SurFACTS in Biomaterials

WINTER 2022
VOLUME 27, ISSUE 1

INSIDE THIS ISSUE

From President Rob Diller

Hello friends of the Surfaces in Biomaterials Foundation. Welcome back from the busy holiday break. The new year is traditionally a time to reflect on the past and look forward to the future. Looking back, I wanted to take a moment to thank Angela DiCiccio, past president of the foundation, for her leadership throughout 2020 and 2021. Angie led the Board, many generous volunteers, and foundation members through unprecedented challenges. Her efforts and a busy couple of years culminated in two successful online BioInterface conferences. These events offered a wide range of amazing speakers as well as the launch of our **first conference mobile app through Socio.**

Looking forward into 2022, as the new Foundation president, I am joined by a talented group of Board members who are very excited about the opportunities in the new year. Planning is already underway for the BioInterface 2022 Workshop and Symposium, which will be held Nov. 2–4 in Portland, Oregon. I encourage each of you to **visit our website** and join **our LinkedIn group** to obtain the latest information about our upcoming **BioInterface conference** as well as other Foundation activities throughout 2022.

Remember, the Foundation relies on strong support from its members and sponsors so that it may continue to serve the scientific and medical device communities. I urge you to consider becoming a member, renewing your membership, or becoming a Foundation sponsor in 2022. We will meet in person for the first time in two years. You can find more information about these opportunities **on our website**. Please feel free to email me directly for more information. On behalf of the Surfaces in Biomaterials Foundation, I wish you and your families a prosperous 2022. I look forward to seeing everyone in person again, in Portland!

Save the Date!

BioInterface 2022 Workshop and Symposium
will take place in person **Nov. 2–4 in Portland, Oregon.**

The planning committee is moving forward
organizing stellar sessions and speakers.

Stay tuned for updates at our website and the
Surfaces in Biomaterials Foundation app.

Development of Novel Antibacterial and Antifungal Dressings for Diabetic Skin Wound Healing

Maoqi Mark Feng and Songqing Lu, Dynamic Entropy Technology LLC (DET), Tri-Cities, WA 99301*

**Corresponding author.*

Introduction

Diabetic ulcers or pressure ulcers are common for patients with diabetes and spinal cord injuries. Without proper management, such wounds often lead to infections such as osteomyelitis or sepsis, resulting in high mortality and morbidity. Besides bacterial infection, diabetic patients are also more susceptible to cutaneous fungal infections. Advanced wound dressings and advanced wound therapies should be applied to ease the severity of infection occurring in diabetic ulcer or even pressure ulcer. Collagen-based medical devices which can be used in skin wound healing, especially in challenging diabetic skin wound healing, especially unique in terms of dual degradation mechanism (enzymatic and hydrolysis), better reproducibility than biological allografts, sustainable anti-fungal drug released as a degradation product, sutureless wound closure due to strong tissue adhesion, and excellent biocompatibility, etc. Built upon DET's unique collagen electrochemical deposition process, the collagen-based biomaterials had been characterized and screened for antimicrobial properties, tissue adhesive properties, cell biocompatibility, in-vitro degradability (Collagenase degradation assay and hydrolysis), and mechanical properties. The developed novel collagen-citrate polymer matrix may significantly accelerate the healing of infected diabetic skin wounds. Further development to a commercial product Collagenized Dressing™ for reducing the health burden of diabetic skin wounds and even regular skin wounds is in progress.

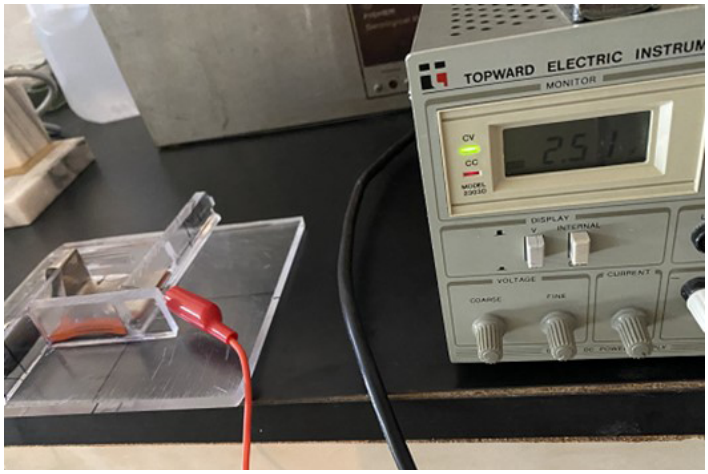


Figure 1. Electrochemical system setup for collagen deposition.

