Replicating Cellular Life Forms in Silica

C. Jeffrey Brinker, Fellow, Sandia National Laboratories, Distinguished and Regent's Professor, the University of New Mexico, Albuquerque, NM USA

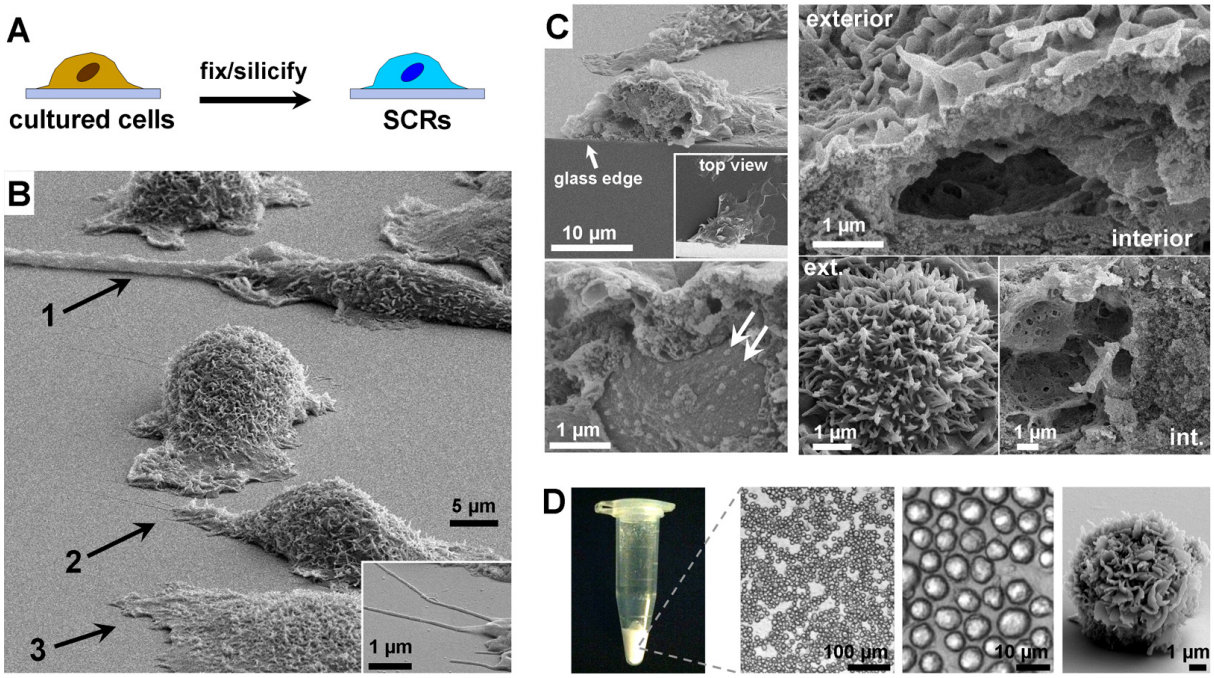

Figure - Generation of Silica Cell Replicas (A) preserves cells features (B) including large (1) and small filopodia (2 and inset) and lamellipodia (3). Fractured SCRs at a glass edge (C) reveal internal features such as nuclear pore assemblies (arrows). Right panels show conservation of complex internal and external features. (D) SCRs derived from solutions of cells provide high-throughput generation of hierarchical, thermo-tolerant particles. Bryan Kaehr et al unpublished.

We have discovered a process, *Silica Cell Replication*, wherein mammalian cells direct their exact replication *in silica*. The silica cell replicas preserve nm- to macro-scale cellular features and dimensions on both the cell surface and interior after drying at room temperature - and largely after calcination to 600˚C. The process appears to be self-limiting and self-healing, and remarkably generalizable to any cells of interest—from red blood cells to neurons. Re-exposure of the SCRs to water provides access to intracellular components, where preliminary experiments show partial retention of enzymatic activity. We envision SCRs as simple, rugged and inexpensive constructs that could be stored dry and then re-activated to enable molecular recognition and selective enzymatic activity. More importantly we propose that SCR could in some cases serve as an alternative room temperature approach to expensive and often impractical cryo-preservation of cellular function.

Background - Natural bioinorganic composites as found in bone, shell, and diatoms have long been heralded as model functional materials that evolved over billions of years to optimize properties and property combinations. Often functionality derives from hierarchical architectures composed of hard and soft components organized according to multiple prioritized length scales. To date it has been difficult to mimic these multiscale designs in synthetic manmade materials. During the past six years we have explored a novel *cell directed assembly* process wherein living

cells direct their integration into 3D solid-state nanostructures. Upon evaporation, acidified suspensions of yeast, bacterial, or mammalian cells plus silicic acid ($Si(OH)_4$) and amphiphilic short chain phospholipids dry to form a conformal, fully 3D bio/nano interface surrounding individual cells. This interface composed of localized lipid bilayers enveloped by a lipid/silica mesophase survives drying and evacuation without shrinkage and preserves aspects of cellular functionality. During the past year we demonstrated that this 'silicification' results in part via a self-catalyzed silica condensation process resulting from an osmotic stress response of the cell that causes a localized pH gradient, along with cell surface protein-directed silica deposition. The role of proteins in silica deposition has been studied extensively in the context of biomineralization of diatoms, single celled organisms that are known to construct exquisite and elaborate silica composite exoskeletons. Although there has been significant progress towards an understanding of the molecular components involved in biogenic silica formation, the whole picture remains vague, as evidenced by a current inability to reproduce diatom-like silica features *in vitro* using synthetic or native silica-associated biomolecules.

Diatom silica biosynthesis is clearly a process by which the chemical microenvironment is tightly controlled through compartmentalization and transport. Taking lessons from nature concerning silica morphogenesis within the acidic diatom silica deposition vesicle (SDV), we wondered if a mildly acidic and highly crowded and confined macromolecular scaffold would prove sufficient for silica deposition. To test this hypothesis, we used multiphoton lithography (MPL) to fabricate protein hydrogel scaffolds. This technique enables microstructures comprised of proteins of choice to be fabricated with arbitrary 3D geometries. We observed that under dilute acidic conditions these scaffolds direct their precise replication to form silica/protein biocomposites with preserved feature sizes that survive calcination. Importantly, proteins of diverse properties (e.g., isoelectric point; pI) directed silica condensation under identical solution conditions, which is to some extent contrary to the existing paradigm that cationic species (e.g., proteins with $pI > 7$) are necessary to direct silica biomineralization.

Based on these results, we recognized that cells are composed of elaborate and functional protein scaffolds and surfaces that are organized over multiple length scales and questioned whether these natural architectures also direct silica deposition under similar chemical conditions? To address this question we fixed cells and treated them with dilute acidic silica solutions as for proteins. We observed that all cellular features are preserved with very high fidelity under what appears to be a self-limiting process (Figure). A fascinating discovery, when viewed in the context of silica sol-gel chemistry, is that these cell replicas are several nanometers thick, self-limiting and withstand drying and calcination with essentially no shrinkage. This mandates that the silica form a contiguous network with nearly a complete extent of condensation. Our current hypothesis is that due to comparable hydrogen bonding strengths silicic acid molecules replace bound water at cellular interfaces and are amphoterically catalyzed by proximal proteins and other membrane bound components to form a self-limiting, defect-free nm-thick silica encasement that resists drying and calcination stress. As the silica layers interact with cell components through strictly non-covalent interactions and preserve dimensional features, de-silicification should 'thaw' the system and allow recovery of bio-functionality.