NanoWestern

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Geomicrobiology at UWO
The Southam Laboratory

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In every natural environment on Earth – from the extremes of hydrothermal geysers and high altitude deserts to temperate soils, streams, lakes and oceans – we find bacteria, microorganisms that normally measure on the micron scale. They are the dominant biomass pool on the planet and require a metabolism to survive, which includes the consumption of chemical substrates for energy, as well as the excretion of reactive waste products and the production of extracellular organic material such as proteins and polysaccharides. Metabolic activity therefore creates a condition of interactive exchanges between bacteria and their surrounding environment, creating disequilibrium perturbations such as alteration of mineral-water equilibria, the changing of mineral surface conditions, as well as a microbiological influence on reaction rates and pathways.

The study of microorganisms and their role in geological and geochemical process is the discipline of geomicrobiology, and can be approached from different scientific perspectives. For example, a microbiologist may be interested in how environmental conditions influence microbial physiology or gene expression of a specific bacterium, whereas a geochemist may focus on how microbial activity controls the precipitation or dissolution of minerals resulting from microbially-induced changes in pH, binding of metals on cell surfaces, or microbial redox reactions. Furthermore,

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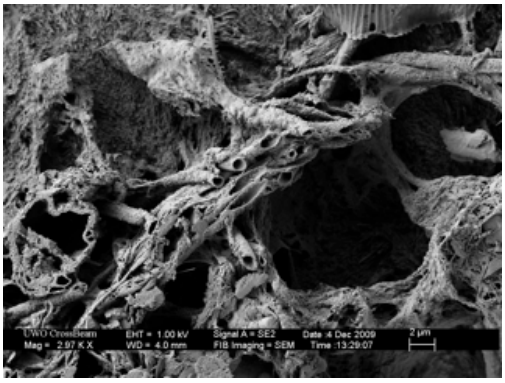

Figure 1 Biologically induced carbonate precipitation – carbonate rocks are widespread on the Earth's surface, and the metabolic activity of microorganisms often leads to geochemical changes in their surrounding microenvironmentthat can induce carbonate precipitation. This image of an acid-etched petrographic thin section reveals filamentous cyanobacteria that were previously entombed within the mineral framework and likely played a role in precipitating these carbonate minerals through photosynthesis.

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geomicrobiology applies techniques in molecular biology, which aid in gaining a more complete understanding of the diversity of microbial communities to target key microorganisms for further experimental studies, be it from the perspective of a microbiologist or a geochemist – and more commonly today, from that of a geomicrobiologist. Success in geomicrobiology requires knowledge in the fields of geology, microbiology, and chemistry, so while students focusing on this discipline are rigorously tested in these areas they emerge with the unique ability to address complex research problems using an interdisciplinary approach.

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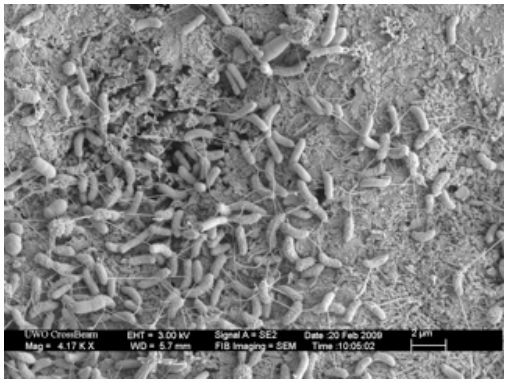

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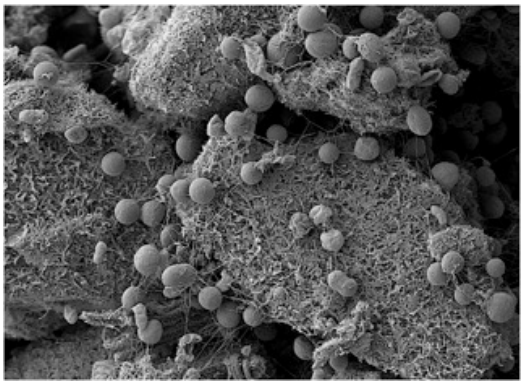

