

SurFACTSin

Biomaterials

Summer 2013 | Volume 18 Issue 3

2013 President's Message:

Dear Fellow Biomaterial Surface Aficionados,

You are invited to join me, your colleagues, academic and industry leaders and invited distinguished scientists in beautiful Minneapolis, Minnesota for The Surfaces in Biomaterials Foundation's Annual BioInterface Conference on October 7-9, 2013 at the Hotel Sofitel. This annual meeting provides an intimate opportunity to meet and network with other professionals while discussing new developments in the biomaterials industry.

The Foundation's mission is to explore the creative solutions to technical challenges at the BioInterface by fostering education and multidisciplinary cooperation among industrial, academic, clinical, and regulatory communities.

Thanks to the efforts of the Program Committee we have a truly remarkable program. The program consists of scientific sessions featuring well-known presenters and invited speakers

discussing cutting edge technologies and recent scientific developments. In addition, this event includes the popular and the highly anticipated Point-Counterpoint Debate session, Student Poster Competition, and Excellence in Surface Science Awardee address. For a full list of session topics please visit www.surfaces.org.

Registration is now open and we encourage you and your colleagues to register early to guarantee your spot at this conference but to also save money with the SIBF early registration rate. Register today at www.surfaces.org.

On behalf of the Program Committee, Foundation's members and Board of Directors, I hope you will join us at BioInterface in October for an enjoyable and valuable experience that will increase your scientific education, professional development, and further the research you are conducting.

Sincerely,
Peter Edelman
President, Surfaces in Biomaterials Foundation

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Members are encouraged to submit articles for future editions of SurFACTS. Please e-mail your report (with all appropriate figures and graphics) to Staff Editor Jazzy McCroskey at jasperm@ewald.com for consideration in a future issue. Deadlines for upcoming issues are posted on surfaces.org.

Altering Biological Interfaces with Gas Plasma: Example Applications

By Khoren Sahagian, Mikki Larner, and Stephen L. Kaplan. Plasma Technology Systems, Belmont, CA

Surface interactions play a critical role in biological and polymeric systems. Reactive gas plasma allows customization of surface chemistry without significantly affecting the bulk. In their energized state, the molecular fragments in a gas plasma will effectively restructure the topmost layer in a single controlled operation.

The exact chemical formulation of gas plasma may vary from simple to complex and there is rarely a single route to achieve a desired surface functionality. Ultimately there are three primary variables that govern the resulting surface modification: plasma chemistry, energy per mol, and cycle time. Judicious selection of these process conditions will yield high quality surface treatment of both organic and inorganic substrates.

A low pressure plasma reactor is a complete chemistry toolbox for altering biological interfaces.

Controlled Surfaces with Enhanced Binding

Active microfluidic devices, sensors and implantable devices often require a specific binding property or binding capacity in order to change reactions with an environment or biological fluid. The most common systems utilize specific functional groups such as amine, hydroxyl, or carboxyl for conjugation to protein, molecule, integrin, or adhesive component.

Gas plasma begins by removing organic surface contaminants by reducing them to volatile compounds. The nascent surface is subsequently reacted to process specific plasma chemistry.

The intensity and duration of a plasma process impacts the resulting surface functionality and density. If the density of a functional moiety is either too high or too low this may hinder an intended surface reactivity. Table 1 illustrates the percentage of elemental nitrogen detected on a gold surface as measured by XPS before and after plasma functionalization¹. This amine is covalently bound to the surface meaning it is permanently incorporated onto the surface. This is exemplified by the persisting nitrogen composition post solvent wash. Figure 1 demonstrates different amine densities resulting from varying plasma process intensity and exposure. It is noteworthy that plasma processing is rarely a linear phenomenon. Additional power or time may not translate into denser species loading.



