Spring 2015

Assessing Innate Immunity in Implant Biointegration Using Surface-Treated, Microporous PDMS Scaffolds

Avery M. Normandin
University of New Hampshire

Assessing Innate Immunity in Implant Biointegration Using Surface-Treated, Microporous PDMS Scaffolds

Avery M. Normandin[1], Kenan Mazic[2], and Kyung Jae Jeong[2]

[1]Department of Molecular, Cellular, and Biomedical Sciences, [2]Department of Chemical Engineering, University of New Hampshire, Durham, NH

In order to devise long-lived, functional implants, it is necessary that the material from which the implant is derived does not conjure destructive immune responses. Innate immune mechanisms, including acute and chronic inflammation and the ensuing foreign body reaction, dictate whether or not biointegration is successful. Thus, when designing biomaterials, it is imperative to cater to known physiological processes which will not trigger undesirable immunological outcomes, but rather support healing processes. This process, called biointegration, involves a seamless physical interconnection between biomaterial and recipient tissue. Many patients who undergo implantation fail to achieve biointegration, cascading to epithelial downgrowth and bacterial infection, subsequent device failure and removal, and in, rare circumstances, sepsis. With this in mind, there exists a pressing need to further optimize modern implants in order to maintain device stability, efficacy, and safety. Because the interaction of host tissue with the biomaterial occurs largely at the material surface, modulation of surface chemistry is an enticing means for improving biointegration. Here, we generated microporous, PDMS bioscaffolds with altered surface chemistries as a model to assess how well modified implants may assuage host immunity *in vitro*. Following surface treatment with polydopamine (PDA) alone or PDA + TiO_2—both promising surface modifications for improving implant outcome—, scaffolds were cultured with either macrophages (MΦ), dermal fibroblasts (DF), or mesenchymal stem cells (MSC) to elucidate how these surface chemistries may either promote or obstruct successful implantation. Understanding how specific surface chemistry modifications like these dictate innate immune mechanisms and wound healing processes will help inform future design of future immunomodulatory biomaterials.

Immune Response to Biomaterials

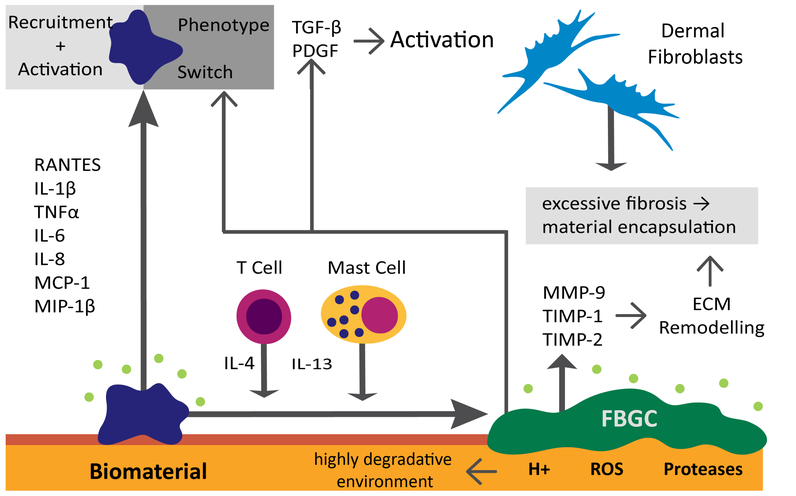


Figure 1. Macrophages are the driving force of chronic inflammation (adapted from X. Wang, 2013).

Methods

PDMS Scaffold Fabrication –

Surface Modification and Characterization – Untreated PDMS discs were immersed in a dilute, aqueous solution of dopamine (2 mg/ml dopamine in 10 mM Tris buffer, pH 8.5) for 24 h with light shaking to generate a layer of PDA. After a thorough wash with deionized water, the PDA-coated sponges were immersed in 0.1M ammonium hexafluorotitanate ($(NH_4)_2TiF_6$) and 0.3M boric acid (H_3BO_3) solution at pH 3.9 overnight on a shaker at room temperature to result in TiO_2 coating.

Cell Culture and Seeding – MSCs, DF, and monocytes were cultured in T75 flasks in the proper medium, prior to seeding onto scaffolds in a 24-well plate. All cells types were incubated at 37˚ C in a 5% CO2 environment. Monocytes were allowed to differentiate into MΦ for 7 days prior to seeding. Seeding densities were as follows (cells/well): MSC $5.7x10^3$; DF $1.9x10^4$; MΦ $1.6x10^4$.

Enzyme-Linked Immunosorbent Assay – ELISAs were performed using kits from Boster Immunoleader. For this measurement, the medium added to cells was collected from each well after 24 h. These samples were centrifuged at 1000 rcf for 5 min and the supernatant was collected for cytokine analysis. For a positive control, one set of cells (N=4) per cell type was spiked with bacterial lipopolysaccharide (LPS) at a concentration of 0.2 ng/mL.

