Pore Size of Implanted Biomaterials Modulates Macrophage Polarity

Eric M. Sussman, Lauran R. Madden, Buddy D. Ratner.
University of Washington, Seattle, WA 98195

Statement of Purpose: The biointegration of implanted medical devices is limited by the body's response to the biomaterial. This response, known as the foreign body reaction (FBR) is complex, involving multiple stages and cell types. The macrophage is likely the orchestrating cell of the FBR. Typically, the long-term response to non-degradable materials leads to a collagenous, avascular foreign body capsule (FBC). For sensors and drug-releasing implants, this outcome is undesireable because the FBC acts as a barrier between the device and tissue. However, the FBR may be altered. For instance, our lab has shown that a spherical pore geometry of optimal pore size and interconnectivity can lead to enhanced vascularity and reduced FBC [1]. The mechanism leading to this outcome is yet to be determined. We hypthesize that these materials function by affecting the polarity of adherent macrophages. In this report, we use immunohistochemistry to show that macrophages *in vivo* adherent to nonporous surfaces have a phenotype more similar to the inflammatory M1 macrophage, while macrophages adherent to porous biomaterials take on a phenotype similar to an anti-inflammatory M2 macrophage.

Methods: Macrophage analysis was performed on poly(2-hydroxyethyl methacrylate) hydrogels of various spherical pore sizes (nonporous, 20, and 30 µm) implanted in rat heart for 4 weeks. Tissue was fixed in methyl carnoys and embedded in paraffin. Antigen retrieval (sub-boiling Tris-EDTA pH 9.0) was performed for 5 minutes. Primary antibodies mouse anti-CD68 (macrophage marker, AbD Serotec) and goat anti-macrophage mannose receptor (MMR, M2 marker, R&D Systems) were diluted to 1:100 and applied overnight at 4 ˚C. These solutions were removed and anti-inducible nitric oxide synthase (iNOS, M1 marker, Abcam) was added for 15 mins at RT at 1:100. For negative controls, isotype-matched IgG was applied to control sections at the same concentration as the corresponding primary antibody. These sections did not reveal any staining. The secondary antibody cocktail consisted of Alexa Fluor 350 donkey anti-goat (1:100, Invitrogen), Alexa Fluor 488 donkey anti-rabbit (1:200, Invitrogen), and biotinylated horse anti-mouse (1:50, Vector Labs) incubated at RT for 1 h. Slides were washed and Alexa Fluor 555-streptavidin (Invitrogen) was applied for 30 minutes at 1:200. Sudan black counterstaining was used to reduce autofluorescence of the tissue. For each implant, 3 to 5 fields containing macrophages were imaged at 40x. Only macrophages (CD68+ cells) were included in the count. Cells were categorized according to the presence of the two polarity markers and the average percentage of each macrophage type per implant pore size was determined.

Results: Variation in macrophage phenotype corresponded to differences in implant morphology. The surfaces of nonporous implants were enriched with M1 type (iNOS+MMR-) macrophages (40%) while the porous materials both had only about 5% M1 macrophages on their surface, regardless of the pore size. Additionally, nonporous implants lacked M2 type (iNOS-MMR+) macrophages while porous materials of 20 µm and 30 µm pores had 5% and 10% M2 macrophages, respectively. A population of cells that stained iNOS+MMR+ also existed on all materials. The number of these double-positive cells increased between nonporous to 20µm and 30µm pores (16, 33, and 67%). The remaining macrophages on the surfaces of the implants did not stain for either polarity marker.

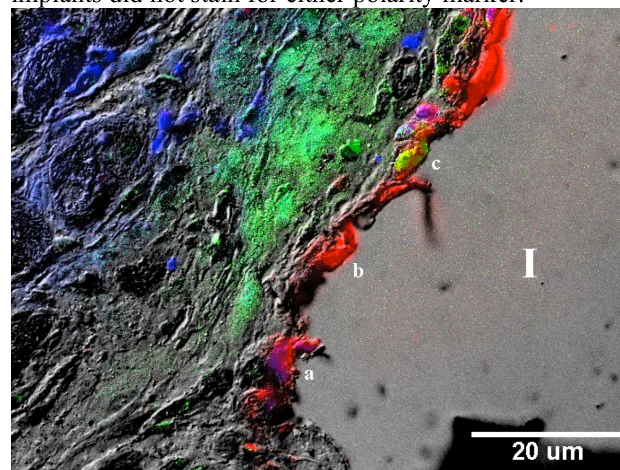

Fig 1. Representative image of macrophage phenotype staining for a 30 µm pore cardiac implant. CD68 = red, MMR = blue, and iNOS = green. (I) denotes the original location of the porous implant, and a, b & c denote iNOS-MMR+. iNOS-MMR-, and iNOS+MMR- macrophages, respectively.

Conclusions: We have demonstrated that the physical characteristics (porosity) of an implanted biomaterial affect the phenotype of macrophages at the interface of the implant and surrounding tissue. Other groups have found that differences in implant characteristics alter macrophage phenotype [2, 3]. Macrophage phenotype differences found here may be responsible for differences in healing observed in our previous studies, namely decreased FBC size and increased vascularity for approximately 30 µm spherical pores. iNOS is a typical M1 marker and MMR has been shown to be present on a subset of M2-type polarized cells [4]. The presence of double-staining iNOS+MMR+ cells could reveal a macrophage phenotype unique to the FBR and additional FBR-associated macrophage phenotypes likely exist. Ongoing studies will focus on further characterizing macrophage phenotype and understanding biomaterial design to guide the future design of medical devices.

References:

[1] Ratner BD. Polym Int. 2007;56:1183-1185.
[2] Anderson JM and Jones JA. Biomaterials. 2007;28:5114-5120.
[3] Brown BA et al. Biomaterials. 2009;30:1482-1491.
[4] Martinez OM, et al. Front Biosci. 2008;13:453-461.