Synthetic cells used to bioengineer new forms of silica

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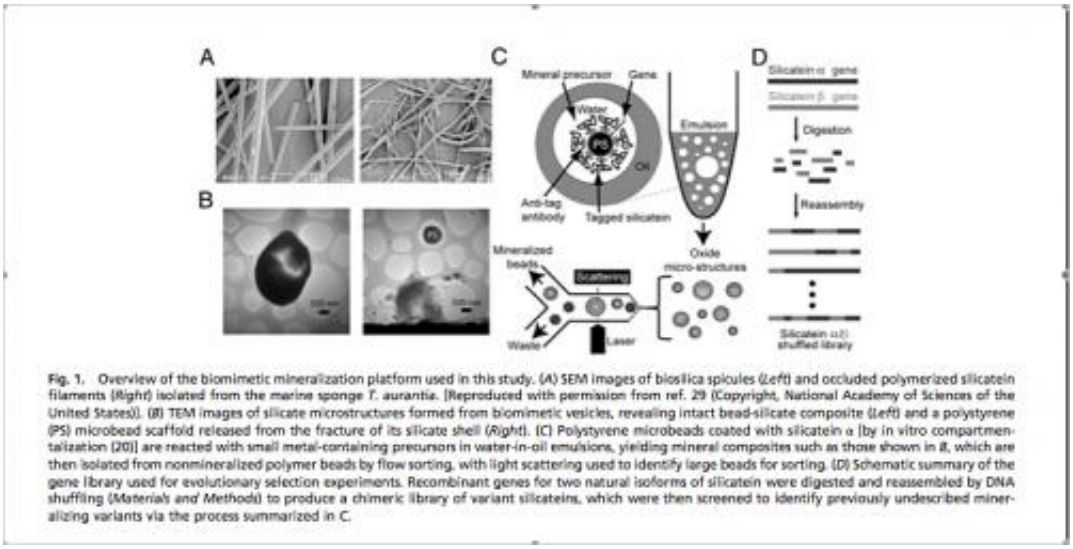

Fig. 1. Overview of the biomimetic mineralization platform used in this study. (A) SEM images of biosilica spicules (Left) and occluded polymerized silicatein filaments (Right) isolated from the marine sponge T. aurantia. [Reproduced with permission from ref. 29 (Copyright, National Academy of Sciences of the United States)]. (B) TEM images of silicate microstructures formed from biomimetic vesicles, revealing intact bead-silicate composite (Left) and a polystyrene (PS) microbead scaffold released from the fracture of its silicate shell (Right). (C) Polystyrene microbeads coated with silicatein α [by in vitro compartmentalization (20)] are reacted with small metal-containing precursors in water-in-oil emulsions, yielding mineral composites such as those shown in B, which are then isolated from nonmineralized polymer beads by flow sorting, with light scattering used to identify large beads for sorting. (D) Schematic summary of the gene library used for evolutionary selection experiments. Recombinant genes for two natural isoforms of silicatein were digested and reassembled by DNA shuffling (Materials and Methods) to produce a chimeric library of variant silicateins, which were then screened to identify previously undescribed mineralizing variants via the process summarized in C.

This is first author Lukmaan Bawazer. Credit: UCSB

(Phys.org) -- Scientists do not fully understand how nature uses proteins to develop new materials and minerals, but learning more about the natural processes could lead to bioengineering methods such as the biological synthesis of solid-state materials for electronics applications. Now researchers in the US have designed a synthetic biological platform to facilitate the study of these processes and genetically engineer new materials.

The scientists, led by Professor Emeritus Daniel E. Morse of the University of California, Santa Barbara, created synthetic cells

containing a polystyrene micro-bead as a nucleus. They then created DNA segments containing genes from two related silicateins along with random mutations and attached a piece of this DNA to each plastic bead. They soaked each bead in a mixture of bacterial proteins required by the synthetic cells to manufacture silicateins, and surrounded the beads with oil to act as the cell membrane.

Silicateins are biomineralizing proteins found in marine sponges that synthesize silica (silicon dioxide) and titania (titanium dioxide) materials. The marine sponge Tethya aurantia, for example, produces silica spicules that make up 75 percent of its dry weight. Silica is commonly used in computer chips, while titania is used in photovoltaic solar cells.

The synthetic cells manufactured silicateins, which appeared on the nuclear bead's surface attached to antibodies. The researchers then ruptured the artificial cells to release the silicateins, and soaked them in a solution containing the silica or titania precursors. The resulting minerals formed a coating on the beads.

The researchers then set out to direct the evolution of the synthetic cells. They first sorted the beads to identify those with DNA coding for proteins making particularly strong minerals. They sorted them by size, with those having the thickest layers of minerals being selected. They then shook the selected beads to break up the minerals, and selected only those beads that survived this process.

Thirty genes randomly selected from the DNA for either silica- or titania-forming enzymes in the selected beads were then sequenced. They found that the genes contained sequences common to the two original silicatein genes, but they also identified new genes that were completely different from the initial genes.

The original genes coded for silicatein alpha, which manufactures silica in clumps of particles. The new genes coded for proteins that produced silica and titania in a dispersed nanoparticle form. One of the new proteins, silicatein X1, manufactured silica in the form of folded sheets of silica-protein fibers.

The process being developed could be extended to other materials. There are other marine sponges, for example, that produce fibreglass, and bacteria that produce magnetic nanoparticles. The new method could also be used to bioengineer novel materials with hitherto unseen structures and to do so in a way that is environmentally benign.

More information: Evolutionary selection of enzymatically synthesized semiconductors from biomimetic mineralization vesicles, *PNAS*, Published online before print June 7, 2012, doi: 10.1073/pnas.1116958109

Abstract
The way nature evolves and sculpts materials using proteins inspires new approaches to materials engineering but is still not completely understood. Here, we present a cell-free synthetic biological platform to advance studies of biologically synthesized solid-state materials. This platform is capable of simultaneously exerting many of the hierarchical levels of control found in natural biomineralization, including genetic, chemical, spatial, structural, and morphological control, while supporting the evolutionary selection of new mineralizing proteins and the corresponding genetically encoded materials that they produce. DNA-directed protein expression and enzymatic mineralization occur on polystyrene microbeads in water-in-oil emulsions, yielding synthetic surrogates of biomineralizing cells that are then screened by flow sorting, with light-scattering signals used to sort the resulting mineralized composites differentially. We demonstrate the utility of this platform by evolutionarily selecting newly identified silicateins, biomineralizing

enzymes previously identified from the silica skeleton of a marine sponge, for enzyme variants capable of synthesizing silicon dioxide (silica) or titanium dioxide (titania) composites. Mineral composites of intermediate strength are preferentially selected to remain intact for identification during cell sorting, and then to collapse postsorting to expose the encoding genes for enzymatic DNA amplification. Some of the newly selected silicatein variants catalyze the formation of crystalline silicates, whereas the parent silicateins lack this ability. The demonstrated bioengineered route to previously undescribed materials introduces in vitro enzyme selection as a viable strategy for mimicking genetic evolution of materials as it occurs in nature.

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