SurFACTS in Biomaterials

SUMMER 2018
VOLUME 23, ISSUE 3

INSIDE THIS ISSUE

Letter from President Chris Jenney

On behalf of the Surfaces in Biomaterials Foundation, we invite all of you to join us at BioInterfaces 2018 in Boulder, Colorado, Oct. 1 through 3. Thanks to an outstanding group of presenters, we have a comprehensive and innovative technical program planned for attendees. As is our tradition, this conference benefits from strong industry participation which nicely complements innovative academic inputs.

Our program begins Monday, Oct. 1, with the "3D Printing in Medical Devices" Workshop, followed immediately by our Keynote Lecture, "Tools for Accelerated Medical Innovation," presented by Brigham and Women's Hospital biomedical researcher Jeff Karp. Afterward, attendees can wind down with a conference-sponsored Monday night social mixer in the heart of downtown Boulder. The schedule on Tuesday and Wednesday, Oct. 2 and 3, includes seven technical sessions, with breaks to hear from our Excellence in Surface Science Award winner, listen to a "Point-Counterpoint" discussion on the future of 3D printing, meet with colleagues, survey the exhibitor hall, and participate in the annual business meeting. This year we are partnering with the BioFrontiers Institute at the University of Colorado, Boulder, to host our poster competition and banquet. To encourage student participation, the first 20 students to submit posters will receive complimentary admission to the technical sessions AND the best student poster award winner will receive $1,000!

Of course, none of this would be possible without the work of many dedicated volunteers and the strong support of our sponsors, including Abbott, DSM, Medtronic, and Gore, our many exhibitor organizations, and our supporting members. For the latest details, please visit our website at www.surfaces.org. We look forward to seeing you in Boulder!

Members are encouraged to submit articles for future editions of SurFACTS. Please email your report (with all appropriate figures and graphics) to Newsletter Committee Chair Melissa Reynolds at melissa.reynolds@colostate.edu *for consideration in a future issue. Deadlines for upcoming issues are posted on* surfaces.org.

October 1-3, 2018, Boulder, Colorado

Workshops/Sessions

MONDAY October 1

BIOINTERFACE WORKSHOP

Theme:
3D Printing in Medical Devices
Co-Chairs: Angela DiCiccio
Verily Life Sciences
Dan Gostovic
Thermo Fischer Scientific

BIOINTERFACE KEYNOTE LECTURE

Theme:
Tools for Accelerated Medical Innovation
Jeff Karp
Brigham and Women's Health Hospital Harvard Medical School

TUESDAY October 2

BIOINTERFACE SYMPOSIUM

Session 1 Topic:
Surface Funtionalization and Thin Film Coatings
Co-Chairs: Daniel Higgs
ALD Nano Solutions
Lijun Zou
W.L. Gore & Associates

Session 2 Topic:
Neurological Devices
Chair: Tim Becker
Northern Arizona University

Session 3 Topic:
Adhesion of Soft Tissues
Chair: Terry Steele
Nanyang Technological University

Session 4:
Point Counterpoint Debate

WEDNESDAY October 3

BIOINTERFACE SYMPOSIUM

Session 5 Topic:
Metallurgy
Co-Chairs: Mallika Kamarajugadda,
Medtronic, plc
Siobhan Carroll, *G. Rau Inc.*

Session 6 Topic:
Metallic Devices
Co-Chairs: Mallika Kamarajugadda,
Medtronic, plc
Siobhan Carroll, *G. Rau Inc.*

Session 7 Topic:
Bioresorbable Materials
Chair: Norman Munroe
Florida International University

Session 8 Topic:
Tissue Engineering and Regenerative Therapies
Co-Chair: Rob Diller
Axolotl Biologix
Roy Biran
W.L. Gore & Associates

For more information and to register go to http://www.surfaces.org/page/2018BioInterface.