Figure 2. Nanowires – minerals such as Fe and Mn oxides are a major potential source of electron acceptors for dissimilatory microbial metabolism. This normally occurs when bacteria are able to access aqueous substrates (e.g., Fe^{3+} and $Mn^{3+,4+}$) , however in cases where there is low electron acceptor availability this extracellular electron transfer can occur through putative nanowires, which effectively shuttle electrons to the mineral surface as well as between microorganisms. Examples of (a) kimberlite and (b) serpentine minerals show extensive nanowire development between bacteria and the underlying surface, the latter showing Methanococcus volate colonization on serpentinized mineral surfaces, which is believe to be enhanced by nanowires. These appendages were possibly used by Earth's earliest microbial life to allow for more efficient electron transfer, thus creating integrated ecosystems.

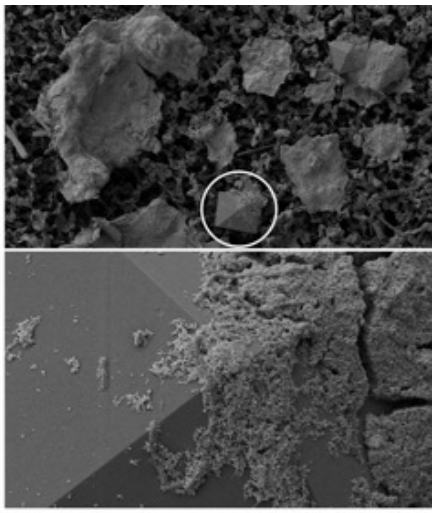

Figure 3. Biofilm development – microorganisms commonly attach to mineral surfaces leading to the development of biofilms that can affect both the aqueous and mineral environments. In this case, consortia of sulfur reducing bacteria have reached a stationary growth phase to create a biofilm. The biofilms are in the initial stages of surface colonization of a precipitated carbonate mineral. This structural microenvironment enables biofilms to concentrate nutrients from surrounding fluid phase and enable further growth.

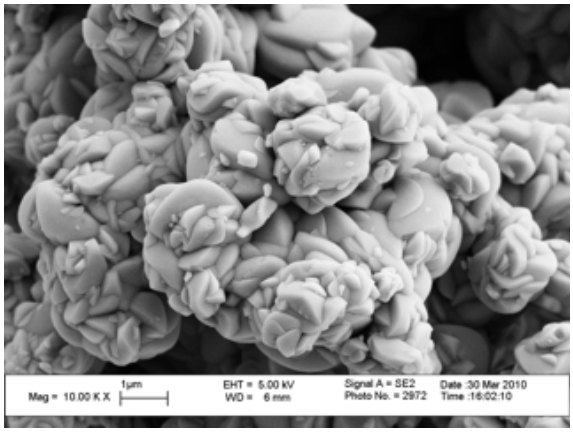

Figure 4. Microbial fossils – The Mazon Creek Fossils, found in Northeastern Illinois, preserve pyritized organisms, sometimes surrounded by pyrite halos, within a siderite ($FeCO_3$) concretion. These pyrite halos may be a two-dimensional view of a three-dimensional biofilm formed at decomposition fronts around the organism. A 10% (w/v) oxalic acid solution was used to dissolve the siderite matrix in order to expose the pyrite fossil and halos. The initial reaction proceeded rapidly, but stopped after about 24 hr of exposure of a piece of concretion to oxalic acid. This image shows the sediments created during dissolution, and indicates that the reaction stops after 24 hr due to passivation of the siderite surface by oxalate crystals.

The University of Western Ontario proudly supports the research of Dr. Gordon Southam, a Canada Research Chair in Geomicrobiology and fearless leader of a laboratory full of aspiring students. Broad in scope, Dr. Southam's research examines the role microorganisms play in a wide array of geochemical processes such as serpentinization, carbonate precipitation, acid mine drainage, and placer gold formation. These studies include field measurements, characterization of natural samples, and laboratory experiments as well as advanced spectroscopic techniques such as synchrotron radiation. However a major emphasis and perhaps most vital to Dr. Southam's research is the visual characterization of these bacteria-mineral processes. High resolution imaging of natural and experimental samples provides us with a microbial perspective of the environment, allowing us to think like a bacterium. The Geomicrobiology Research Group applies a diverse suite of microscopy techniques at UWO and relies heavily upon the scanning electron microscopes housed in the Western Nanofabrication Facility. While we could go into scientific detail about the theory and hypotheses driving our research, it seemed more appropriate to present some data pertaining to our ongoing research in the form of images captured with these instruments, along with brief explanations of what is being shown.