Experimental Section

Materials and chemicals. Collagen, 97% type-I, pepsin-soluble collagen extracted from fetal bovine hide, was purchased from Collagen Solutions LLC, San Jose, CA. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and other chemicals were all purchased from Sigma-Aldrich.

Collagen wound matrix was deposited on a platinum electrode sheet using a patented electrochemical process. This process allows the co-deposition of dialyzed collagen with one or more secondary components, thus resulting in the formation of a composite collagen matrix, as described in the literature [1-2]. The collagen deposition voltage was 2.5 V and the deposition time was 40 minutes to two hours.

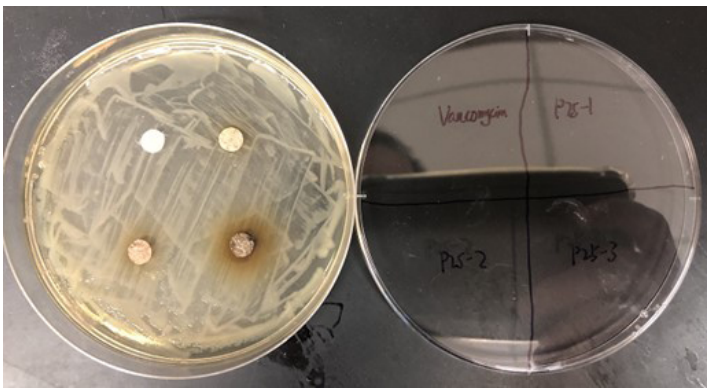


Figure 2. Collagen sheets from electrochemical deposition.

For deposition of collagen matrix with antifungal iCMBA prepolymer (AiC), dialyzed collagen was mixed with salt-free AiC prepolymer at different collagen/AiC mass ratios (e.g., 90:10; 75:25; 50:50; 25:75, and 10:90). Each mixture will be placed inside the electrochemical chamber (2.5 V DC between two platinum electrodes, 80 min deposition time) for deposition into thin sheets. The electrochemically deposited collagen-AiC prepolymer will be placed into a silver nitrate containing Tris-HNO3 buffer (pH 8.5) (for approximately 1 g of pre-polymer, use 1 mL of silver nitrate solution 0.15 g/mL).

Figure 2 shows the collagen sheets from electrochemical deposition. Here the collagen is pepsin soluble, and each disk contains 6 mg.

Continued on page 4

Development of Novel Antibacterial and Antifungal Dressings for Diabetic Skin Wound Healing ...

continued from page 3

Oxidation and consequently the cross-linking reaction of catechol-containing AiC prepolymer was triggered to form AbAf iC polymer inside a collagen matrix. In addition, the entire collagen/AbAf iC citrate polymer biomaterial matrix itself was further chemically crosslinked using our previously tested crosslinking agent [3], biocompatible 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) solution at three different concentrations (0.1, 0.5 and 1 w/v%). EDC chemistry crosslink COOH groups with amine groups. Thus, it can not only crosslink collagen itself, but also link collagen that is rich in NH2 groups and AbAf iC citrate polymer that is rich in COOH groups.

Table 1 lists the conditions of two groups (P32 and P25) with different mass ratios tested. The EDC and collagen sheet were mixed for overnight, and then treated with AgNO3 for 5 minutes. The samples from each group of collagen-AbAf were sent to PSU for further treatment.