Precision Biomaterials for In Vitro Modeling of Chronic Pulmonary Diseases

Researchers at the University of Colorado, Anschutz Medical Campus (Aurora, Colorado) have developed a new class of photo-addressable poly(ethylene glycol) (PEG)-based hydrogel biomaterials that afford investigators on-demand spatiotemporal control over local mechanical properties, while limiting hydrolytic degradation. These materials have been designed to retain their structure, composition and tunable mechanical properties over long periods of time under cell culture conditions with the goal of engineering models of chronic progressive pulmonary diseases in vitro. Idiopathic pulmonary fibrosis (IPF) is an example of a particularly devastating chronic pulmonary disease with surprisingly few therapeutic treatment options. As IPF progresses tissue deep within the lungs become thick, stiff and scarred resulting in decreased respiratory function that worsens over time until respiratory failure occurs, typically within 3 to 5 years. One way to learn more about the progression and treatment of a disease like IPF is to study how cells within the lung respond to signals outside the body. This research can be done more rapidly and inexpensively in the laboratory than in animal or human studies but has traditionally been accomplished in a Petri dish. These dishes are flat and significantly stiffer than the tissue in our lungs and recent discoveries have revealed that these cells cultured upon these dishes can behave considerably different than what is expected within the body. For this reason, we propose to create three-dimensional (3D) cell culture platforms with mechanical and chemical properties that are inspired by real lung tissue.

To achieve this goal, we aim to design three sequential, orthogonal chemical reactions that will result in the ability to control hydrogel micromechanical properties in a way that emulates the in vivo progression and resolution of fibrosis in vitro. Human lung tissue ranges in stiffness from 1-5 kPa (healthy) to >20 kPa (fibrotic). Each sequential reaction will drive a change in hydrogel modulus as follows: Stage 1, non-stoichiometric thiol-ene, Michael addition of a multi-arm PEG- α-methacrylate (hydrolytically resistant) with a photodegradable dithiol crosslinker, should create an initially low modulus hydrogel in the range of healthy pulmonary tissue (1-5 kPa); Stage 2, α-methacrylate homopolymerization, should produce an approximately 4-fold increase in modulus to levels measured in fibrotic lung tissue (>20 kPa); and Stage 3, photodegradation should reduce the modulus back down to healthy levels (1-5 kPa). Indeed, preliminary results show that 8-arm, 10 kg/mol PEG-α-MA (10 wt%) reacted at an 8:5 ratio with a short, nondegradable crosslinker (DTT) can be stiffened in situ to successfully achieve Stage 1 and Stage 2. We envision that when combined with advanced biomanufacturing techniques these materials may not only be used to mimic the complex geometry of lung tissue, but also to mimic disease progression in vitro (Figure 1).

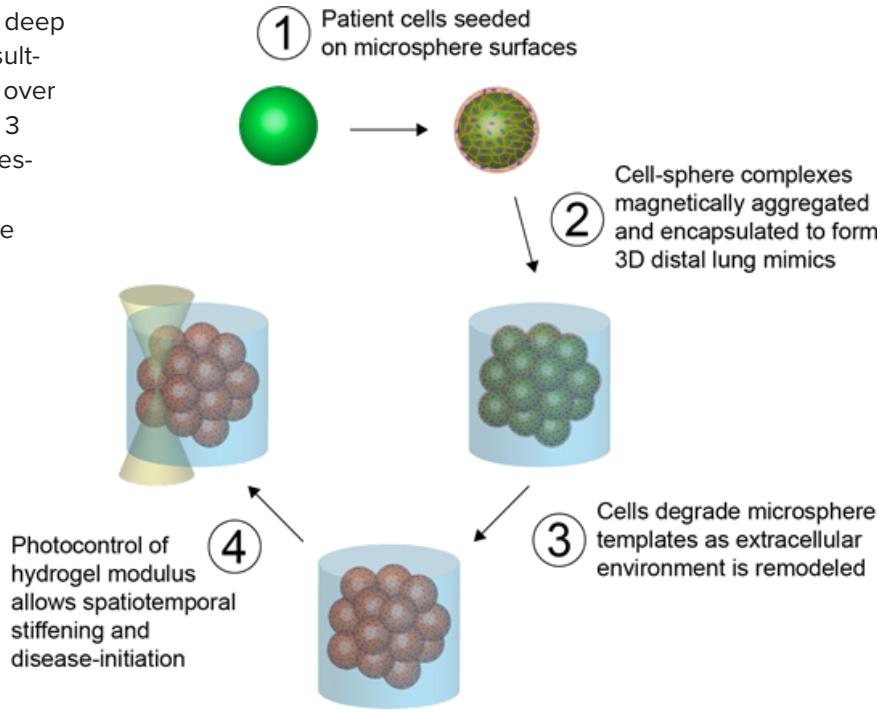

Figure 1. Engineering strategy for the creation of 3D in vitro models of pulmonary fibrosis.

