Response of Marrow Stromal Cells to Multi-Functional Peptide-Reinforced Cell-Adhesive Nanocomposite Scaffolds

Esmaiel Jabbari, Alireza S. Sarvestani, Xuezhong He
Biomimetic Materials and Tissue Engineering Labs, Chemical Engineering Dept, University of South Carolina, SC, USA

Statement of Purpose: Hydrogel/apatite nanocomposites are the ideal biomaterial to mimic the physio-chemical and biologic properties of the bone. We hypothesized that synthetic peptides with multiple biologically active sequences, separated by inert spacers and covalently attached to degradable scaffolds, can coordinate multiple regenerative functions *in situ* including temporary matrix stability, cell attachment and migration, and matrix degradation. The objective of this work was to investigate the effect of a multi-functional peptide sequence consisting of a sequence derived from osteonectin protein (Glu6) and an integrin-binding RGD sequence on material properties of the degradable poly(L-lactide-*co*-ethylene oxide-fumarate) (PLEOF)/apatite composites. Results demonstrated that the Glu6 sequence significantly improved the mechanical properties and the RGD sequence affected the attachment of bone marrow cells to the degradable PLEOF scaffold.

Methods: PLEOF was synthesized by condensation polymerization of ultra-low molecular weight PLA and PEG with fumaryl chloride (FuCl). The structure of PLEOF macromer was characterized by ^{1}H-NMR and FTIR. The PLEOF macromer with PLA and PEG molecular weights of 1.2 kDa (PI of 1.4) and 4.3 kDa (PI of 1.1), respectively, had M_n of 7.4 kDa and PI of 2.3.
The structure of the multi-functional peptide is shown in Figure 1. The peptide was synthesized manually in the solid phase using a method developed in our laboratory on the Rink Amide NovaGel™ resin [1]. Resin was swollen in DMF; six protected glutamic acid derivatives were coupled successively to the resin; then the Fmoc-mini-PEG linker was coupled followed by a protected lysine. The protected aspartic acid, glycine, and arginine amino acids were coupled successively to the lysine residue to form the RGD integrin-binding sequence on the peptide chain. After coupling the last amino acid, the Fmoc- protecting group was deprotected. Next, an acrylate group was coupled to the peptide in the solid phase by coupling acrylic acid to the amine group of the lysine residue. The product was purified by HPLC and characterized by mass spectrometry. Porogen leaching technique was used to fabricate the scaffolds. The polymerizing paste (PLEOF, apatite nanoparticles (20x80nm whiskers), multi-functional peptide as crosslinker, initiator and accelerator) was transferred to a mold and crosslinked. Shear modulus was measured by a rheometer and bone marrow stromal cells, isolated from Wistar rats, were used for cell attachment and migration.

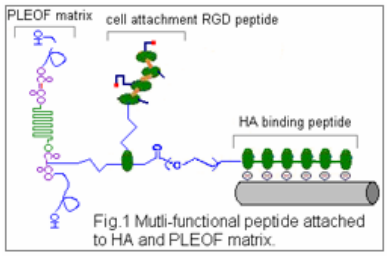

Fig.1 Mutli-functional peptide attached to HA and PLEOF matrix.

Results / Discussion: A sterile scaffold was placed in each well and seeded with BMS cell suspension in primary media ($2x10^4$ cells/cm^2). Subsequently, the plate was incubated for 24 h, washed, and stained with cAM for live cell visualization with a confocal fluorescent microscope. Figure 2 shows a 45 µm thick section near the disk surface without (a) and with (b; 1x 10-2 M) peptide. Attachment of the BMS cells to the scaffold with peptide (b) can be observed.

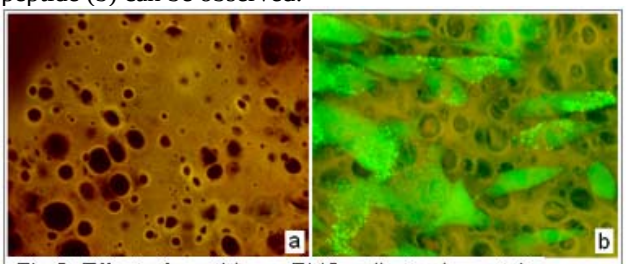

Fig.2. Effect of peptide on BMS cell attachment (a: without and b: with peptide)

BMS cells were seeded on the top surface of the scaffolds ($2x10^6$ cells/scaffold). The scaffold/cell constructs were placed in 12 well plates and incubated for 1 week. Cells were stained with cAM and EthD dyes visualized with confocal microscopy. Figure 3 shows the live cell distribution at a depth of 160µm. The surface of a scaffold pore is filled with migrated cells, which demonstrates that peptide-treated scaffolds support migration of BMS cells.

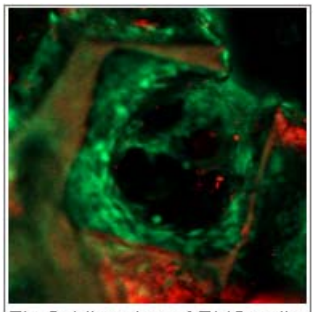
Fig.3. Migration of BMS cells in peptide-treated scaffolds.

The composite mixture was injected on the Peltier plate of a rheometer for rheological and gelation measurements. Figure 4 shows the normalized shear modulus of the PLEOF/HA composite with and without the peptide linker. The experimental results as a function of apatite concentration demonstrate an order of magnitude increase in shear storage modulus for peptide-treated apatite nanoparticles versus untreated composites.

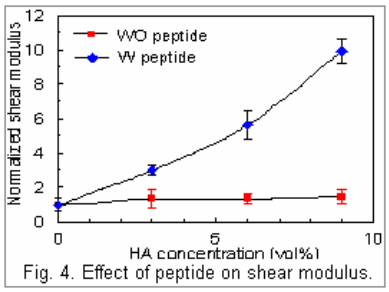

Fig. 4. Effect of peptide on shear modulus.

Conclusions: Results demonstrated that the peptide linker significantly improved modulus and well as cell adhesion.

Reference:

[1] X. He, E. Jabbari, Protein Pept. Lett. 13 (2006) 715.