Table 1. Different mass ratios of EDC to collagen sheets tested

Parameters/Sample	P32	P32A	P32B	P32C	P25-1	P25-2	P25-3
EDC/Collagen mass ratio	90/10	90/10	50/50	10/90	82/18	34/66	5/95

The above collagen-citrate polymer biomaterials were placed between two porous plastic plate mold (to prevent curl up/deformation after drying) and frozen at -20 °C and lyophilized into thin, flat sheets for eight hours. A Labconco freeze dryer was used in the tests.

Figure 3 and Figure 4 shows the freeze-dried collagen sheets fixed at 3 mg for each half disk.

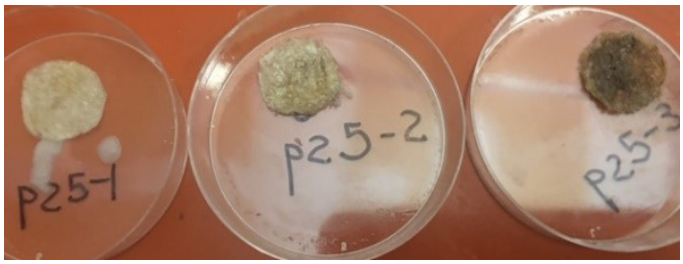


Figure 3. Freeze-dried collagen sheets from electrochemical deposition.

In vitro antimicrobial testing. The broad-spectrum antibacterial properties of collagen-AbAf iC polymer matrix was tested using a Zone-of-Inhibition test against clinically relevant Methicillin-Resistant

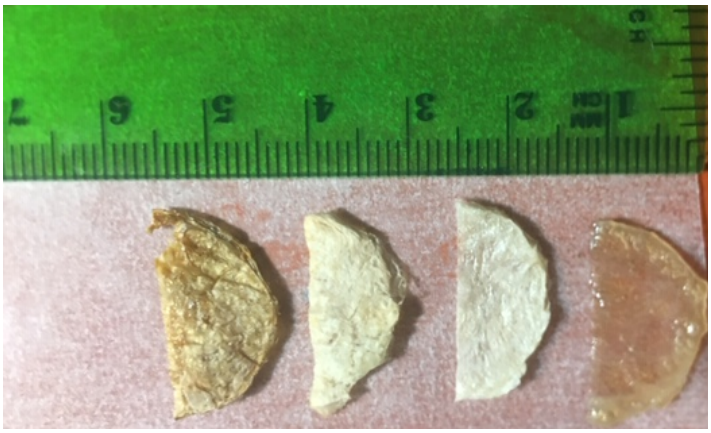


Figure 3. Part of the freeze-dried collagen sheets from electrochemical deposition.

Staphylococcus aureus (MRSA, ATCC 33592) and Pseudomonas aeruginosa (ATCC 15442). Each group of matrix was cut into circles (e.g., 5 mm diameter) and placed into the center of agar plates (N=3 per group) cultured with bacteria, after 48 hours, the zone of inhibition (killing of bacteria due to release of silver ions) will be measured and quantified. For antifungal testing, Candida albicans (C. albicans, ATCC® 10231™) was used following established safety protocols. YM medium broth (Lot #:1964C030) and YM agar (Lot #: 1964C030) was used for fungi (C. albicans) culture. For experiments, C. albicans was craped from YM agar plates and dispersed in Tween 20 (0.2 wt%) containing YM broth medium, counted with a hemocytometer, and diluted into a final fungi concentration of 0.5-1×107 cells/mL. The actual measure of fungal survival was determined using a colony growth assay on YM agar plates. The Anti-fungal effect of direct exposure to hydrogels was performed by following our published procedures [4,5]. The fungal survival ratio was calculated using the following equation:

Fungal survival ratio (%) = 100 × Ns /Ncon (1)

Where Ns stands for the number of fungal colonies for samples, and Ncon stands for the number of fungal colonies for YM broth blank control. For each sample, 6 plates were casted (N = 6 per sample group), and the fungal survival ratio numbers were averaged. The anti-fungal performance of collagen-AbAf iC polymer matrix of different compositions was also tested by the halo test method. 4 mLs of YM broth medium containing 0.5-1×107 cells/mL C.