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Sample	C	N	O	Cu	Zn	Au	F
Control	46.5	-	10.8	1.6	-	39.7	-
Amine	72.9	16.5	8.1	0.1	-	2.2	-
Amine washed	73.6	15.7	10.0	-	0.4	0.2	0.1

Table 1 XPS measurement of amine incorporation onto a gold surface. The modification is permanently bound to the surface and persists after a solvent wash

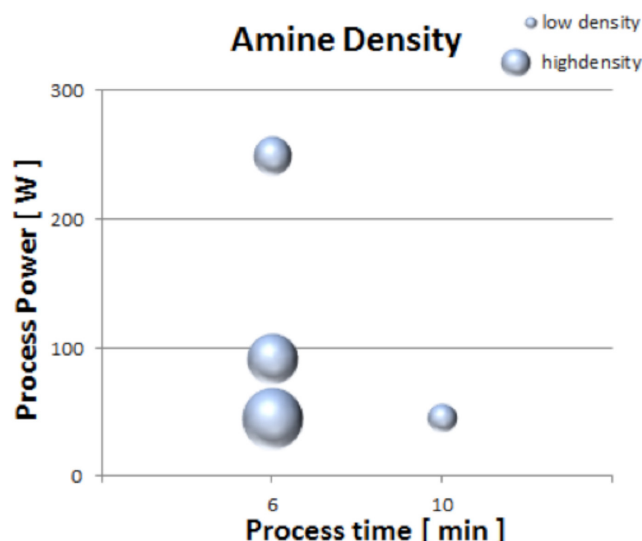


Figure 1 The relative amine density on a surface as related to process power and duration of a plasma surface modification

Figures 2 and 3 illustrate impact of variation in elemental composition to plasma power and precursor concentration for a quartz nano-pipette.

The nano-pipette is a device used for electrical detection of DNA-functionalized nanoparticles^{2,3}. The ability to tailor surface properties of such nano-scale devices are essential to avoid undesired adsorption of biological material onto the walls of a nano channel since it can alter flow properties as well as electrical properties of the channel.

Plasma-enhanced chemical vapor deposition (PECVD) of mercaptopropyltrimethoxysilane was investigated as an alternative method to silanize the internal surfaces of these nano-pipettes. The mercaptosilane in a subsequent step was coupled to bovine SA.

An interesting feature is observed. Sulfur content

appears to reach its maximum relative composition at a power of 50 Watts (W). A minimum in C:S ratio of 3.3 is found at 50 W and increases with power suggesting that higher plasma power lead to extensive fragmentation of the carbon-sulfur bond in mercaptopropyltrimethoxysilane.

