Immobilization of BMP-2 on Metal Surface Using Chitosan Derivative With Dual Functions

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Statement of Purpose: This study aims to achieve two go als: 1) to coat titanium implants with Az-P-LM-O-CMC a nd 2) to immobilize growth factors. Phosphonated UV-cur able low-molecular-weight chitosan is also called azidoph enyl phosphonated low molecular-Ocarboxymethyl chitos an (Az-P-LM-O-CMC). Chitosan is appropriate as a coati ng material for implants because it has anti-bacterial funct ions and can protect bacterial infection during implantatio n. It is known that phosphonate groups interact with titani um via coordinate bonds. Azido groups are involved in cr osslinking reactions as they form a nitrene intermediate by UV irradiation such that protein drugs could be photo-im mobilized. With these two processes, it is possible to short en the period of osseointegration.

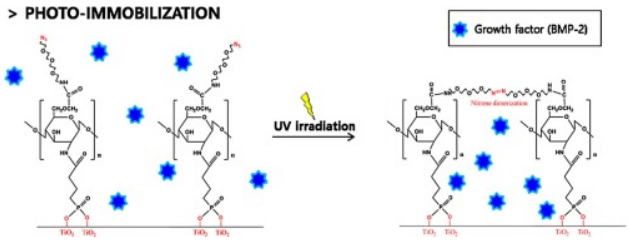

Figure 1. Photo-immobilization of Az-P-LM-O-CMC

Methods: LM-O-CMC was prepared according to depoly merization method and carboxymethylated method. Az-P-LM-O-CMC was prepared with 4-phosphonobutyric acid and 11-azido-3,6,9-trioxaundecan-1-amine. To evaluate pr otein immobilization, Az-P-LM-O-CMC solution containi ng FITC-BSA was cast onto a titanium plate. The samples were covered with a photomask and UV irradiation was c arried out. After washing, the samples were observed by fl uorescence microscopy. To characterize titanium surfaces coated with each sample, water contact angle measuremen t was carried out. To test protein release, a Human BMP-2 ELISA Kit was used. The protein release test was carried out according to the concentration of Az-P-LM-O-CMC a nd the number of coated layers. ALP activity was evaluate d using an ALP assay kit. The concentration of calcium io ns was measured using a calcium assay kit.

Results: Az-P-LM-O-CMC was prepared by coupling 4-p hosphonobutyric acid and 11-azido-3,6,9-trioxaundecan-1 -amine. Phosphonate groups form coordination bonds wit h titanium in the same way, and azido groups facilitate pro tein immobilization. Through micropatterning, Az-P-LM-O-CMC is suggested that the azido group have the ability to immobilize protein. Water contact angle was confirmed that on hydrophilic surfaces, cell adhesion and growth are improved. Photo-crosslinking via azido groups affects the hydrophilicity of the titanium surface. Protein release test was verified that the drug release rate could be controlled by adjusting the number of layers. Cytotoxicity was confi rmed that Az-P-LM-O-CMC did not affect the viability of cells. ALP activity is well known as a marker of bone for mation. the group in which BMP-2 was photo-immobilize d using Az-P-LM-O-CMC showed good ALP activity. Th e calcium colorimetric assay measures the calcium that is generated when cells are differentiated into osteoblasts. T he group in which BMP-2 was photo-immobilized using A z-P-LM-O-CMC showed higher concentrations of calcium . In addition, the concentration of calcium was higher in th e group with BMP-2 photo-immobilized using Az-P-LM-O-CMC than in the group with free BMP-2.

Conclusions: Many methods of modifying the surface of titanium are known. The main purpose of this study was to coat titanium surfaces and simultaneously immobilize B MP-2 using Az-P-LM-OCMC. Chitosan has been reported to have anti-microbial properties, but lacked the ability to promote osseointegration compared with other coatings. I n implants, osseointegration is very important. Therefore, the results of this study supplemented the disadvantages o f chitosan. The structure of Az-P-LM-O-CMC was confir med by FT-IR, ^{31}P-NMR, and GPC. The ability for Az-P-LM-O-CMC to be coated on titanium surfaces was checke d by measuring water contact angle. Protein immobilizatio n was identified with fluorescence microscopy using micr opatterning. Protein release tests revealed that the rate of p rotein release could be controlled by adjusting the number of coated layers. Biocompatibility was observed with cyt otoxicity testing. The effects of drug immobilization and r elease effects were evaluated by the ALP assay and calciu m colorimetric assay. Biocompatible Az-P-LM-O-CMC c ould be coated onto titanium and simultaneously used to i mmobilize BMP-2. The significant disadvantage of impla nts is the long period of time required for osseointegration , and Az-P-LM-O-CMC is expected to be a good coating material for implants.

Reference:

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