We are currently working toward creating engineered 3D in vitro models of fibrotic disease that can be modified with exposure to light to replicate key aspects of the disease not otherwise reproducible in existing bench top cultures or animal models. These improved models of healthy or diseased lung tissue can be used to study lung cell responses in a more natural environment that will not interfere with the experimental conditions and allow researchers to address complex questions associated

Precision Biomaterials for In Vitro Modeling of Chronic Pulmonary Diseases ... continues on pg. 6

Preventing Thrombus in Small Diameter Vascular Grafts

By Sue James, Hieu Bui and David Prawel, Colorado State University

Researchers at Colorado State University are trying to solve an old problem. How to prevent blood from clotting inside small diameter vascular grafts made from ePTFE. Cardiovascular disease has been the leading cause of death for over a century in the United States. Bypass surgery remains the preferred treatment in many cases and the number of patients undergoing such surgeries is expected to increase with a growing aged population. However, there are sourcing and sizing problems with the gold-standard auto- and allografts used in these surgeries, while early thrombotic occlusion of the alternative small diameter (<4mm) synthetic vascular grafts reduces their 5-year patency rate to lower than 30%. Therefore, there is an urgent clinical need to develop thromboresistant small diameter vascular grafts. As tissue engineering/regenerative medicine approaches remain far from clinical practice, a more immediate solution would be thromboresistant synthetic vascular grafts with long-term patency. Thrombosis is a complication faced by any blood-contacting synthetic material because the only fully hemocompatible material is a healthy endothelium. Thus, to mitigate risks, current vascular graft patients are treated with systemic anticoagulant and/or antiplatelet drugs. Unfortunately, the efficacy of these therapies is limited in regions of high shear stress typical of coronary arteries, increasing the risk for bleeding due to their systemic nature. An endothelial layer that mimics the lumen of native vessels should reduce thrombotic complications. While there has been some success with preseeding endothelial cells on VGs in vitro before implantation, the expense, complexity and safety concerns such as contamination, degradation and infection prevent this technology from achieving widespread use.

Synthetic materials such as expanded polytetrafluoroethylene (ePTFE) are relatively successful as large diameter vascular grafts. ePTFE is preferred over PET by vascular surgeons due its superior mechanical strength and thromboresistance. However, despite its biostability, ePTFE's hydrophobic surface is known to promote protein adsorption and platelet activation, which cause failure, usually due to early stage thrombotic occlusion, especially in small diameter vascular grafts.

We have modified the inner lumen of ePTFE grafts with high molecular weight hyaluronan (HA), a non-branching, anionic, and nonsulfated polysaccharide that is found in the inner lumen of blood vessels. HA has demonstrated reduced activation and adhesion of platelets and proteins, reduced calcification and anticoagulation properties, motivating its use in cardiovascular biomaterials. We chose HA over heparin, a sulfated polysaccharide used on current ePTFE grafts to decrease thrombosis, because it has been shown that HA modification promotes hemocompatibility and endothelialization better than heparin modification. This is likely because heparin only prevents coagulation, not platelet adhesion and activation. Furthermore, while heparin has been shown to reduce risk factors for stroke, heparin coatings have not improved small diameter vascular graft clinical performance.

Briefly, our approach to enhancing the ePTFE with HA, is as follows. Sodium hyaluronate (750 kDa) and cetyltrimethylammonium bromide (CTAB) were dissolved in distilled (DI) water to form a HA-cetyltrimethylammonium (HA-CTA) precipitate, which was dried and dissolved in ethanol creating a 0.4% (w/v) solution. The inner lumen of commercial ePTFE VGs (Bard IMPRA® 10S10) were sprayed with the HA-CTA/ethanol solution using an airbrush. The HA-CTA-treated graft was dried and the HA-CTA then crosslinked in a 2 % (v/v) poly(hexamethylene diisocyanate) (HDI)/acetone solution. Following this, all un-crosslinked HDI, the CTA complex and any uncrosslinked HA were carefully removed.

