256c Synthesis and Characterization of Apatite Nanoparticles Grafted with Unsaturated Hydrophilic Macromers

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Tissue engineering strategies for the regeneration of damaged orthopedic tissues involve the use of highly porous and interconnected scaffolds with acceptable mechanical properties to serve as a substrate for adhesion, spreading, migration, proliferation, and differentiation of osteoblastic cells. One approach to bone replacement involves the use of prefabricated scaffolds for cell transplantation to promote three dimensional tissue growth, nutrient diffusion, matrix production, and vascularization. These prefabricated scaffolds present a large surface area for cell growth and reduce the diffusional barriers for material transport.

Calcium phosphate ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP) have shown to promote bone ingrowth, are biocompatible and osteoconductive. The major drawback of ceramics based on HA, TCP, or their combination is that the initial mechanical properties are less than cancellous bone which leads to difficulty in maintaining the composite within the defect during surgery. In particulate form, they offer increased mechanical strength to polymeric composite materials primarily in compression, but are less effective in enhancing resistance to torsional and bending forces. To improve shear and tensile strength and fracture toughness of ceramics, composite biomaterials based on natural or synthetic polymers and ceramics in particulate form have been developed. The use of calcium phosphate ceramics reinforced with natural or synthetic polymers has improved the fracture toughness of these composites and has enabled faster and more aggressive rehabilitation. We hypothesize that surface grafting of apatite nanoparticles (with large surface to volume ratio) with hydrophilic unsaturated macromers will improve their dispersion in the aqueous phase and enhance bonding at the interface between the filler and the aqueous matrix in polymer/ceramic composites and subsequently increase the resistance of the composite biomaterial to torsional and bending forces. In this work, we describe a novel method for grafting unsaturated hydrophilic macromers to the surface of apatite nanoparticles for use as filler in synthetic bone substitute.

HA nanoparticles were grafted with hydrophilic unsaturated poly(ethylene glycol) oligomers to improve their suspension stability and interfacial bonding in the aqueous hydrogel solution. The grafting reaction was carried out in two steps. In the first step, poly(ethylene glycol) methacrylate (PEGMA) was condensed with 3-isocyanatopropyltrimethoxysilane (iCPTMS) to form a PEGMA-PTMS urethane with unsaturated methacrylate and trimethoxysilane end-groups. In the second step, the trimethoxysilane end of the urethane was reacted with reactive phosphate and carbonate groups on the HA surface using ammonium hydroxide and methanol as the catalysts to produce HA with grafted PEGMA oligomers (gHA). In a typical reaction, PEGMA was dried by azeotropic distillation and hydroquinone was added as a radical scavenger to prevent free radical polymerization of methacrylate groups of PEGMA. DMF and iCPTMS was added dropwise with stirring to the reactor and the condensation reaction was allowed to proceed under reflux conditions and with excess PEGMA. The reaction was allowed to cool to ambient conditions, HA was added to the reactor with stirring, and the reaction mixture was sonicated to break up the aggregate of HA nanoparticles using a probe sonicator. The suspension was sonicated for 30 min with an Ultrasonic Processor with a power and frequency of 10 Watts and 20 kHz, respectively, in the continuous mode. Ammonium hydroxide and methanol were added to the reaction, the mixture was sonicated for an additional 30 min, and grafting reaction was allowed to continue under reflux conditions. The grafted HA was washed with methylene chloride, centrifuged, and re-dissolved at least 5 times to remove all unreacted components and dried under vacuum.

gHA was characterized with FTIR, TGA, and TEM. A Thermo Nicolet FTIR Nexus 470 was used to measure the absorption spectrum of gHA powder in the IR region. Samples were dried under vacuum for at least 12 h at 50°C before acquisition of FTIR spectrum. The unmodified and grafted HA powders were packed and pressed firmly in the reference and sample compartments of the FTIR cell and spectrum was collected under a dry nitrogen atmosphere with 16 averaged scans and a resolution of 4 cm^{-1}. The broad absorption band with peak position at 2880 cm^{-1} was due to symmetric and asymmetric C-H vibrations of the $–CH_2-$ group of PEG in PEGMA and propyl group in iCPTMS. The shoulder at 2950 cm^{-1} was due to symmetric and asymmetric C-H stretching vibration of the $–CH_3$ groups of methacrylate and methoxy silane. These absorption bands were absent in the spectrum of untreated HA. The absorption with peak position at 1720 cm^{-1} and a shoulder at 1740 cm^{-1} were due to the C=O stretching vibration of the ester group of methacrylate in PEGMA. This absorption was absent in the spectrum of HA grafted with PEG terminated with methoxy (mPEG) in place of methacrylate group. The absorption with peak location at 1670 cm^{-1} was assigned to carbonyl absorption band of the urethane group (amide I) and the broad absorption with peak location at 1580 cm^{-1} was collectively attributed to N-H bending and C-N vibration (amide II) of the urethane group. These peaks were absent in the spectrum of untreated HA. The absorption with peak location at 1360 cm^{-1} was assigned to the C-H bending vibration of the alkene group of methacrylate in PEGMA which was absent in the spectrum of HA grafted with PEG terminated with methoxy group. The absorptions with peak locations at 1300 cm^{-1} and 1250 cm^{-1} were due to the C-N and N-H bending vibrations and to C-O bending vibration of the urethane group, respectively, formed by the reaction of PEGMA and iCPTMS. The absorptions with peak locations at 1420 and 1460 cm^{-1} were attributed to vibrations of the phosphorous/oxygen/silicon complex (P-O-Si) on the surface of HA by the reaction of methoxysilane with phosphate groups. The absorptions in the FTIR spectrum of gHA with untreated HA as the reference confirmed the grafting of PEGMA-PTMS urethane on the surface of HA.

A Perkin Elmer thermogravimetric analyzer was used to measure the amount of grafting on the HA surface. The experiments were carried out under helium atmosphere. The sample was heated to 120°C, allowed to equilibrate for 15 min to remove water content, and heated to 900°C at a rate of 10°C/min. Samples included untreated HA (HA), HA grafted with PEGMA-PTMS urethane without sonication (gHA-WOS), and HA grafted with PEGMA-PTMS urethane with sonication (gHA-WS). The weight loss of HA, gHA-WOS, and gHA-WS after reaching 900°C was 5.0%, 8.0%, and 46.0%, respectively. After subtracting the weight loss of 5.0% due to bound water, the percent grafting of gHA-WOS and gHA-WS was 3.0% and 41.0%, respectively. These results demonstrates that sonication of the HA nanoparticles during the grafting reaction reduces the aggregate size and substantially increases the extent of grafting and that the extent of grafting can be controlled by sonication.

The morphology of the HA nanoparticles were examined using a JEOL 100 CXII transmission electron microscope at an accelerating voltage of 100 kV. The nanoparticles in the gHA sample without sonication had whisker like morphology, similar to untreated HA, with long and short axis of 100 and 20 nm, respectively, while those in the gHA sample with sonication had a more rounded morphology with long and short axis of approximately 20 nm. Our results demonstrate that sonication of the suspension during the grafting reaction breaks the nanoparticles along the long axis, forming rounded nanoparticles, and creating new surface area for grafting. This explains the substantially higher extent of grafting for gHA samples sonicated during the grafting reaction.