Modification of Chitosan Scaffolds by Electrokinetic Particle Treatment

Stephanie Tully-Dartez [1,2], and Rachel E. Gade[1] and P. Sidney Sit[1,2]
[1]Biomedical Engineering and [2]Institute for Micromanufacturing
Louisiana Tech University, Ruston, LA 71272

Statement of Purpose: Tissue engineering utilizes porous scaffolds within which embedded cells are allowed to develop into a functional tissue substitute [1]. There exists a need for additional processing or modification, such as incorporation of growth factors/particulates, after initial scaffold fabrication due to the fabrication condition and limitation on the ways the scaffold material can be processed. Hydroxyapatite particles have been shown to increase adhesion and proliferation of osteoblasts [2] whereas iron oxide particles have demonstrated potential for increasing membrane permeability of endothelial cells [3]. Previously, electrochemical impedance spectroscopy (EIS), in which a current is allowed to pass through the porous materials under alternative current setup, has been successfully applied to examine pore characteristics of chitosan scaffolds [4]. The purpose of the current study is to examine the use of electrokinetics for the modification of chitosan scaffolds with particulates.

Materials and Methods: Chitosan scaffolds were fabricated by dissolving 2% wt per vol chitosan powder (Sigma) in 2% acetic acid (Sigma), heated at 50 ˚C, followed by lyophilization. Iron oxide microparticles (15 μm in diameter, 2% wt/vol, Sigma), micron-sized hydroxyapatite (HA, 100-300 μm in diameter, 2% wt/vol Berkeley Advanced Biomaterials) and nanometer-sized HA (200 nm in diameter, 0.5% wt/vol, Sigma) were used as model particles. Particle suspensions were prepared in phosphate-buffered saline (PBS).

Cylindrical chitosan scaffold (3 cm in diameter, 1.5 cm in thickness) was placed in a plexiglass box (5 cm in length in each side) filled with particle suspension. Occasional stirring was performed to maintain particle suspension. A central electrode was inserted into the center of the scaffold whereas a radial electrode was placed around the periphery. For HA particles, the central electrode was set to negative whereas the radial electrode is positive because the net surface charge is positive. The opposite arrangement was used for iron oxide particles since they carry net negative charge. A direct voltage of 4V was used to electrokinetically deliver particles into the interior of the chitosan scaffolds. The run times for nanosized HA, micron-sized HA and iron oxide were 0.66 hr, 6 hr and 43 hr, respectively. For control experiment, no electrokinetic treatment was applied.

Electrokinetically particle-treated scaffold was sectioned to expose the interior for visual observation by bright-field optical microscopy. Dried scaffold was sectioned and sputter-coated with gold for scanning electron microscopy (SEM). In mercury intrusion porosimetry (MIP), the applied pressure and the corresponding volume of mercury penetrating into the scaffold were recorded. The MIP software then automatically converted the data into porosity data and mathematically derived the average pore diameter. The EIS was run with a constant current of 0.1 mA between two titanium electrodes at a frequency sweep of 10^6 to 10^{-4} Hz. The results were compared to scaffolds without electrokinetic particle treatment.

Results and Discussion: Optical microscopic observation revealed evenly distributed nanosized HA particle whereas no micron-sized HA particles were found within the scaffold. This is expected because the latter is too large to penetrate the pore of the scaffold whose average size is about 107 μm. In comparison, more iron oxide particles were found to infiltrate the scaffold with electrokinetic particle treatment as compared to control. SEM corroborates with optical microscopic results. Moreover, presence of calcium within the scaffold was confirmed from the results of energy dispersive X-ray analysis.

The EIS produced a Nyquist plot of the real (x-axis) vs. imaginary impedance (y-axis) which showed a curve with x-intercept at approximately 10,600 ohms, corresponding to the bulk resistance of the unmodified scaffold. With the presence of iron oxide particles, the bulk resistance increased slightly resulting in significant decrease in porosity (70.2%) when comparing to control (71.0%; $p<0.05$). For unmodified scaffold, the overall porosity of the chitosan scaffold as determined by MIP was about 72.90±0.49%. With the presence of iron oxide particles, the porosity was significant reduced to 71.98±0.36% ($p<0.05$).

Conclusions: A protocol for electrokinetic particle intrusion was successfully developed for the modification of chitosan scaffolds. Iron oxide particles with size in the 15 μm range were delivered electrokinetically into the interior of the chitosan scaffolds as compared to simple exposure to the particle suspension without the potential difference. The modification procedure can be performed in physiologically tolerable conditions making it possible for further modification. The electrokinetic particle modification protocol has the potential for the delivery of a large number of candidate agents by optimizing the treatment conditions.

Acknoledgement: Partial funding was provided by NIH Grant P20 RR16456. Stephanie Tully-Dartez was a NSF GK-12 Teaching Fellow supported by a grant awarded to Dr. David Mills of Louisiana Tech University (DGE 0638730). The authors would like to thank Dr. Henry Cardenas and Mr. Faisal Syad of Louisiana Tech University for the use of electrochemical impedance spectroscope. Technical support on SEM was provided by Dr. Karen Xu.

References: 1. Principles of Tissue Engineering. R Lanza, R Langer, J Vacanti, Ed, 2nd ed, (2000). 2. Barrias et al., Key Eng Mater, (2004) 254-256:877. 3. Apopa et al., Particles and Fiber Toxicol, (2009) 6:1. 4. Tully-Dartez et al., Tissue Eng: Part C. (2009) In press.