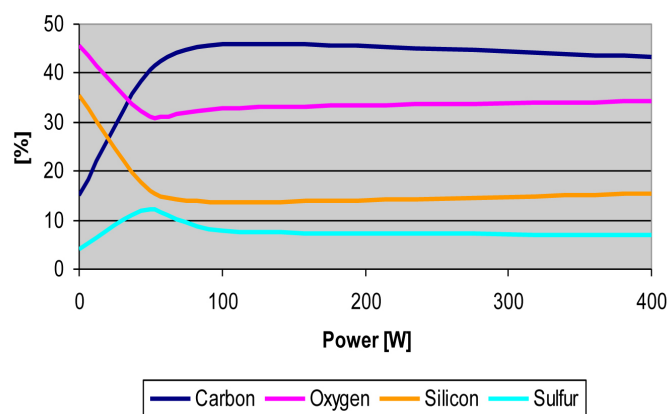


Figure 2. Impact of power on sulfur species incorporation on glass nano-pipetter

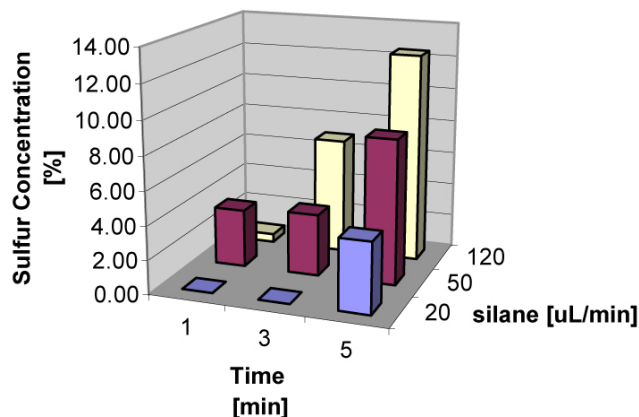


Figure 3 Sulfur content as a percentage of time and concentration

Bioactive Surfaces

Dynamic interactions exist between surfaces and living organisms. Biocompatibility is loosely achieved if a device functions without eliciting an unfavorable response in a living system. Surface energy, ionic interaction, and intermolecular forces all play a role in the adsorption of proteins. A host's immune system may respond to implantable devices by marking the surface with communicative molecules. Immunological response may be suppressed by

altering surface chemistry. S. Kane et al. demonstrates the feasibility of plasma polymerized polyethylene glycol (PEG)-like hydrogels on the surfaces of implantable ultra high molecular weight polyethylene (UHMWPE)⁴. Figure 4 compares the protein adsorption via optical fluorescence on surfaces of varying plasma deposition. A positive correlation exists between plasma coating thickness and protein resistance. The ether (C-O) content in the plasma coated hydrogel was measured between 82.1-83.2%. This nearly matches the 100% ether content of conventional bulk polymerized polyethylene glycol. Additionally some mechanical properties of the ultra-thin film were characterized using atomic force microscopy. Unlike alternative hydrogels, the plasma deposited coating may be covalently coupled to the surface.

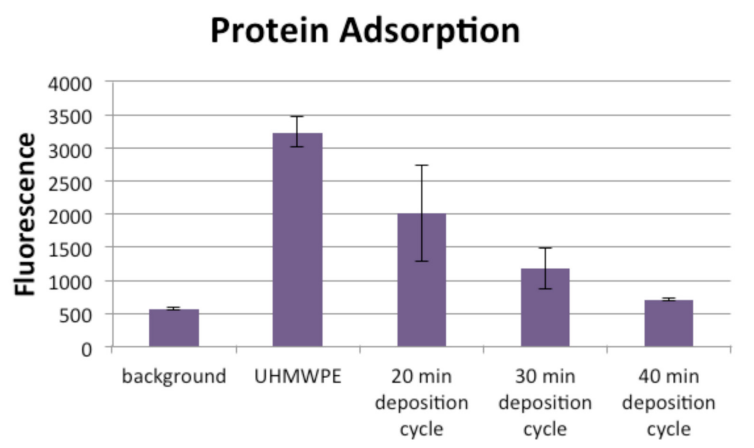


Figure 4 Protein adsorption on the surface of PEG-like plasma polymerized coatings as related to increasing deposition times.

Dry Lubricious Coatings and Anti-blocking

Without a surface treatment many categories of elastomers adhere to themselves or other surfaces when exposed to pressure, temperature, or humidity. Anti-blocking refers to the ability of a surface to not stick. In the medical device arena, anti-blocking agents such as waxes and oils are often unacceptable solutions in the management of adhesion. Such modifiers may be unapproved for device use due to the potential for elution into a working fluid or disruption of organism function. Plasma polymerized coatings form densely crosslinked polymer networks that are covalently bound to a surface. Some of these coating chemistries have also been optimized

for performance as flexible gas and/or liquid barriers⁵. Figure 5 demonstrates plasma processes which reduce static friction as deposited on a fluoroelastomer surface. Plasma treated components were then soaked in oil for 48 hours and the change in coefficient of friction was noted in yellow. Processes 4, 5 and 6 exhibit a threefold decrease in the coefficient of friction and minimal change after the oil exposure.

