Hydroxyapatite Mineralization on Porous Silicon Surfaces and in 1-D Gels for Biomimetic Applications

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Statement of Purpose: Development of biomimetic materials for mineralized tissue and interface applications relies on understanding the carefully controlled interaction of cells, proteins, matrices, and mineral. We have developed a gel-based double-diffusion system (DDS) that allows for *in vitro* integration of cells and nucleating surfaces to study the effects of gradients, surface chemistry and proteins on the formation of hydroxyapatite (HA; the mineral component of bone). Porous silicon (pSi) disks serve as nucleating surfaces with tunable surface chemistries.[1,2] The integration of gels and functionalized surfaces into a DDS allows us to modify crystal size, morphology, and surface coverage, all of which affect are key variables for determining materials and biological properties. We can also introduce temporal and spatial gradients of proteins and ion/small molecule gradients to further tune the mineralization.

Methods: A modified double diffusion system (DDS) was used for gel-based mineralization studies.[3] Agarose 1B was purchased from Sigma. Gel mineralization experiments were conducted at calcium and phosphate reservoir concentrations of 100mM (Tris Buffer, pH 7.4). Porous silicon surfaces were fabricated using Siltronix p-type 0.001-0.003 Ωcm silicon. Electrochemical etching methods generated pSi surfaces and pSi lift-off films.[4] The pSi was functionalized with oxide, methyl, alcohol and carboxylic acid chemistries via standard methods.[1] Surface functionality was verified by using ATR (attenuated total reflection) geometry on a Bruker v80 FTIR spectrometer. Solution-based experiments were conducted at 25°C, ambient atmosphere as well as at 37°C, 5% CO_2 for 7 days. For both temperatures, pSi disks were placed in 2.5mM Ca^{2+}/1.5mM PO_4^{3-} and 5mM Ca^{2+}/3mM PO_4^{3-} (Tris buffer, pH 7.4). In all solution-based experiment the pSi disks were suspended, functionalized-side down, in solution to ensure heterogenous surface nucleation. Mineralized gel samples were analyzed after grinding of frozen lyophilized gel. Mineral morphology was characterized using a FESEM. Powder and GADDS x-ray diffraction (XRD) analysis was used to confirm mineral polymorph.

Results: HA formation in the gel DDS was confirmed via SEM and XRD (Fig. 1). FTIR spectroscopy confirmed surface functionality on oxide (Si-OH), dodecane (-CH_3), undecylenic alcohol (-OH), and undecylenic acid (-COOH) functionalized surfaces.

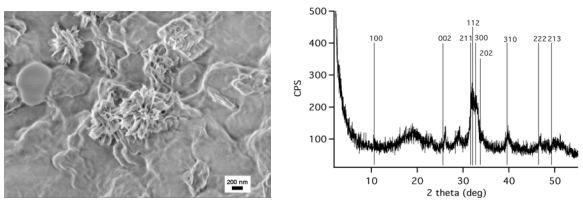

Figure 1: *SEM image and XRD results for HA grown in agarose gel. 200 nm scale bar.*

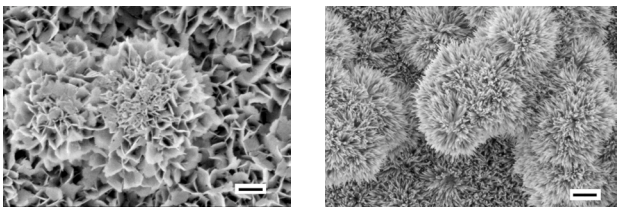

Figure 2: *SEM images demonstrating surface chemistry effect on crystal morphology (left -CH_3 pSi, right -COOH pSi). Both scale bars are 400 nm.*

Solution-based HA formation on pSi and single crystal silicon surfaces was confirmed for 5mM/3mM Ca^{2+}/PO_4^{3-} concentrations via SEM and GADDS XRD. Platelet crystals in the rosette-like aggregates on the -CH_3 surface are larger than the smaller, needle-like crystals grown on the -COOH surface (Fig. 2). The pSi lift-off films are currently being integrated into the gel-based DDS system to examine the combination of gel and surfaces on mineral morphology (Fig. 3).

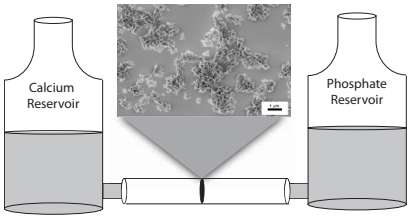

Figure 3: *DDS system diagram with placement of nucleating surface and HA SEM on oxide surface. 1μm scale bar*

Conclusions: This system allows for the study of mineralization behavior in a biologically-relevant environment, in which nucleating surfaces are integrated into a gel-base DDS system. HA formation on functionalized pSi surfaces and in agarose gels was confirmed by SEM and XRD. Surface functionality on pSi surfaces, obtained via standard surface chemistry techniques, was shown to affect crystal size and morphology. Future studies in the DDS will include introduction of pSi surfaces (with varying surface chemistries), cells (e.g., articular chondrocytes, breast cancer cells), and ion gradients. Ultimately, we will examine the effects of cells on mineralization (and mineralization on cells). The integration of multiple systems (nucleating surfaces, gels, gradients, and cells) provides insight into the biological mechanisms affecting mineralization as well as the development of biomimetic strategies to repair hard tissues and treat diseases.

References:

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