The Fabrication and Characterization of Fluoropolymer Micropatterned Glass Substrates for Selective Cell Adhesion

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The spatial control of cell adhesion and growth has made contributions to many different areas, including cell biology, cell-based biosensors, and tissue engineering.[1-4] In numerous biological processes such as proliferation, differentiation, and apoptosis, the cell-cell interactions such as cell signalling are extremely important. As such, the ability to manipulate these interactions with higher precision, organization, complexity, and control than traditional cell culture systems is extremely desirable and is a major topic of interest. In order to control the cellular environment, the positioning must be manipulated at the single-cell level. To accomplish this, cell patterning is commonly used to precisely control the positioning of cells across a surface, by exploiting the interactions between cells, proteins, and surfaces.[4, 5]

Typically, there are many different methods used to fabricate these cell positioning substrates, including microcontact printing[6-8], stencil patterning[9, 10], photolithography[11], photochemically generated patterns[12], and plasma lithography[13]. These techniques provide control over a variety of factors that are known to affect cell adhesion, mainly differences in wettability of the surface, electrostatic interactions, surface roughness, and surface chemistry. In this research[14], conventional glass microscope coverglasses are used as the underlying substrate. Glass is one of the main materials generally used in cell culture, so cells are known to be capable of adhering and surviving on these surfaces. Glass also has the benefit of being optically transparent, which allows for analysis using optical and fluorescence microscopies. As the cell-repelling surface that will promote the cells to locate solely on the glass features, a hydrophobic surface is typically the most effective, due to the low surface energy. To this effect, fluoropolymers are used, for their chemical stability and biological inertness due to the hydrophobic nature.[15-18]

Plasma lithography is one such method that is particularly successful for the fabrication of patterned cell culture substrates. It combines the high resolution patterning of photolithography, with the chemical versatility of plasma polymerization.[19] A photoresist mask with the desired patterned design that will promote cell adhesion is first deposited on the glass coverslips. This resist mask protects the underlying glass areas from any other modification steps. Next, a fluoropolymer film is deposed onto the surface of the patterned substrate, and finally, after a lift-off step by immersion in a photoresist solvent, the photoresist mask and overlying polymer is removed from the surface. The final architecture of the substrate has a fluoropolymer film surface with patterned areas wherein the glass surface is revealed. Depending on the feature shape and dimensions of the patterned glass areas, cells of all kinds have the potential to adhere, proliferate, and form interconnected networks across the surface (Figure 1).

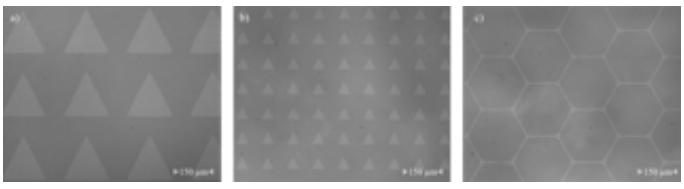

Figure 1. Optical images in bright field of the fluoropolymer patterned glass surfaces, exhibiting numbers patterning designs.

Investigation into the wettability of the final fluoropolymer and glass surfaces was performed by contact angle goniometry, where the glass was found to hydrophilic with a contact angle of 45 ± 2 °, while the polymer is hydrophobic with a measurement of 98 ± 3 °. This difference in wettability can be observed in Figure 2, where the substrate was immersed in water and imaged. The triangular shaped droplets verified the hydrophilic nature of the glass compared to the fluoropolymer, as the water localized in the windows and avoided the polymer.

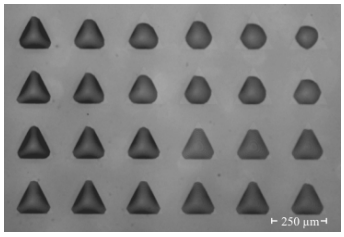

Figure 2. Bright field optical image of water droplets localized in the triangular glass areas.