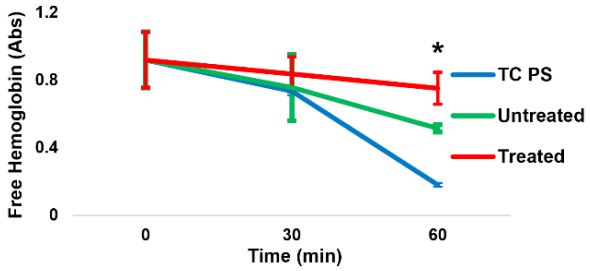

Figure 1: Whole blood clotting showing HMW-HA treatment significantly reduces blood clotting on the surface of ePTFE after 60 minutes* (n=3, α=0.05).

We used static whole blood clotting and measured platelet adhesion and activation to initially test the thrombotic potential of the hyaluronan treated ePTFE grafts. Absorbance is inversely correlated

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Magnetically Powered Microbots for Rapid Ablation of Blood Clot

Dante Disharoon, David W.M. Marr, and Keith B. Neeves; Colorado School of Mines

Researchers at the Colorado School of Mines (Golden, CO) have developed microbots that are powered by magnetic fields to target and lyse blood clots. This technology brings the Fantasic Voyage out of the realm of science fiction and into the clinic to target blood clots in vessels that cannot be reached by catheters. One example of this type of clot is called a lacunar stroke, which refers to occlusion of a small penetrating arteries of the brain. These and other types of strokes can be treated by a class of drugs called plasminogen activators, with recombinant tissue plasminogen activator (tPA) being the most common, and only FDA approved drug. However, because these clots are often far upstream of major arteries, the delivery of tPA relies on diffusion, which is slow and usually ineffective in lacunar strokes. By coupling tPA to magnetically powered microbots we show a faster and more effective rate of delivery and clot ablation.

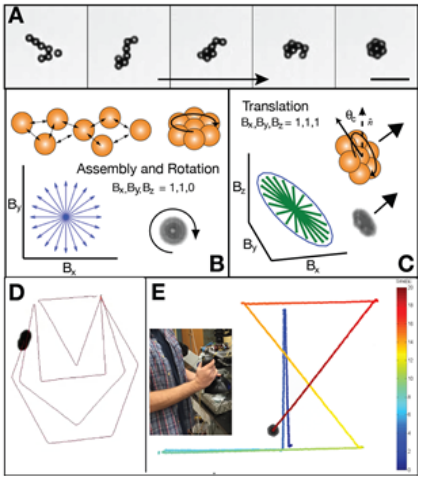

Fig. 1. A-B. With application of a rotating magnetic field in the surface plane, magnetic beads assemble and rotate. Scale bar = 20 µm. C. When the field rotation axis is oriented off the surface, wheels "stand up" and roll along the surface. D,E. Programmed trajectories or manual joystick control are achieved with a simple shift in the magnetic field.

Assembly and Translation of Microbots

At the microscopic scale, viscous forces dominate motion, which at the human scale would be like swimming through molasses. Microorganisms overcome these viscous forces with flagella or cilia that shed sticky fluid off of their appendage to create propulsion. Researchers have used microorganisms as their inspiration to create microswimmers with rotating or undulating features. Here, we take a different approach, inspired by one of man's inventions, the wheel, to create rolling microbots.

The bots are made up of superparamagnetic beads that are ~1 µm in diameter. Superparamagnetic materials only have a dipole in the presence of a magnetic field, thus their magnetic properties can be instantaneously turned on and off. These beads consist of polystyrene with embedded iron oxide domains that gives them their superparamagnetic properties.