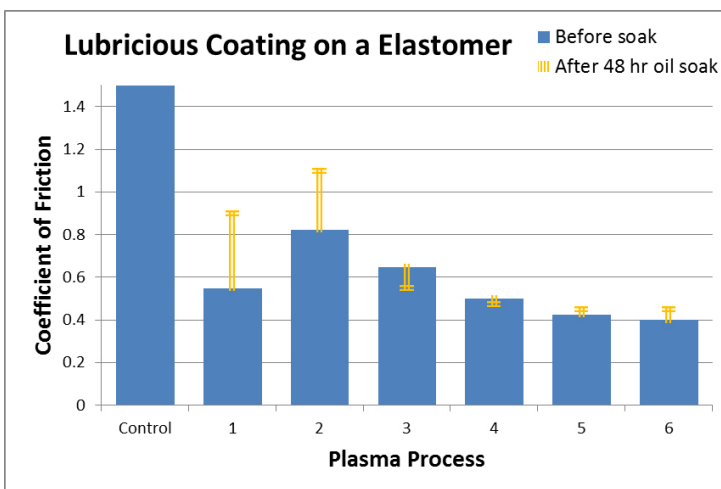


Figure 5 Friction reducing plasma surface treatments before/after oil soak. The coatings are leach free and some exhibit good compatibility in oil as demonstrated by the soak test.

Device Hydrophilization

As markets improve the accessibility of point of care diagnostic devices to their consumers, product evolution drives material selection towards the use of low cost commodity polymers such as polypropylene and polyethylene. Many of these plastics, however, lack the surface polarity and stability that typically makes a surface compatible with an aqueous solution or biological reagents for the required product shelf life.

There is sometimes confusion relating wetting, surface energy, and chemical functionality. One general misconception is that 70 dynes/cm is synonymous with a hydrophilic surface. In general a high surface energy does correspond to low contact angle with water however dyne solutions are not water, they are solvent mixtures. Functional groups of the plasma treated samples may interact with the hydroxyl, ether, or amine groups present in the solvents⁶. This would effectively confound the

ideal relationship between low water contact angle and high surface dyne. A study was conducted on polyethylene (a hydrophobic polymer) by varying exposures of power and pressure using three plasma chemistries known to add oxygen moieties to a surface. Figure 6 presents measurement made 48 hours, following treatment, on the polyethylene surface using a surface dyne solution and contact angle measurement using distilled water. Sample Processes 1 and 2 exhibit expected correlation of low surface energy and high dyne values, however, Processes 3, and 4 demonstrate instances where high surface dyne energy is not accompanied by hydrophilicity. Reliance on the contact angle or dyne solution alone is not an accurate guide for wettability.

Conclusion

Plasma is a versatile tool that is capable of designing controlled interfaces on a variety of materials. This includes streamlining approaches where conventional multi-step wet chemistries are employed. Plasma gives the design engineer the freedom to separate mechanical, optical, and fabrication techniques from the surface requirements. Freedom of choice usually results in significant cost savings. With plasma surface treatment, the choices and capabilities are expansive. Plasma surface treatment is not one process, but an entire chemistry tool box.

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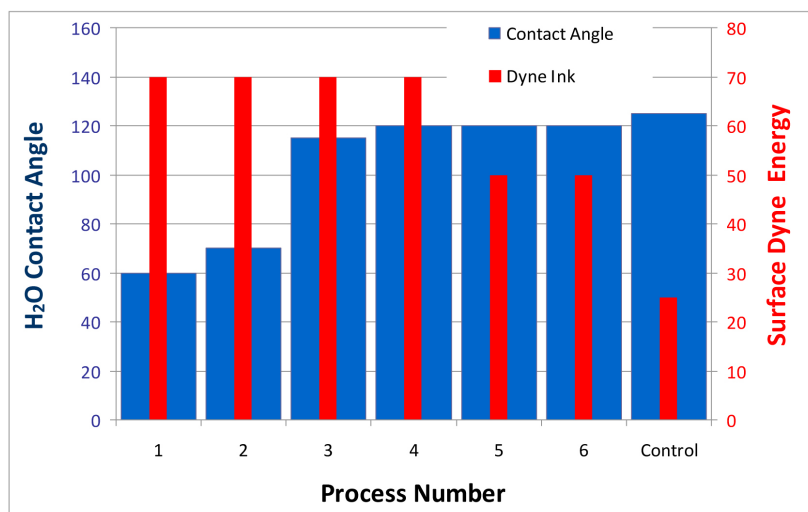