The chemical composition of the fluoropolymer film was examined with the use of FTIR spectroscopy. The spectra in Figure 3 shows the typical CF_x vibrations in the 1400 – 950 cm^{-1} region. There is a broad peak around 1200 cm^{-1} with contributions from the CF_2 asymmetric and symmetric stretches at 1223 and 1170 cm^{-1}, respectively. There is also a CF stretch at 1340 cm^{-1}, a CF_3 vibration mode at 980 cm^{-1}, and finally a small peak at 740 cm^{-1}, which is the repeating structure of $–CF_2–CF(CF_3)–$ in the film. The dominance of the CF_2 species suggests that it is a fundamental unit of polymer formation.

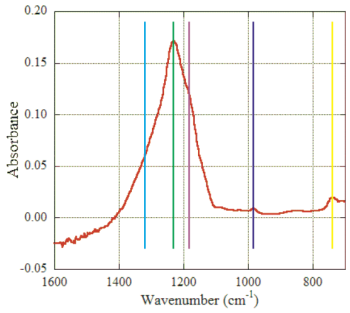

Figure 3. FTIR spectrum of the fluoropolymer film deposited on a silicon wafer.

The topography of the patterned substrate was studied using atomic force microscopy (AFM), across one of the triangular glass windows. This image is shown in Figure 4a. The results suggests two major pieces of information, the first demonstrating the efficiency of the lift-off process. The glass surface is fairly smooth with no residue left behind from the photoresist mask. This is important, as any material remaining on the glass would act as a contaminant for the cells that adhere in this area. Also, by regarding the topographical cross section image across the surface (Figure 4b), the thickness of the polymer film was determined. The thickness measurement shows that the polymer film is approximately 25 nm, which corresponds to the ellipsometry measurements of 28 nm.

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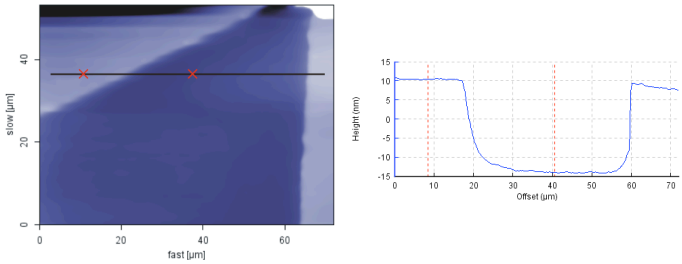

Figure 4. AFM image (a) and cross-section image (b) taken across a triangle window in the fluoropolymer background.

Finally, preferential cell adhesion onto the patterned areas has been examined using mammalian mouse myoblast C2C12 cells. These cells were plated onto the patterned surfaces, and after an appropriate incubation time that allowed the cells to adhere and proliferate, were immobilized and imaged. In Figure 5, the mammalian cells are observed to be located almost exclusively within the glass triangular regions, while any that were partially attached to the fluoropolymer display unhealthy morphologies, as the membrane edges are raised. As an application of these cell patterned substrates, subcellular components have been fluorescently labelled and imaged with confocal microscopy, in order to determine cell structure and adhesion. As observed in Figure 5c and d, the cell nuclei (blue), actin cytoskeleton (red), and focal adhesions (green) can all be visualized. It has been verified that the cell morphology that is observed on typical cell culture substrates is present with cell adhered to the patterned substrate, along with many points of adhesion to the underlying glass.

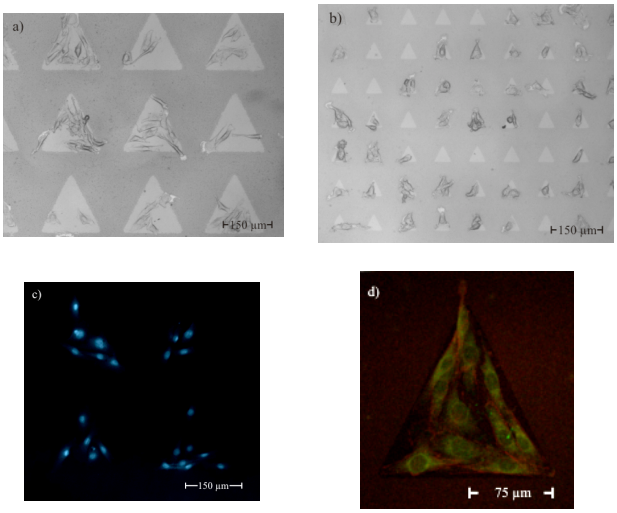

Figure 5. a) and b) Bright field optical images of C2C12 cells adhered to the glass windows with different sized triangular features. C) Fluorescent image of nuclei of the cells, and d) confocal image of the actin (red) and focal adhesion complexes (green) of the cytoskeleton of the cells.

