Development of a Novel Silica Sol Vapor Deposition System for use in Interfacial Tissue Constructs

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Statement of Purpose: Mild processing conditions and inherent biocompatibility make silica sol-based materials popular for use in orthopedic tissue engineering. These materials also have the potential to serve as delivery vehicles for various organics (i.e. proteins, enzymes) and inorganics (i.e. constituent apatite species Ca^{++} and PO_4^-). Our long-term goal is to develop composite sol nanoparticle-nanofiber-microporous polymer scaffolds for interfacial tissue engineering. By controlling the chemical, physical, and mechanical properties of these materials and incorporating gradients of directive factors, such as Ca^{++}, PO_4^- and growth factors, to mimic native tissues, we hope to generate tissue scaffolds that promote appropriate cell behavior as a means for bone and interfacial tissue regeneration. The objectives of this preliminary study were to develop reliable methods for creating vaporized sol nanoparticles and to characterize their suitability for use in tissue scaffolds.

Methods: Vaporized sol-materials were generated using tetramethyl orthosilicate (TMOS)/H2O hydrolyzed with HCl and rotary evaporated to remove excess methanol. A novel vaporization chamber attached to a jet-type nebulizer was used to create thin film coatings of sol droplets with pressurized air (35-40psi). Droplet morphology and size distribution on various surfaces was analyzed using FESEM and BioQuant™ software. Cytotoxicity was assessed by direct culture (L929 fibroblasts) and a live/dead fluorescence assay (calcein-AM/ethidium bromide).

Sol solutions were doped with 5v/v% Ca^{++} and PO_4^-, vaporized onto polystyrene plates (90 seconds) and incubated in SBF (1 week). Apatite-like mineral formation was assessed using FESEM and EDS.

Composite materials were constructed by depositing the sol vapor onto the surface of electrospun fibers and porous polymer scaffolds (60, 90 and 180 seconds). FE-SEM was used to assess the spatial distribution and uniformity of the sol vapor.

Results: Vaporized sol droplets were successfully generated with the nebulizer system and easily controlled to create simple patterns. Droplet size ranged from <5μm to >20μm (data not shown). Cytotoxicity measures (**Fig. 1**) showed that cells cultured with stable sol vapor were viable and morphologically normal. Droplets were stable for >72 hours in culture (<5% loss in droplet volume).

Particles doped with Ca^{++} and PO_4^- were capable of inducing stable apatitic mineral formation (**Fig. 2 right**), compositionally similar (Ca/P ratio) to bone specific mineralites (**Fig. 2 Table left**).

Electrospun fibers and porous scaffolds were successfully vapor-coated to generate composite materials (**Fig. 3A-D**). The sol vapor had no affect on the structure or stability of substrate materials and could be grossly patterned using simple masking techniques (**Fig. 3A**).

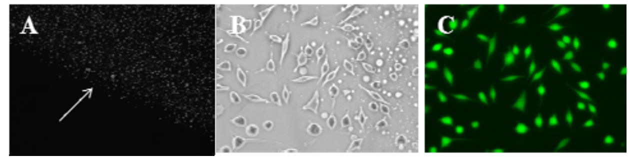

Fig. 1. Cells viability in culture with sol particles. (**A**) Patterned Rhodamine B-labeled particles (arrow at vapor-line) (5x). (**B, C**) Phase contrast and live-dead fluorescent image of fibroblasts seeded at vapor-interface (10x). Cells were viable and morphologically normal

Mineral Crystal	**Ca/P**
Tricalcium Phosphate	1.50
Mineralized Sol Crystals	***1.63***
Hydroxyl Apatite (HAp)	1.67

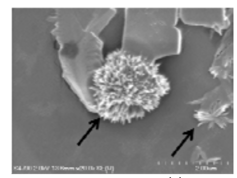

Fig. 2. Apatite-like mineral formation with Ca^{++} and PO_4^- doped sol. (**right**) FESEM image of apatite-like mineral crystallites (arrows). (**Table left**) EDS analysis showed a Ca/P ratio similar to bone mineral species.

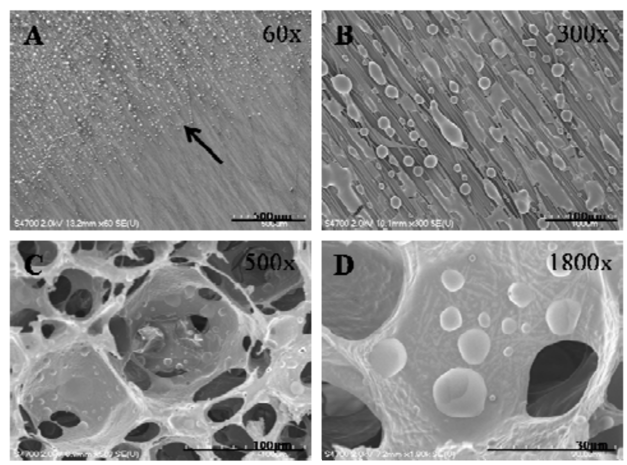

Fig. 3. Composite scaffolds. FE-SEM images of vapor coated (**A,B**) electrospun PLLA nanofibers (arrow indicates vapor line created by masking) and (**C,D**) fibrin scaffolds. Particles deposited onto the surface and also penetrated between fibers and into scaffolds.

Conclusions: We have shown that: (1) vaporized sol particles can be generated quickly under mild processing conditions, (2) particles are stable and biocompatible, (3) inorganic ions can be delivered and induces apatite-like mineral formation and (4) two- and three-dimensional patterned composite scaffolds can be generated without affecting the underlying substrate. Further experiments are underway to develop controlled release of inorganic and organic molecules from particles as well as assess the cell response to generated composite scaffolds.