Development and Characterization of 3D, Nano-Confined Multicellular Constructs for Advanced Biohybrid Devices

LDRD
LABORATORY DIRECTED RESEARCH & DEVELOPMENT

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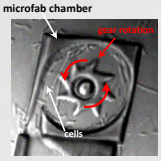
Problem

Inspiration for the design of new materials and devices is increasingly found in biological systems where sensitive detection, energy conversion and molecular/nano machinery have been continually improved upon by evolution. However, to impart the useful properties of biological systems into devices requires new ideas and technologies. Although there has been much focus on material functionalization using biomolecules, incorporation of self-sustaining and self-replicating components (e.g., biological cells and bio-catalysts) into solid-state platforms has received scant attention. Moreover, in order to bridge the organic structures and functionalities of cells to the inorganic, solid-state materials of modern devices, functional biotic/abiotic interfaces are required. The proposed research will address these problems using a breakthrough approach for the rapid-prototyping of 3D bio-interfaces and catalytic architectures. This will enable deployment high value cellular behaviors (e.g., material synthesis, nanomachinery, etc.) in device settings.

Cells employed for:

Material synthesis

Propulsion

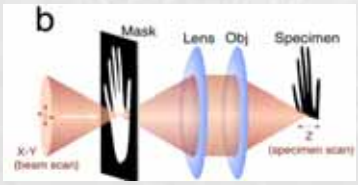


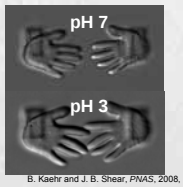
Integrated into devices

Approach

In order to better understand and broadly employ living cells and biological processes for applications involving sensing, material synthesis, microactuation and energy conversion requires the development of new strategies to both explore and optimize interfaces between living and non-living components. Multiphoton fabrication, an inherently three dimensional direct-write fabrication approach, provides a potential route for these interfaces to be rapidly prototyped. Microstructures can be fabricated with arbitrary 3D geometries and from biological components (e.g., proteins, enzymes) that retain functionality, enable precise scaffolding and direct mechanical responses to chemical stimuli.

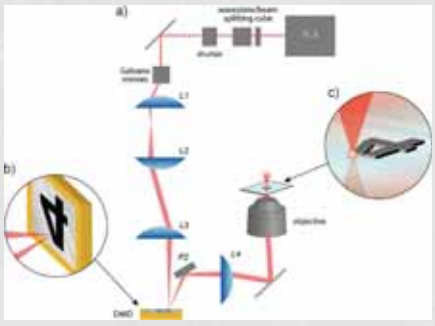
Masked-Directed Multiphoton Lithography of Biological Materials



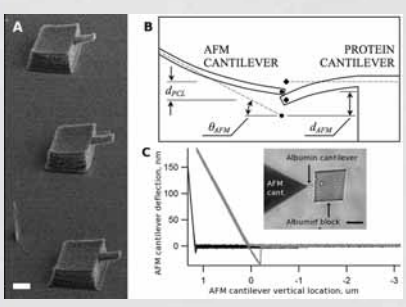


B. Kaehr and J. B. Shear, *PNAS*, 2008.

DMD-directed MPL. a) The output from a titanium sapphire (Ti:S) laser is attenuated using a waveplate and beam-splitting cube and is raster scanned using a pair of galvanodriven mirrors. Three relay lenses direct the focused beam onto the face of the DMD. After reflection off the DMD the beam is guided by a periscope (P2) and is recollimated using a tube lens before entering the microscope objective. b) Close-up showing interrogation of the DMD dynamic-mask using a scanned laser focus in which the region corresponding to the character "4" reflects the scanned laser light toward the microscope. c) A protein replica based on the mask image in (b) is fabricated in the microscope specimen plane.



In situ analysis of microfabricated 3D hydrogels



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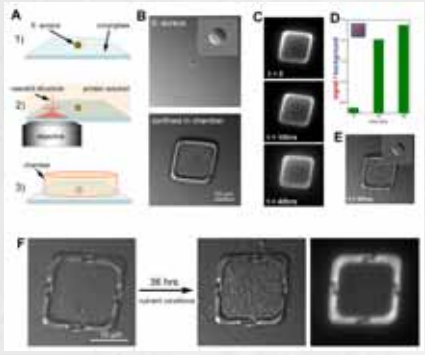
Multiphoton fabricated protein microcantilevers investigated using AFM. Protein microcantilevers (**A**; SEM image, scale bar, 5 µm) are tested in aqueous media by pressing with an AFM cantilever (**B**). An AFM force curve with the recorded cantilever deflection on the ordinate and the AFM z position on the abscissa is shown in **C**. The black force curve was collected by pressing the AFM tip on the glass substrate, and consequently the slope = 1. The grey curve was collected by pressing the AFM tip upon the tip of the protein microcantilever (insert) and the slope < 1 due to the additional compliance of the protein microcantilever. The insert shows an optical microscope image of the AFM cantilever (dark triangle) positioned at the tip of a protein cantilever (scale bar, 10 µm).