Figure 6 Contact angle measurements, 48 hours after processing, in comparison to dyne-cm measurements, conducted on polyethylene after exposure to various plasma processes



BioInterface 2013

07 Oct - 09 Oct 2013

Hotel Sofitel

5601 West 78th Street

Bloomington, MN 55429 USA

This year's highlights include our workshop entitled "Intelligent Surfaces in Biotechnology"; our interesting and lively Point-Counterpoint session; the presentation of our prestigious Excellence in Surface Science Award; our Student Poster competition; and, of course two full days of solid technical sessions.

Keynote Lecture:

Marcus Textor, ETH Zurich, Switzerland

"Bioinspired Surface Functionalization: Concepts and Applications"

Register online at: <http://surfaces.org/cde.cfm?event=403219!>

Ex-situ Lift-out and EXpressLO™ for Site Specific Surface Analysis

By Lucille A. Giannuzzi

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Introduction

Focused ion beam (FIB) lift-out techniques were first developed for site-specific transmission electron microscopy (TEM) [1]. Since then, FIB and lift-out methods have been used to prepare samples for site-specific surface analysis such as Auger electron spectroscopy (AES), secondary electron mass spectrometry (SIMS) [2,3], and X-ray photoelectron spectroscopy (XPS) [4]. These site specific samples are typically about 5-50 μm in length by 5-10 μm wide and ~ 20 nm to several μm in depth. The FIB is used to ion mill and remove all but the region of interest from within a bulk material leaving the region of interest within the FIB milled trenches. Lift-out methods are used to excise the sample left behind by the FIB milling process. FIB lift-out methods have been successfully used to analyze several classes of inorganic and organic materials, biomaterials, and composites [5].

As the name implies, ex-situ lift-out (EXLO) is performed outside of the FIB instrument where a light optical microscope and glass rod manipulator system is used to pluck the specimen from within the trench and transfer it to a carbon coated TEM grid for TEM or surface analysis [1,2,4], or directly onto clean silicon or another suitable substrate for surface or other analysis only [3]. Electrostatic forces are presumed to aid the lift-out process and Van der Waals forces secure the specimen to the carbon film or smooth substrate. Early references simply refer to EXLO as "lift-out." The lift-out and manipulation process via EXLO is extremely fast and reproducible, generally taking < 5 minutes per sample at a > 90 % success rate. In addition, EXLO does not require expensive FIB time for the lift-out process and supports multiple FIB instruments since lift-out is performed outside of the FIB.

Over the years, many in the TEM specimen preparation community abandoned EXLO because the carbon film inhibits certain TEM techniques and

makes it difficult to further thin or plasma clean the specimen. Recently, a new method called EXpressLO™ has been introduced which combines the ease and throughput of EXLO with a new grid design that does not require a carbon film [6-8]. This new grid design exploits the notion that the specimen will adhere to a smooth and clean substrate via Van der Waals forces. EXLO and EXpressLO are detailed below.

EXpressLO™ Techniques

Figure 1 shows (a) a low magnification image and (b) higher magnification image of the newly designed copper patent pending EXpressLO™ grid. The half grid allows for further FIB or ion milling of the specimen from the open end of the half grid. The slots in the grids provide support for the specimen for surface analysis and/or TEM analysis. Different grid materials are currently being explored.

