Biomimetic Fibrous Hydrogels with Aligned Hierarchical Structure Promote Biomineralization in Vitro

Akhil Patel[1]*, Vinayak Sant[1], Shilpa Sant[1,2,3#]

[1]Department of Pharmaceutical Sciences, School of Pharmacy; [2]Department of Bioengineering; [3]McGowan Institute of Regenerative Medicine, University of Pittsburgh, PA. #: Corresponding author

Statement of Purpose: Bone and teeth are two major hard tissues in human body. These hard tissues are formed by a natural process of mineral deposition called biomineralization, which generally provides toughness to the hard tissues. In extracellular matrix (ECM) of hard tissues, collagen acts as an organic template to guide mineral deposition. Hydroxyapatite is naturally occurring calcium apatite which acts as inorganic phase. In bone and teeth, these organic and inorganic phases interact at molecular level forming hierarchical nanocomposites. However, most of the materials currently being used for hard tissue regenration are just physical blends of organic and inorganic phases. Thus, there is need to fabricate biodegradable, bioactive materials which match structural hierarchy of natural tissues at nano/micro scale to actively guide the mineral deposition and promote regeneration of damaged hard tissues in a natural manner.

Methods: Oppositely charged natural polysaccharides were used for preparing aligned fibrous hydrogel scaffolds. In order to study role of amount of charge and charge imparting groups in biomineralization, we chose three different pairs, each with two oppositely charged polymers. Appropriate concentrations of such polysaccharides were allowed to self-assemble in a microfluidic chamber at constant rate. Aligned hydrogel fibers were collected on a plastic coverslips to fabricate fibrous scaffolds. Hierarchical structure at different scale lengths (nm to cm) of fabricated hydrogels was investigated using light and electron microscopy. These fibrous hydrogels were further incubated in Simulated body fluid (SBF) at 37°C for three days to study their mineralization potential in vitro. Chemical and crystalline structure, alignment and spatial distribution (inter/intrafibrillar) deposition of deposited minerals were investigated using fourier transform infra-red spectroscopy (FTIR), X-ray diffraction (XRD), scanning and transmission electron microscopy.

Results: We developed hierarchical fibrous hydrogels mimicking collagen at different length (nano/micro-) scales and studied their potential for biomineralization. Self-assembled hydrogel scaffolds mimic collagen-like hierarchy, which was confirmed using light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). TEM images revealed collagen-like dark and light bands in all three scaffolds. All the different hydrogel scaffolds promoted biomineralization, although to varied degree. Morphology and nature of mineral deposits also varied among the different groups based on the amount of negative charges and charge imparting group. SEM images of mineralized scaffolds revealed mineral deposition on the surface of the scaffolds. Mineral deposition on the surface was also found to be aligned along the length of hydrogel fibers in scaffolds. TEM of thin sections of mineralized scaffolds showed mineralization inside scaffolds. Mineral deposition, both on surface and inside the scaffolds, was orderly rather than random. FTIR and X-ray diffraction studies revealed apatite-like chemical composition of deposited minerals.

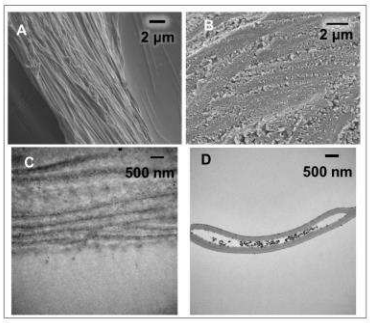

Figure 1. **A.** Hydrogel scaffolds with fibrous hierarchy; **B.** SEM of mineralized scaffold; **C, D**. TEM images showing intra- and interfibrillar mineralization.

Conclusions: Fibrous hydrogel scaffolds with collagen-mimetic hierarchy were fabricated successfully for all three chosen pairs of polysaccharides. All three scaffolds showed ability to sequester minerals after as short as three days incubation in SBF. Physicochemical properties such as electrostatic charges and charge-imparting group of these fibrous hydrogels played important role in dictating mineral size and structure. These differences further led to different mineral deposition behavior on the surface and interior of the scaffold. Interestingly, mineral deposition was orderly rather than random.

These studies collectively prove collagen-mimicking ability of aligned fibrous hydrogels to guide mineral deposition in vitro. They also suggest potential of these scaffolds for hard tissue regeneration in vivo.

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