Continued on page 5

Development of Novel Antibacterial and Antifungal Dressings for Diabetic Skin Wound Healing ...

continued from page 4

albicans were evenly cast onto YM agar plates (85 mm diameter 6 mm depth). The matrix discs (around 3 mm diameter) were placed on the agar plate and the constructs will be incubated at 37°C for 24 h in the dark before being examined for a "halo" or "zone of inhibition" surrounding the matrix disc. Three samples per group were used and the controls was pure collagen and pure AbAf iC polymer.

Mechanical testing. The mechanical properties of the collagen-AbAf iC polymer matrix (prepared using a dogbone-shaped electrochemical chamber) were tested at both wet and dry condition. The controls were pure collagen and pure AbAf iC polymer.

Direct gelation of collagen. A non-electrochemical collagen gelation method was also tested. Table 2 lists the gelling agent and antibacterial agent concentration for the tests. Typically, to a 6-cm diameter plastic dish, 10 wt% collagen (6 grams) was added together with 1.2 wt% agarose and 0.5 wt% gelatin as gelling agent, and 1 mL of 0.015 M antibacterial agent zinc acetate or zinc salicylic acid, the mixture was solidified in air after two hours.

Table 2. Parameters for direct gelation of collagen

Collagen	Gelling agent	Anti-bacteria agent
10 wt%	1.2 wt% agarose	none
10 wt%	1.2 wt% agarose	Zinc acetate 0.015 M
10 wt%	0.5 wt% gelatin 1.2 wt% agarose	Zinc acetate 0.015 M
10 wt%	1.2 wt% agarose	Zn salicylic acid 0.015 M
10 wt%	0.5 wt% gelatin 1.2 wt% agarose	Zn salicylic acid 0.015 M

Figure 5 shows the three samples prepared according to the recipe in first three rows of table 2. The right-hand side image is a freeze-dried sample.

Antibacterial test. The frozen DH5α (E. coli) was in tube with 2 mL LB and placed at 37°C overnight. The bacteria number was about 5x106 bacteria/mL. For each dish, 0.1 mL (2.5x103 bacteria) diluted bacteria was added and spread evenly. The dish was placed in the air at 37°C for 5 hours.

Results and Discussions

We have previously shown that ester containing

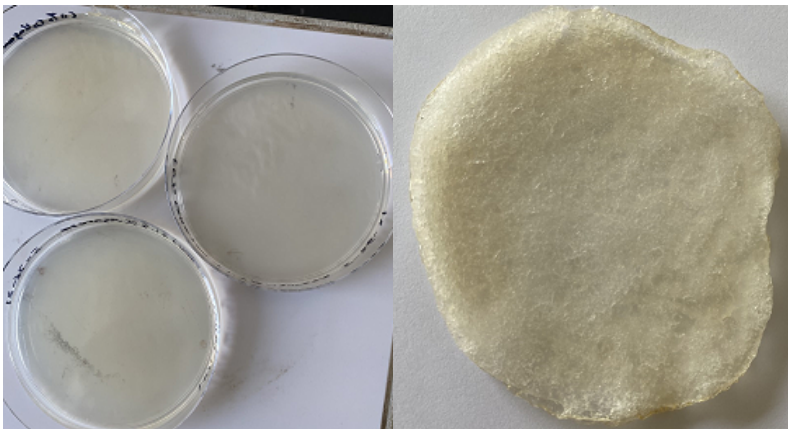


Figure 5. Gelled collagen sheets from non-electrochemical method (left). Right: Freeze-dried gelled collagen.

PLGA can be encapsulated inside the collagen matrix using our electrochemical process [1], thus we expect that AiC pre polymer which possess ester bonds should also be encapsulated inside to form collagen-AiC polymer composite. In the case that AiC prepolymer cannot be efficiently incorporated inside collagen using the electrochemical process, an alternative approach was adopted by forming electrochemically deposited, lyophilized, porous collagen sheet first, then AiC polymer solution can penetrate collagen sheets or casted together, and then crosslinked into a biphasic scaffold.