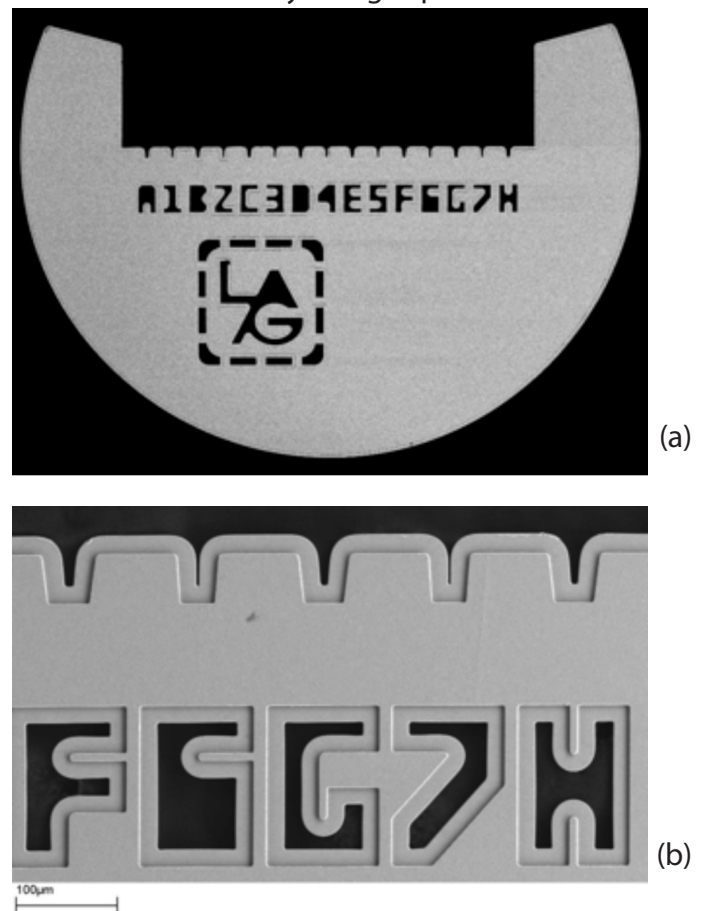


Figure 1. (a) A low magnification image of the EXpressLO™ grid. (b) Detailed view of the slotted specimen support region of the EXpressLO™ grid.

Figure 2 shows a series of still images from a movie of the EXpressLO™ process as viewed with the light optical microscope [9]. The lift-out steps are shown in figures 2a-2c. Figure 2a shows the lift-out specimen centered within the FIB trenches. Figure 2b shows the glass manipulator lifting out the specimen. Figure 2c shows the specimen attached to the glass needle manipulated up and away from the original sample surface. Manipulation of the specimen to the EXpressLO™ grid is shown in figures 2d,2e. The specimen attached to the glass needle approaches the grid in figure 2d. The specimen is manipulated and positioned across the open slot of the EXpressLO™ grid in figure 2e. Once the specimen is placed on the grid it may then be taken to any surface characterization tool for analysis as before [2-4].

