**Design of Biomaterial Bioinorganic Interfaces Related to Mineralization**

**Davoud Ebrahimi1, Zaira Martin-Moldes2, David L. Kaplan2, Markus J. Buehler1**

1Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

2Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA

Biomineralization at organic-inorganic interfaces is critical to biological-material functions, in some cases in terms of preventing mineralization (e.g., in calcific aortic valve disease) while in other applications in promoting the mineralization process (e.g., in osteogenesis) in the body. Bioengineered silks are attractive biomaterial substrates as they provide scaffolds for tissue regeneration due to biocompatibility, the ability to fine tune properties through sequence modification and processing, and the potential to engineer these proteins to incorporate diverse and selective functional domains. High performance supercomputing simulations were utilized to synergistically identify relationships between the sequence design of silk protein-silica binding peptides and the effect on silicification. In addition, intracellular pathways involved in the process of mineralization when stem cells were grown on these silica substrates were also assessed.

In this integrated experimental-simulation approach, six silk sequence constructions were pursued, based on designs with three key domains: a core silk domain for materials assembly, a histidine tag for purification, and a silica binding peptide domain for silicification. The results showed that the addition of the silica and histidine domains reduced β-sheet structure in the recombinant protein materials, and increased solvent-accessible surface area to the positive charged amino acids, leading to higher levels of silica precipitation. Moreover, the simulations showed that the location of the charged biomineralization domain had a minor effect on protein folding and consequently the surface exposure of charged amino acids, in agreement with the experimental data. To determine key intracellular pathways involved in cellular responses to the silk-silica materials during osteogenesis, gene expression levels of integrins, MAPK (mitogen-activated protein kinase), Runx2 (Runt-related Transcription Factor 2) transcription factor (TF) and osteoblast markers were analyzed by qRT-PCR. In a first approach we identified the integrin αVβ3 as the protein responsible for sensing the surface to promote differentiation. Moreover, simulations showed activation of integrin αVβ3 when this protein was in contact with the silica surface. Cells growing on the silk-silica films showed induction of MAPK, TF and osteogenic markers, while cells growing on silk films without silica showed no induction of these genes. The use of a blocking antibody against integrin αVβ3 showed an abolishment of the induction of genes observed for the cells cultured on silk-silica films related to MAPK and TF, confirming involvement in osteogenesis. The location of the biomineralization domain with respect to the core silk protein domain resulted in no differences in gene expression.

This integrated modeling and experimental approach provides insight into sequence-structure-function relationships for control of mineralized protein biomaterial structures.

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