Collagen Scaffold Fabrication and Collagen-Citrate Polymer Modification. We have developed an electrochemical process that can assemble collagen molecules into a robust, densely packed nanofibril network conforming to the shape/contours of the cathodes, due to electrophoretic deposition, isoelectric focusing, and pH-induced collagen gelation under electrolysis of water. With the help of Dr. Jian Yang at PSU, an antibacterial and antifungal injectable citrate-based

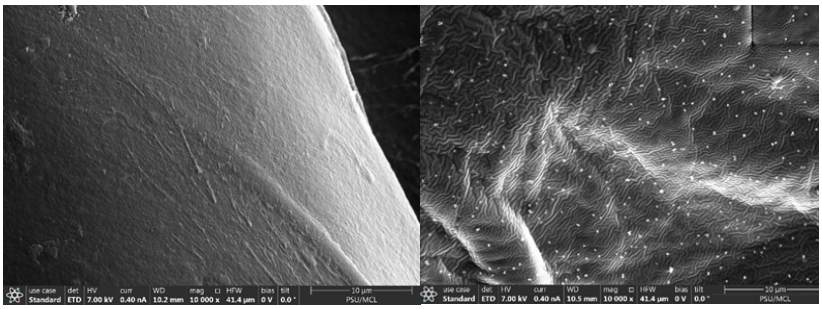


Figure 6. SEM images of DET's Collagen Scaffold with densely packed nanofibril network (A) and Collagen-Citrate Polymer Scaffold (B).

Continued on page 6

Development of Novel Antibacterial and Antifungal Dressings for Diabetic Skin Wound Healing ...

continued from page 5

mussel-inspired bioadhesives (AbAc iCMBA) were prepared. The morphology and antimicrobial properties of the collagen-citrate polymer scaffolds were evaluated. In the meantime, the collagen deposition processes and various ways to incorporate AbAc iCMBA polymers into collagen scaffolds were systematically evaluated.

As shown in Figure 6(A), the electrochemically deposited collagen wound scaffold showed densely packed and aligned nanofibril structures. After incorporation of AbAc iCMBAs, the collagen scaffold was evenly coated with a layer of AbAc iCMBA polymer and the coating does not affect the aligned nanofibril structures as shown in Figure 6(B).

Antimicrobial Evaluation of AbAc iCMBA-modified Collagen Scaffolds. The iCMBA-collagen scaffolds were fabricated at various weight ratios and their antimicrobial properties were evaluated against the clinically relevant Staphylococcus aureus (S. Aureus). Zone of inhibition studies showed all AbAc iCMBA-Collagen scaffolds showed a clear inhibition zone. The use of Ag crosslinkers seem resulting in enhanced zone of inhibition (Figure 7A). Both iCMBA-Collagen scaffold (5 wt% and 10 wt% AbAc iCMBA) showed potent bactericidal effects (>99%) against S. Aureus. Our results supported that the incorporation of AbAc iCMBA resulted in collagen scaffolds with potent antimicrobial properties as proposed that are promising for the use in the treatment of infected wound.