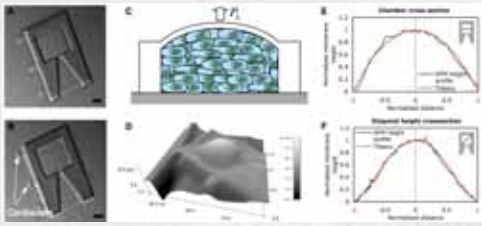
Results

In situ microfabrication enables cell isolation/incubation



In situ confinement of single cells and groups leads to outcomes dependent on the diffusivity of the microchamber. (A) Schematic of entrapment. S. aureus carrying the QS reporter agr: P3-gfp (1) are confined in a protein microchamber fabricated from a biocompatible protein solution (2, 3). B) Optical microscopy images of a single cell confined in a low diffusivity chamber. (C, D) At 16 hrs and 40 hrs the cell reports QS via fluorescence and forms a cell-division septum (E). (F) A group of ~7 reporter S. aureus cells entrapped in a high diffusivity chamber (left panel, achieved via chicane ports on the sidewalls), replicates to fill the chamber over time (center panel) but does not report QS (right panel).

Mechanical Analysis of Dynamic Cell/Material Interactions



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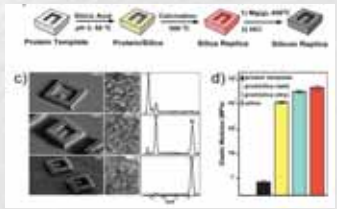
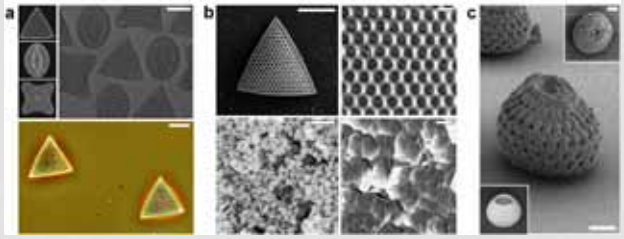
Motile bacteria are captured from the surrounding environment and confined in a protein microchamber (**A, B,** images show the glass substrate/chamber interface and the chamber ceiling respectively; scale bars, 10 µm); bacterial cells reproduce, filling the chamber and deforming the chamber ceiling (**B**, **C**, **D**). The deflection of the membrane, measured by AFM, is compared to a theoretical model for the lateral (**E**) and diagonal (**F**) cross-sections to yield a pressure of (2.7±1.3 kPa) exerted by the cell colony.

Protein-Directed Assembly of Arbitrary 3D Inorganic Architectures

Protein microstructures (light gray cartoon of microcantilever) template silica condensation to form protein/silica hybrid structures (yellow). Calcination removes the protein component leaving a silica replica (red) that is converted to silicon via magnesio-isothermic reduction of silica (dark gray). c) SEM images of protein, silica, and silicon microcantilevers (left panels, top to bottom respectively; scale bars, 10 µm), higher resolution surface topography (middle panels, scale bars 1 µm) and corresponding EDS spectra (right panels show relative intensity). d) Elastic modulus of protein, protein/silica (wet and dry), and silica materials.



Khripin, C.Y., D. Pristinski, D. R. Dunphy, C. J. Brinker, **Kaehr, B.** (2010, under review).



Microfabrication of artificial diatom and radiolarian frustules. a) Images of diatom frustules (upper left panels) direct the fabrication of BSA protein microstructures (DIC micrograph, upper right panel; scale bar, 20 µm) using MDML (lower panel; scale bar, 20 µm). Phase micrograph of diatom-like silica microstructures. b) Characterization of hierarchical features displayed by microfabricated diatom structures using SEM and AFM. The smallest constituent particles, on the order of ~16 nm in size, are visible in the AFM phase image, bottom right panel (scale bars clockwise from top left, 20 µm, 2 µm, 200 nm, 50 nm) c) CAD designed microfabrication of artificial radiolarian frustules using MDML (scale bars, 10 µm). Upper inset, top view; lower inset shows 3D rendering generated from the image sequence used to direct multiphoton fabrication.

Significance

This work represents the development of essentially a new class of materials with potential applications exceeding those initially proposed, benefiting DOE/DHS/and national security missions involving bioterrorism, biomedical and bionic technologies. Breakthrough developments reported here for rapid-prototyping of inorganic device materials in 3D will further enable Sandia thrust areas in photonics, plasmonics, and metamaterials

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