When the beads are subject to a static magnetic field they form linear chains. Upon rotation of the field, the chains reconfigure into compact wheel-like structures in order to reduce drag forces (Figure 1A). We call these structures microwheels. When lying on a surface, these microwheels can do mechanical work, like pumping fluids in microfluidic devices, 1,2 but they do not move (Figure 1B). To get them to translate from one point to another, the magnetic field is oriented at an angle with respect to the surface, which in turn inclines the microwheels off the surface (Figure 1C). Now, the rotating assembly experiences friction with the surface and rolls like a wheel. These wheels can roll at speeds of greater than 100 µm/sec, which is comparable to the fastest microorganisms.3 To steer the microwheels we change the angle of the magnetic field with respect to the surface, which is akin to turning a bike by leaning your weight to one side. The wheels can be programmed to follow a specific path or controlled by a user with a joystick (Figure 1D-E).

Lysis of Blood Clots

To turn these microwheels into clot busting machines we immobilize tPA on to the surfaces of the individual beads. The steps in the drug delivery strategy is depicted in Figure 2. First, individual tPA-coated beads are injected into the body intraarterially or intravenously. Next, a magnetic field is used to pull individual beads and aggregates of beads from a flowing artery into the occluded vessel. These microwheels roll through the occluded vessel to the interface of the clot. Then, microwheels mechanically burrow into the clot, dissolving it biochemically via tPA from the inside out. This combined biochemical and biomechanical lysis leads to much faster ablation compared to lysis by tPA alone.

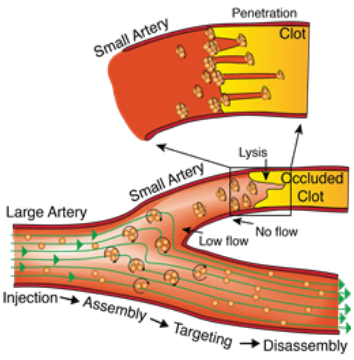

Fig. 2. Approach to tPA-microwheel induced reperfusion of occluded small arteries.

Magnetically Powered Microbots for Rapid Ablation of Blood Clot ... continues on pg. 6

Magnetically Powered Microbots for Rapid Ablation of Blood Clot ...

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A demonstration of the efficacy of microwheel clot ablation is shown in Fig. 3. 4 Here, we track the dissolution of the clot over time for tPA alone and tPA-coupled microwheels. When we drive microwheels directly into a clot they dissolve the clot at a rate approximately five-fold higher then tPA at therapeutic concentrations. Lysis can be further enhanced using a corkscrew motion to bore into the clot.

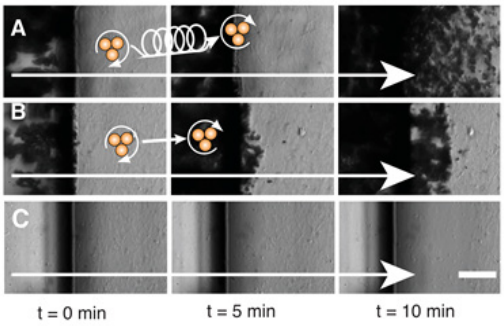

Fig. 3. Dissolution of a blood clot using tPA-coated microwheels moving in a corkscrew (A) or direct motion (B). compared to soluble tPA alone (C), scale bar = 40 µm. Microwheel move from left to right and are visualized as a swarm of black particles to the left of the clot.

There are several advantages to this drug delivery strategy. The systemic tPA concentration when coupled to the beads can be lower than the therapeutic concentration, which is not possible with tPA alone. This is important because the primary risk of tPA is bleeding, and most dangerously, hemorrhaging into the brain. While the systemic concentration is low, the local tPA concentration can be quite high because microwheels accumulate at the clot interface. In contrast, delivery of tPA alone relies on diffusion, which means that the local concentration can never exceed the systemic concentration. Following clot dissolution, the microwheels instantaneously disassemble into individual beads once the magnetic field is removed. The beads are small enough that they can pass through the smallest of blood vessels, but not so small that they can cross the vascular wall. Over time, these beads can then be removed by the body's natural foreign body response.

