Integrative Design of a Poly(ethylene glycol)-Poly(propylene glycol)-Alginate Hydrogel for Three Dimensional Biomineralization

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Statement of Purpose: A mineralized polymeric matrix has been extensively studied to understand biomineralization processes and to further regulate phenotypic functions of various cells involved in osteogenesis and physiological homeostasis. It has been often proposed that several matrix variables including charge density, hydrophobicity, and pore size play vital roles in modulating composition and morphology of minerals formed within a three dimensional (3D) matrix. However, the aspects have not yet been systematically examined because a tool enabling the independent control of the matrix variables is lacking. This study presents an advanced integrative strategy to control morphology and composition of biominerals with matrix properties, by using a hydrogel formulated to independently control charge density, hydrophobicity, and porosity.

Methods: The hydrogels were made by radical co-polymerization of poly(ethylene glycol) monomethacrylate (PEGmM), poly(propylene glycol) monomethacrylate (PPGmM), and methacrylic alginate (MA) in various formulations, so the charge density and hydrophobicity of the hydrogel can be separately controlled with mass fractions of MA and PPGmM, respectively. Also, hydrogels which present only nano-sized pore (nanoporous) are lyophilized and rehydrated to prepare the hydrogels containing micro-sized pores (micropores). The resulting hydrogels were incubated in simulating body fluid to induce mineralization within the hydrogels. Various analytical tools, such as scanning electron microscopy (SEM), X-ray diffraction spectroscopy (XRD), Fourier transform infrared spectroscopy (FT-IR), as well as quatitative analyses were used to characterize the minerals formed inside the hydrogels. We further evaluated the viability of cells laden into mineralized hydrogels to show the effect of mineralization on the cell viability.

Results: The amount of apatite minerals formed inside the hydrogels increased with increasing fractions of MA, and PPGmM, which suggest that the increases in charge density and hydrophobicity promoted the deposition of minerals. In addition, increasing the pore size from nano-scale to micro-scale significantly increased overall amounts of apatite in all conditions. These conditions which improve the extent of mineralization were related to the supersaturation with respect to apatite. Morphological analysis using SEM and spectroscopic analyses using XRD and IR confirmed that increasing charge density, hydrophobicity, and pore size resulted in significant increase of apatite mineral deposition (Fig 1). Interestingly, in nanoporous hydrogels, the majority of minerals formed inside the hydrogels were rhombohedral calciate minerals, which indicate that the influx of bigger and more negatively charged phosphate ions were limited in nanoporous hydrogels. Then, we further evaluated the viability of cells laden into mineralized microporous hydrogels, and showed that the viability was significantly promoted with mineralization.

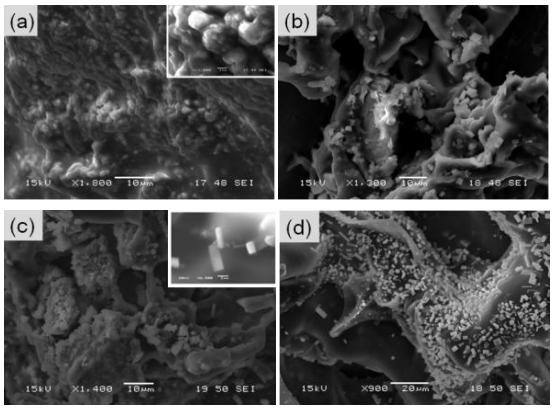

Fig. 1. Scanning electron microscopic (SEM) images of mineralized hydrogels. More extensive deposition of spherulitic minerals was observed in microporous hydrogel with higher PPGmM (a) than that with lower PPGmM (b). The inset in (a) shows the magnified view of spherulitic mineral particles. In contrast, the nanoporous hydrogel with higher PPGmM presented more extensive deposition of rhombohedral minerals (c) than that with lower PPGmM (d). The inset in (c) shows the magnified view of rhombohedral mineral particles.

Conclusions: Taken together, we conclude that charge density, hydrophobicity, and pore size of a hydrogel are major variables that should be considered in the integrative design of a three dimensionally mineralized matrix. The charge density and hydrophobicity of the hydrogel, tuned with mass fractions of MA and hydrophobic PPGmM and micro-sized pores of the hydrogel facilitate the transport of calcium and phosphates into the hydrogel matrix, and subsequent ion of calcium and phosphates to the matrix and further determine the composition and morphology of minerals. Furthermore, the apatite formed within the hydrogel greatly contributes to elevating the viability of cells cultured within the gel matrix, likely because of the enhanced adsorption of cell adhesion proteins onto the apatite. Overall, the mineralized matrix created throughout this study would provide a better understanding of the critical roles of the properties and structure of a natural extracellular matrix in regulating the biomineralization process and the cellular activities. In addition, the results of this study will be highly useful in designing a wide array of mineralized synthetic materials used for various biological and industrial applications.