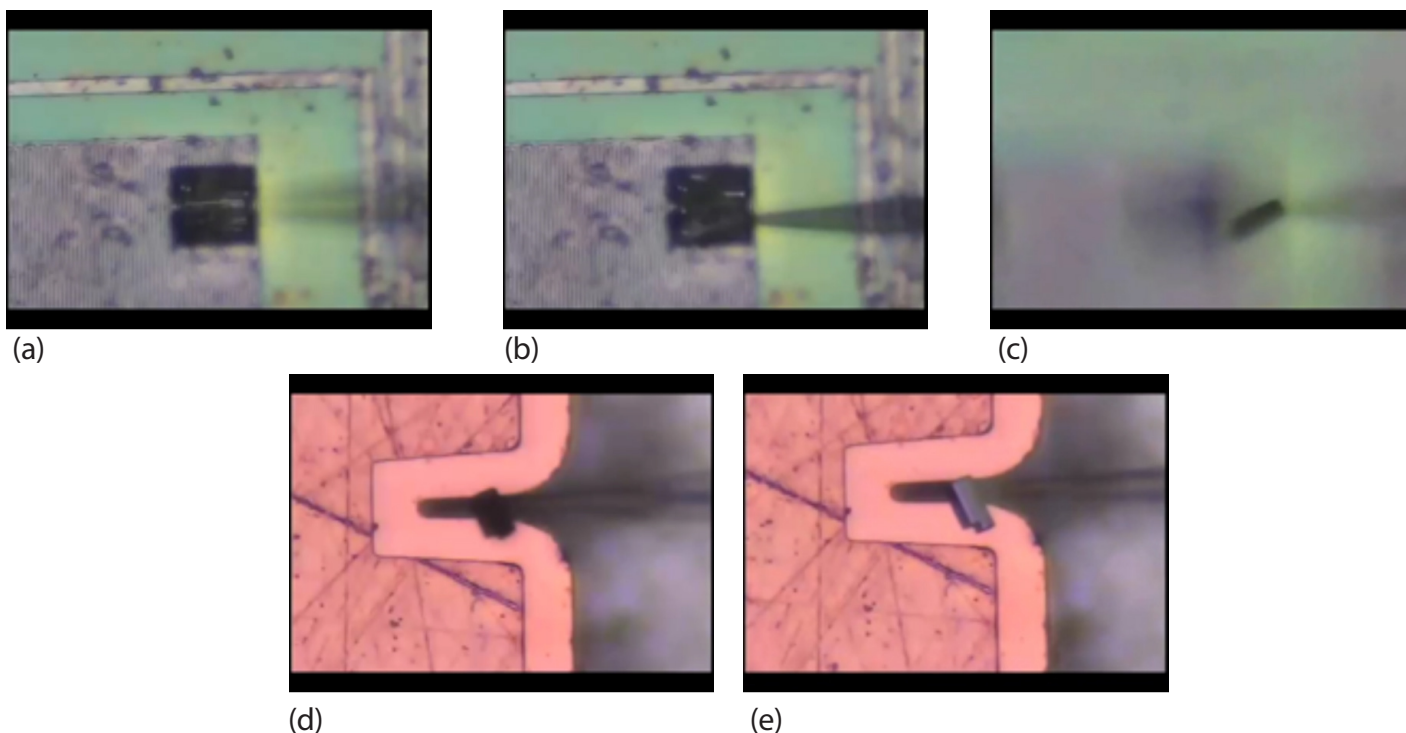


Figure 2. Time series of the EXLO and EXpressLO™ process. (a) the FIB milled free lift-out specimen. (b) EXLO of the specimen via a glass needle manipulator. (c) the lift-out specimen on the glass needle. (d) manipulating the specimen to the EXpressLO™ grid. (e) the specimen positioned on the EXpressLO™ slotted grid.

Regulatory Update

By Phil Triolo PhD RAC

The proposed changes to regulations and regulatory requirements that would have the most significant effect on US manufacturers are under review in the EU. On September 26, 2012 the EU Commission proposed that the current three directives on active implantable medical devices (AIMD), medical devices (MDD) and in vitro diagnostic medical devices (IVDD) be replaced by two regulations, one covering all medical devices except in vitro diagnostic devices, the other addressing only IVDDs. The changes would bring the CE Marking process much closer to the US system for regulatory clearance and approval, and is being actively discussed by the European Parliament's Environment, Public Health and Food Safety Committee. A vote on the amendments, originally scheduled for July 10, has been delayed until November. The proposals are controversial and negotiations are underway which will determine the extent of the changes, and consequently, the relative desirability, from a regulatory perspective, of launching products in the EU as opposed to the US. Stay tuned...

The FDA's latest draft biocompatibility guidance, Use of International Standard ISO- 10993, Biological Evaluation of Medical Devices Part 1: Evaluation and Testing has been released www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM348890.pdf. For all of us working with materials and biological safety evaluations, the guidance is a must-read. It includes new recommendations for the evaluation of products that are, or include nanoparticles and of products that incorporate polymers that polymerize in situ. The draft guidance is disappointing in that, despite a commitment to end

animal testing, it still references USP <151> Rabbit Pyrogen Test to evaluate the highly unlikely potential for material-mediated pyrogenicity, and use of the in vivo thrombogenicity (canine) study to evaluate the thrombogenicity of blood-contacting devices. As always, FDA prudently recommends that sponsors discuss their biological safety evaluation plans with ODE, especially before initiating long-term testing.