In summary, magnetically powered microbots are a novel drug delivery strategy for treating blood clots in vessels that cannot be reached by catheters or where systemic delivery is ineffective. Compared to other field-based drug delivery strategies like ultrasound, magnetic fields do not attenuate in tissue, and thus are particularly well-suited for deep penetrating arteries of the brain. Moreover, because microwheels move by rolling rather than swimming, they are ideal for environments with a high surface area to volume ratio, like small arteries. Technical challenges that need to be addressed for this technology to be translated into humans include targeting in complex, three-dimensional geometries and real-time imaging. For the former, a combination of rotating and permanent magnetic fields can be applied so that microwheels roll on surfaces that are not perpendicular to gravity. For the latter, because iron oxide is embedded in the beads, they have significant contrast in both x-ray and magnetic resonance imaging. Next steps in the development of this technology are to test microwheels in animal models of stroke and in 3D printed human brain vasculature models.

1. Terray A, Oakey J, Marr DWM. Microfluidic control using colloidal devices. Science. 2002;296(5574):1841-1844. doi:10.1126/science.1072133.

2. Sawetzki T, Rahmouni S, Bechinger C, Marr DWM. In situ assembly of linked geometrically coupled microdevices. Proceedings of the National Academy of Sciences. 2008;105(51):20141-20145. doi:10.1073/pnas.0808808105.

3. Tasci TO, S HP, Neeves KB, Marr DWM. Surface-enabled propulsion and control of colloidal microwheels. Nat Commun. 2016;7:10225. doi:10.1038/ncomms10225.

4. Tasci TO, Disharoon D, Schoeman RM, Rana K, Herson PS, Marr DWM, Neeves KB. Enhanced Fibrinolysis with Magnetically Powered Colloidal Microwheels. Small. 2017;13(36):1700954. doi:10.1002/smll.201700954.

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with chronic pulmonary diseases. We can use these models to test how lung cells respond to stiffening during disease progression and to new therapies in a high-throughput manner, eventually leading to a better understanding of how to cure fibrotic diseases as well as rapid development of therapeutics. If successful, cells from individual patients could be grown in these new platforms to create precision therapies for personalized medicine.

Preventing Thrombus in Small Diameter Vascular Grafts ...

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to blood clotting — more free hemoglobin (higher absorbance) means less clotting. There was significantly less clotting on HA treated ePTFE versus untreated ePTFE and tissue culture polystyrene (TCPS) after 60 minutes (Figure 1). Platelet activation state was quantified using the established method of dividing their morphology into six stages. Platelet activation results (Figure 2) indicate that HA treated ePTFE significantly reduces platelet activation on the surface, an important process in thrombus and fibrin formation. Most of the platelets found on HA treated ePTFE were in the early stage of dendritic extension and spreading while on untreated ePTFE most of the platelets aggregated. Our HA treatment produces consistent, conformal surfaces on commercial ePTFE graft material that are significantly less thrombotic than plain ePTFE in static conditions. Our future work will focus on dynamic testing of thrombosis and exploring the use of different molecular weight hyaluronans and ePTFE microstructures to tailor the in vivo response to prevent thrombus, control endothelialization and avoid intimal hyperplasia.

Recently published paper on this work:

Hieu T. Bui, David A. Prawel, Emily Li, Aidan Friedrich, Susan P. James, Hyaluronan Enhancement of Expanded Polytetrafluoroethylene Cardiovascular Grafts, Journal of Biomaterials Applications 2018, DOI: 10.1177/0885328218776807, Vol. 33(1), 52–63.

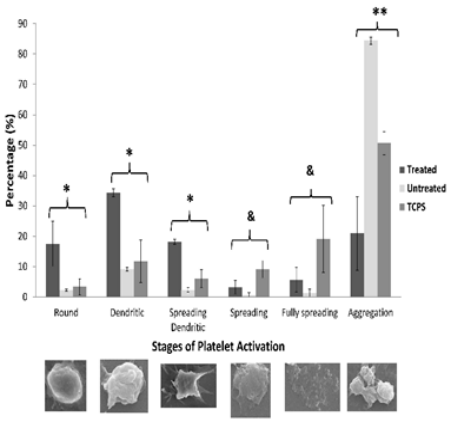

Figure 2: Quantitative analysis of the platelet activation study showing that HMW-HA treatment promotes significantly less later-stage activation (*) are different from other groups, TCPS (&) are significantly different from the other groups, and all groups are significantly different from each other (**). (n=3, p < 0.05)

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