Surface Modification & Characterization

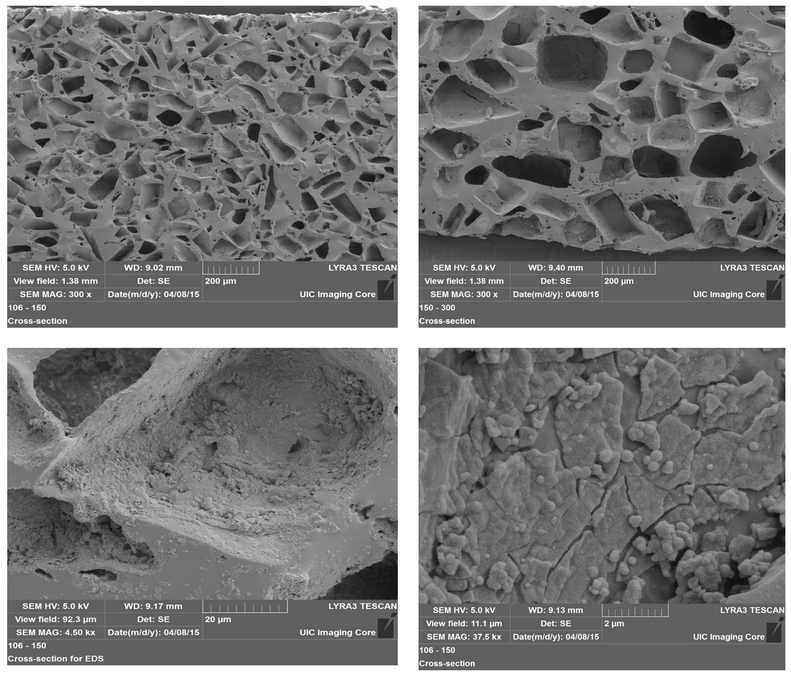


Figure 2. Scanning electron microscopy (SEM) images of microporous scaffolds with altered surface chemistries and varying porosities.

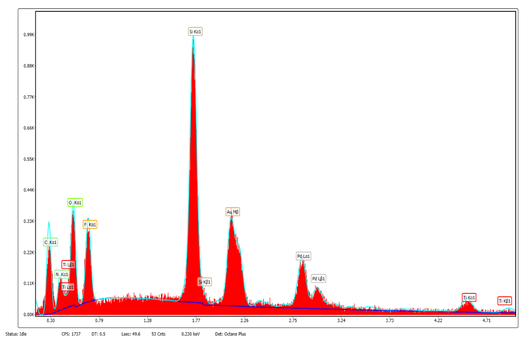


Figure 3. Energy dispersive x-ray spectroscopy (EDX) of surface-treated scaffold. Many elements present in the spectrum, such as Au, Si, C, and Pd, result from Au/Pd coating for SEM. There is a peak at Ti demonstrating that coating was successful.

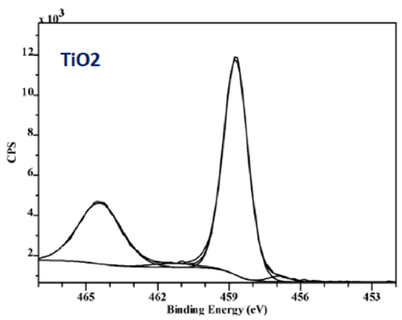
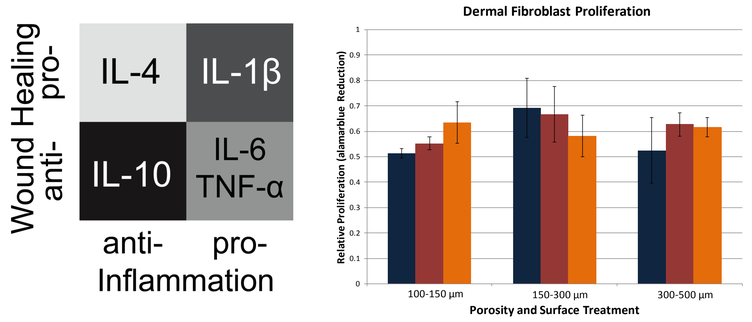


Figure 4. X-ray photo electron spectroscopy (XPS) of surface-treated scaffold. This demonstrates the oxidation state of deposited metal on the scaffold surface (*e.g.,* whether it is metallic Ti, for example, or metal oxide (TiO_2). Here, there is a peak at TiO_2.

In vitro Cytokine & Proliferation Analysis



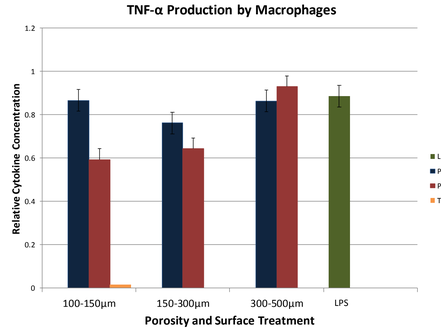


Figure 5. (Above Left.) A chart illustrating the role of cytokines in the foreign body reaction (adapted from Brodbeck *et al.* 2013). **Figure 6.** (Above right.) DF proliferation in response to PDMS. **Figure 7.** (Left.) Production of pro-inflammatory cytokine TNF-α by macrophages in presence of scaffolds.

Conclusions & Future Studies

Here, we have established a successful mechanism for generating microporous, PDMS scaffolds with altered surface chemistries—including deposition of PDA and TiO2—as a model for characterizing success of implant biointegration.

TiO2 coating on PDMS results a marked decrease in production of the pro-inflammatory cytokine tumor necrosis factor α in macrophages, making it a suitable candidate for biomaterial modification.

In the future, a larger panel of cytokines, including those vital for both wound healing and inflammation, must be assessed. DF and MSC proliferation and adherence to these scaffolds should also be examined. Lastly, *in vivo* experimentation is necessary to examine overall tissue reaction to implantation.

Acknowledgements & References

We thank all member of the Jeong Lab—Alison Deyett, Shujie Hou, and Mohammadjavad Mohammadi—for assistance in experiments and for critical feedback of this work. We also thank Shilana Kowack and Paige Normandin for assistance in scaffold fabrication.

References: Xiaohong Wang (2013). Overview on Biocompatibilities of Implantable Biomaterials, Advances in Biomaterials Science and Biomedical Applications, Prof. Rosario Pignatello (Ed.), ISBN: 978-953-51-1051-4, InTech, DOI: 10.5772/53461.