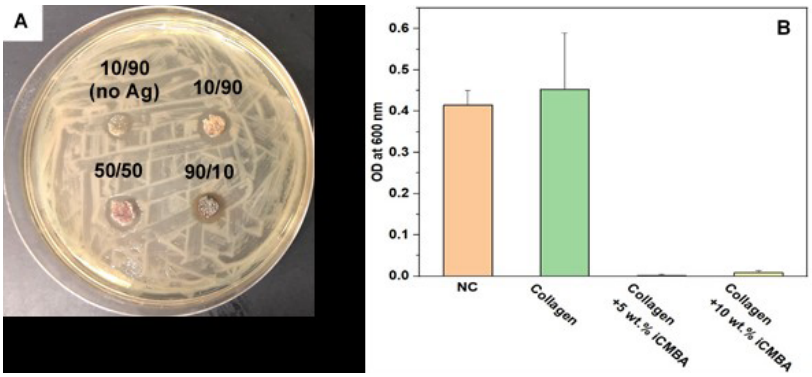


Figure 7. Antimicrobial evaluation of collagen and AbAc-iCMBA Collagen Scaffolds cultured with S. Aureus. A) Zone of inhibition of AbAc-iCMBA scaffold at various weight ratios of AbAc-iCMBA/Collagen of 10/90, (no crosslinker Ag), 10/90, 50/50, and 90/10. iCMBA was crosslinked with AgNO3 unless otherwise specified; B) Bactericidal effects of collagen and AbAc-iCMBA-modified collagen scaffolds (5% iCMBA and 10% iCMBA) cultured with S. Aureus.

Direct collagen gelation results. Collagen hydrogel prepared by the gelation method is a much easier pathway to prepare collagen dressing. This method can be easily scaled up for mass production using existing equipment.

Bacteria growth and anti-bacterium test. Figure 8 compares collagen hydrogel without (left in left picture) and with (right in left) antibacterial agent after 48 hours growth of bacteria on the surface. The results clearly show that 0.015 M zinc acetate and zinc salicylic acid suppress the DH5α growth completely (no bacterium is seen while in control dish can see hundreds of bacteria, see the white dots).

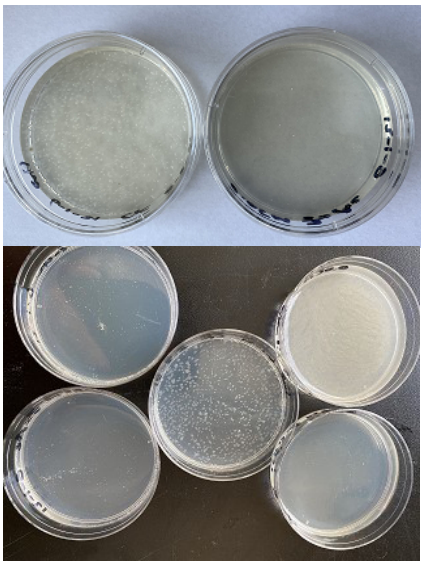


Figure 8. Antibacterial test for the collagen sheets.

Acknowledgement

We thank Dr. Xingguo Cheng and Prof. Jian Yang for the technical guidance and the financial support from NIH (Project Number 1R41DK123949-01).

References

[1] X. Cheng, V. Z. Poenitzsch, L. Cornell, C. Tsao, and T. Potter, "Electrochemical Bioencapsulation of Nanomaterials into Collagen for Biomedical Applications," J. Encapsulation Adsorpt. Sci., vol. 3, no. 1, pp. 16–23, 2013.

[2] X. Cheng et al., "Platelet-derived growth-factor-releasing aligned collagen–nanoparticle fibers promote the proliferation and tenogenic differentiation of adipose-derived stem cells," Acta Biomater., vol. 10, no.3, pp. 1360–1369, 2014.

[3] N. Edwards, D. Feliers, Q. Zhao, R. Stone, R. Christy, and X. Cheng, "An electrochemically deposited collagen wound matrix combined with adipose-derived stem cells improves cutaneous wound healing in a mouse model of type 2 diabetes," J. Biomater. Appl., vol. 33, no. 4, pp. 553–565, Oct. 2018.

Continued on page 7

Development of Novel Antibacterial and Antifungal Dressings for Diabetic Skin Wound Healing ...

continued from page 6

[4] J. Guo et al., "Synthesis and characterization of anti-bacterial and anti-fungal citrate-based mussel inspired bioadhesives.," Biomaterials, vol. 85, pp. 204–217, Apr. 2016.

[5] T. E. Swanson, X. Cheng, and C. Friedrich, "Development of chitosan–vancomycin antimicrobial coatings on titanium implants," J. Biomed. Mater. Res. Part A, vol. 97A, no. 2, pp. 167–176, May 2011.