Similarly, if the size of the triangular features is decreased to the dimensions of a single cell, very small numbers of cells can be positioned in each feature (Figure 5b). This allows for the possibility of single-cell positioning, for individual cell analyses.

As a result, the plasma lithography method for fabricating a successful cell positioning substrate was effective, as it allowed for healthy cell adhesion, proliferation, and can be further modified for potential studies into the single-cell patterning of cells and the more complex neurons.

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References:

1. Veiseh, M.; Wickes, B. T.; Castner, D. G.; Zhang, M., Biomaterials 2004, 25, 3315-3324.
2. Iwanaga, S.; Akiyama, Y.; Kikuchi, A.; Yamato, M.; Sakai, K.; Okano, T., Biomaterials 2005, z26, 5395-5404.
3. Schmalenberg, K. E.; Uhrich, K. E., Biomaterials 2005, 26, 1423-1430.
4. Rosenthal, A.; Macdonald, A.; Voldman, J., Biomaterials 2007, 28, 3208-3216.
5. Khademhosseini, A.; Suh, K. Y.; Yang, J. M.; Eng, G.; Yeh, J.; Levenberg, S.; Langer, R., Biomaterials 2004, 25, 3583-3592.
6. Kam, L.; Boxer, S. G., J Biomed Mater Res 2001, 55, 487-495.
7. Jiang, X.; Ferrigno, R.; Mrksich, M.; Whitesides, G. M.; Frederick, M.O.; Mulder, J. A.; Tracey, M. R.; Hsung,R. P.; Huang, J.; Kurtz, K. C. M., J Am Chem Soc 2003, 125, 2366-2826.
8. Chen, C. S.; Ostuni, E.; Whitesides, G. M.; Ingber, D. E., In Extracellular Matrix Protocols, Springer: 2000; Vol. 139, pp 209-219.
9. Folch, A.; Jo, B. H.; Hurtado, O.; Beebe, D. J.; Toner, M., J Biomed Mater Res 2000, 52, 346-353.
10. Jimbo, Y.; Robinson, H. P. C.; Kawana, A., IEEE T Bio-Med Eng 1993, 40, 804-810.
11. Bhatia, S. N.; Balis, U. J.; Yarmush, M. L.; Toner, M., Biotechnol Progr 1998, 14, 378-387.
12. Dillmore, W. S.; Yousaf, M. N.; Mrksich, M., Langmuir 2004, 20, 7223-7231.
13. Goessl, A.; Bowen-Pope, D. F.; Hoffman, A. S., J Biomed Mater Res 2001, 57, 15-24.
14. Varma, S.; McLachlan, J.; Leclair, A. M.; Galarreta, B. C.; Norton, P. R.; Lagugné-Labarthet, F., Analytical and Bioanalytical Chemistry 2010, 396, 1159-1165.
15. Ruardij, T. G.; Goedbloed, M. H.; Rutten, W. L. C., IEEE T Bio-Med Eng 2000, 47, 1593-1599.
16. Chen, M.; Zamora, P. O.; Som, P.; Peña, L. A.; Osaki, S., J Biomat Sci-Polym E 2003, 14, 917-935.
17. Heitz, J.; Svorcik, V.; Bacakova, L.; Rockova, K.; Ratajova, E.; Gumpenberger, T.; Bauerle, D.; Dvorankova, B.; Kahr, H.; Graz, I., J Biomed Mater Res A 2003, 67A, 130-137.
18. Hopp, B.; Kresz, N.; Kokavecz, J.; Smausz, T.; Schieferdecker, H.; Doring, A.; Marti, O.; Bor, Z., Appl Surf Sci 2004, 221, 437–443.
19. Goessl, A.; Garrison, M. D.; Lhoest, J. B.; Hoffman, A. S., J Biomat Sci-Polym E 2001, 12, 721-738.

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