256d Aqueous Based Hydrogel/Apatite Nanocomposite Scaffolds for Guided Bone Regeneration
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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.

Bone matrix is a composite material consisting of aqueous and inorganic phases. The inorganic component contributes approximately 65% of the wet weight of the bone. The organic component usually contributes a little more than 20% of the wet weight and water can contribute as much as 15% by wet weight in cortical bone. The aqueous component gives bone its form and contributes to its ability to resist tension, while the inorganic, or mineral, component primarily resists compression. Bone that has been demineralized is flexible, pliable, and resistant to fracture. Bone that its aqueous phase has been removed is rigid and brittle and a slight deformation factures it. The bone mineral crystals are classified as apatite and contain both carbonate ions and acid phosphate groups. The carbonate and acid phosphate groups of the mineral crystals are very labile and play important roles in the interaction of the crystals with the surrounding extracellular fluid and with the aqueous components of the matrix. Recent studies demonstrate that there is a significant physical and chemical interaction between the aqueous and mineral phases of the bone matrix.

The aqueous phase of the bone matrix, even though it constitutes only 15% of the matrix, plays a central role in regulation of collagen fibril mineralization, modulation and control of cell division, cell migration, cell differentiation, cell maturation, maintenance of matrix integrity, growth factor modulation, signaling from the cell to the nucleus, and the extent of mineral-collagen interactions. A plethora of noncollagenous proteins reside within the aqueous phase which control the cellular function and the rate of bone turnover. These include glycoproteins, small integrin-binding ligands, and matrix extracellular proteins. We hypothesize that hydrogel/apatite nanocomposites are the ideal biomaterial to mimic the physio-chemical and biologic properties of the bone matrix and to fabricate scaffolds for bone regeneration. In this work, we describe synthesis, characterization, and fabrication of hydrogel/apatite nanocomposite scaffolds for bone regeneration.

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). 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. 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 thermogravimetric analyzer was used to measure the amount of grafting on the HA surface. When sonication was used to disperse the nanoparticles during the grafting reaction, grafting as high as 40% by weight was measured. The

morphology of the HA nanoparticles were examined with TEM. 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.

Poly(lactide-ethylene oxide-fumarate) (PLEOF) unsaturated terpolymer was synthesized by condensation polymerization of low MW PLA and poly(ethylene glycol) (PEG) with fumaryl chloride (FuCl) and triethylamine (TEA) as the catalyst. PLEOF macromers were synthesized using PEG with Mn ranging from 1 to 5 kD and PLAF with Mn ranging from 1 to 7 kD. The weight ratio of PEG to PLA was varied from 100/0 to 85/15 to produce hydrophilic water-soluble terpolymers. The structure of PLEOF macromer was characterized by ^{1}H-NMR and FTIR.

Hydrogel/apatite porous scaffolds were prepared using PLEOF as the degradable macromer, methylenebisacrylamide (MBIS) as the crosslinking agent, a neutral redox initiation system, and sodium chloride crystals as the porogen. The redox system consisted of ammonium persulfate (APS) and tetramethylethylenediamine (TMEDA), respectively. Salt crystals were sieved and the fraction retained on the 300 um mesh sieve was used for scaffold fabrication. Grafted-HA based on total weight of the hydrogel (sum of the weight of PLEOF, PBS, MBIS, initiator and accelerator solutions, and the graft weight of HA) was added to the polymerizing mixture, transferred to a 5 mm diameter x 3 mm height Teflon mold and pressed manually to maximize packing. The mold was placed in a conduction oven to facilitate crosslinking and the porogen was leached out by soaking the scaffolds in distilled water for 2 days, during which time water changes occurred every 8 h. The scaffolds were dried in vacuum for at least 12 h before use. The pore morphology was studied with an environmental scanning electron microscope (ESEM) equipped with an electron backscattered detector and an integrated x-ray energy dispersive analysis system. Macropores created by the porogen and micropores created by the partial phase separation of hydrophilic (PEG) and hydrophobic (PLA) domains of PLEOF hydrogel were observed in the ESEM micrographs.

Scaffolds were sterilized with ethanol, washed with PBS and seeded with neonatal heart fibroblast cells, and incubated for 48 h to study cell attachment to these composite surfaces. Attached cells were fixed, rinsed with PBS, and permeabilized by soaking in PBS with 0.1% triton x-100 and 0.1M glycine. Cell nucleus was stained with 4',6-Diamidino-2-phenylindole or SYTOX Green and counterstained with Texas Red-X Phalloidin. Since the PLEOF hydrogel is an inert non-interacting macromer, cells did not attach to this model surface and had rounded morphology. However, when the nanocomposite scaffold was treated with collagen type I, having integrin binding RGD domains, cells adhered to the surface and had extended morphology with focal point adhesion. The cell morphology was further examined by SEM, demonstrating strong attachment of the fibroblast cells to the scaffold surface mediated by the integrin binding sequences on the collagen fibrils. Our results demonstrate that hydrogel/apatite nanocomposites are an attractive alternative as a biomaterial for hard tissue regeneration.