Establishing an In Vitro Immunologic Testing Framework for FDA Safety Standards for Implantable Devices: A Biomaterials Perspective

Arely Mendiola and Shreya Raghavan, Department of Biomedical Engineering, Texas A&M University

The Food and Drug Administration (FDA) provides guidance for the medical device design process to ensure the safety and efficacy of implantable devices. Established classifications categorize medical devices which provide developers direction for testing their devices and providing sufficient evidence of safety and efficacy. FDA regulations cover the entire device design process from mechanical testing and biocompatibility to packaging and shelf life. The test recommendations vary based on the nature of the device. Medical devices are sorted into three different classes based on the risk associated with the use of the device: Class I, II, and III [1]. All implantable devices are considered Class III, as placing foreign objects in the body involves significant risk [1]. Before Class III devices can be sold, they must undergo Premarket Approval (PMA), which entails the most stringent pathway to market [1]. As expected, implantable devices require more profound examination in all aspects, including biocompatibility. Various biocompatibility tests which are recommended by the FDA exist, including several immunologic tests [2].

Although there are immunologic tests used to assess medical devices, these tests are almost exclusively used as supporting evidence for mechanisms of toxicity, and not risk assessment [3]. This means that the immune response to medical devices is used as a secondary metric for biocompatibility. Immune inquisition could be a valuable tool for FDA guidance because it can provide new data about the body's response to different materials. By isolating and evaluating the immune response of the body to implantable devices, a new framework can be created for bio-responsive materials that will allow for a more holistic examination of safety and efficacy. Immune responses to implantable materials are a complex interplay that involves multiple cell types, and soluble biochemical mediators (like cytokines, chemokines and antibodies) orchestrated in a spatio-temporally precise manner. These types of responses are often explored and investigated in animal models, and human immune responses are derivatives thereof. We posit that an in vitro approach to study immune responses to implantable materials will allow an investigation of human immune responses to biomaterials. This in turn can cascade in two differing directions: i) offering insights into biomaterial device design; and ii) risk assessment in medical device design. Furthermore, an in-vitro test bed offers practicality due to experiment repeatability and cost effectiveness, as animal studies are significantly more expensive than their in vitro studies. Importantly, an in vitro test bed offers tremendous flexibility due to its ability to isolate specific variables of study. These variables can be an immune cell/response of interest, or they could be biomaterial properties like surface roughness or functionalization. The permutations and combinations therefrom can offer increasing levels of complexity, all within a human immune response framework. Thus, establishing an immunologic testing framework will be a significant move forward in developing better medical devices.

Here, we describe one such in vitro immunologic testing framework we recently developed to test why shape memory polymer foam coated aneurysm occlusion devices resulted in better histological scores of healing in animal models. In designing and developing this study, we used the human monocytic cell line, THP1s, that are capable of differentiating into macrophages, the phagocytic cells of the innate immune system and a big player in the foreign body response associated with implanted biomaterials[4]. Macrophages tend to adopt plastic phenotypes that range

Continued on page 8

Establishing an In Vitro Immunologic Testing Framework for FDA Safety Standards for Implantable Devices: A Biomaterials Perspective

continued from page 7

from inflammatory to pro-regenerative, switching dynamically between the two states even during the foreign body response. These states are associated with distinct cytokines, and gene and protein expression profiles. Activation of macrophages can be triggered by any number of variables, including surface roughness, porosity, and chemistry of the biomaterial they encounter. By systematically characterizing macrophage phenotypes using molecular biology tools like flow cytometry, quantitative real-time polymerase chain reaction, and microscopy and image-based analysis, one can theoretically assess how macrophages activate in response to a biomaterial. Indeed, our work demonstrated that shape memory polymer foam coated aneurysm occlusion devices programmed towards a pro-regenerative phenotype within 72 hours of device contact at much higher rates compared to bare platinum coil aneurysm devices, which could in part explain better histological healing scores obtained in animal models[4]. The advantage of this particular study was the ability to isolate macrophages in vitro, in a human context, and understand how they respond to the biomaterial. Similar assays can be developed using other cell types like neutrophils, the first responders in a foreign body response following blood-biomaterial interaction. These assays can also be designed with mounting complexity, including several immune cell types and adaptive cell types to gain an even more global picture of human immune responses that involve cross-talk between adaptive and innate immunity. Should the study require focus on healing, other cell types including fibroblasts and mesenchymal cells can be incorporated to investigate fibrosis and matrix deposition.