Of interest to those designing and/ or manufacturing combination products are FDA's draft guidance on the evaluation of supplied heparin and a final rule about to go into effect on Current Good Manufacturing Practice for Combination Products. The final rule on cGMPs (FDA-2009-N-0435), effective July 22, clarifies which cGMP requirements apply when drugs, devices, and biological products are combined to create combination products. The accompanying guidance document <http://www.fda.gov/RegulatoryInformation/Guidances/ucm126198.htm> and an informative webinar <https://collaboration.fda.gov/p83277278/?launcher=false&fcsContent=true&pbMode=normal> are available.

Heparin for Drug and Medical Device Use: Monitoring Crude Heparin for Quality <http://www.fda.gov/downloads/Drugs/G.../UCM291390.pdf> provides suggestions for the characterization and acceptance of supplied heparin and the routine auditing of heparin suppliers.

Should you be interested in having some specific regulatory topic addressed in upcoming editions of SurFACTS, or if you would like to write an article about a recent regulatory experience or "challenge," please email me at philt@philt.com.



Surface TechDays One-Day Symposiums in October!

Detroit | Chicago | Minneapolis/St. Paul

Focus of this one-day symposium, held in three separate cities of the industrial Mid-West:

Better adhesion for successful bonding and sealing. Get a leg up on the competition by learning how new technologies can enable you to produce at lower cost with added safety and environmental benefits.

Oct 1 - Detroit, MI
Oct 2 - Chicago, IL
Oct 4 - Minneapolis/St. Paul, MN

Representatives from technology leaders from the United States and Europe will be present to give demonstrations and answer your questions.

WHO SHOULD ATTEND:
Manufacturers, Integrators, Process Engineers,
Manufacturing Engineers, Design Engineers,
Production Managers, Quality Control

Surface Science Calendar of Events

ESB 2013 - 25th European Conference on Biomaterials

08 Sep 2013
Madrid, Spain

4th International Symposium on Surfaces and Interfaces for Biomaterials

24 Sep 2013
Rome, Italy

Surface TechDays One-Day Symposiums, October 1 (Detroit)

01 Oct 2013
VisTaTech Center, Schoolcraft College
18600 Haggerty Road
Livonia, MI 48152 USA

Surface TechDays One-Day Symposiums, October 2 (Chicago)

02 Oct 2013
Surface Tech Center Plasmatrete,
2541 Technology Drive, Suite 407
Elgin, IL 60124 USA

Surface TechDays One-Day Symposiums, October 4th (MPLS/STP)

04 Oct 2013 MacMillan Auditorium- U of M
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07 Oct 2013 - 09 Oct 2013
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Polymers For Medicine and Biology 2013

09 Oct 2013 - 12 Oct 2013
Santa Rosa, CA USA

TCT 25

27 Oct 2013 - 01 Nov 2013
San Francisco, CA USA

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10 Feb 2014 - 13 Feb 2014
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Wanted: Members

To be leaders in the surface science community

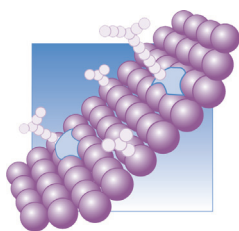
- Join a forum that fosters discussion and sharing of surface and interfacial information
- Have your voice heard and your interests represented within the surface science and biomedical community
- Help shape workshops and symposia that further the world-wide education of surface science
- Promote understanding of interfacial issues common to researchers, bio-medical engineers and material scientists.

Benefits of Membership:

- Discounted registration at BioInterface, the annual symposium of the Surfaces in Biomaterials Foundation.
- Your logo and a link to your website in the member directory on the official website of the Foundation, www.surfaces.org.
- Complimentary full page ad in SurFACTS, the Foundation's newsletter and discounts on all advertising.

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