Continued on page 9

Establishing an In Vitro Immunologic Testing Framework for FDA Safety Standards for Implantable Devices: A Biomaterials Perspective

continued from page 8

A unique challenge that comes with using in vitro testbeds such as ours to understand immune responses is creating benchmarks to determine what would be considered an acceptable level of immune reactivity for a particular cell type. Wider adoption of in vitro assays with an intention of creating such benchmarks, and a close collaboration between the biomaterials design team, immunology and immune engineering team, along with regulatory and clinical experts will no doubt accelerate in vitro testing into everyday practice for biomaterials device design and testing. Delving into the human immune response as a tool for risk assessment will offer a more well-rounded understanding of the benefits and drawbacks of employing numerous materials to implantable devices and will guide the device design process to mitigate potential adverse effects.

[1] Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process", in: F.a.D. Administration (Ed.) 2020.

[2] Immunotoxicity Testing Guidance, in: F.a.D. Administration (Ed.) 1999.

[3] D. Germolec, R. Luebke, A. Rooney, K. Shipkowski, R. Vandebriel, H. van Loveren, Immunotoxicology: A brief history, current status and strategies for future immunotoxicity assessment, Curr Opin Toxicol 5 (2017) 55-59.

[4] S.M. Chau, S.M. Herting, D.A. Noltensmeyer, H. Ahmed, D.J. Maitland, S. Raghavan, Macrophage activation in response to shape memory polymer foam-coated aneurysm occlusion devices, J Biomed Mater Res B Appl Biomater (2022).

Follow the Surfaces in Biomaterials Foundation on Social Media!

Like us on Facebook

Visit our LinkedIn page

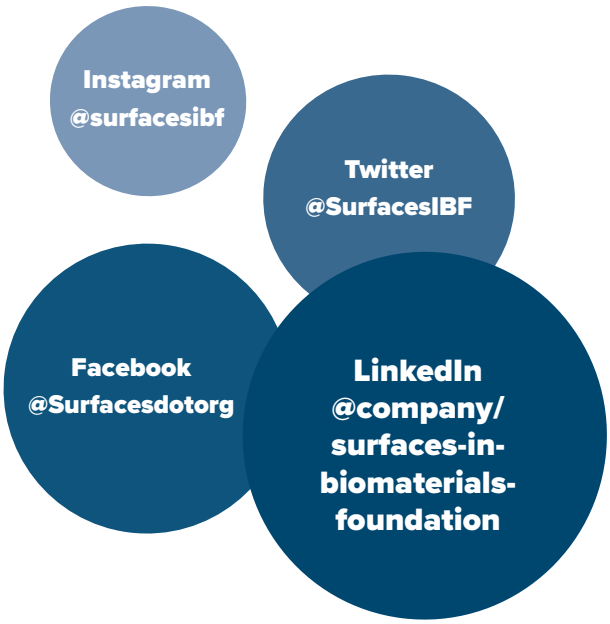
Follow us on Twitter

Check out our Instagram

We have a new logo!

SIBF

Surfaces in Biomaterials Foundation



SurFACTS in Biomaterials is the official publication of the Foundation and is dedicated to serving industrial engineers, research scientists, and academicians working on the field of biomaterials, biomedical devices, or diagnostic research.

SIBF

Surfaces in Biomaterials Foundation

Thank You to
OUR MEMBERS

APS AMERICAN PRECLINICAL SERVICES NOW PART OF NAMSA®

AST PRODUCTS, INC.

BAUSCH + LOMB

BIOCOAT® INCORPORATED

CARMEDA GORE

Elkem

SCHOOL OF BIOMEDICAL ENGINEERING
COLORADO STATE UNIVERSITY

GORE

ISurTec®
Innovative Surface Technologies, Inc.

Medtronic

Learn more about membership at www.surfaces.org/Member-Benefits