Analysis of macrophage activation on polymerized lipid bilayers

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Statement of Purpose: The mechanism for host-material interaction generally starts with protein adhering and denaturing onto the surface of the material. This is followed with migration and activation of macrophages to try and remove the foreign object. If the substrate is not degradable, then a frustrated phagocytotic cycle is started in which the macrophages are chronically activated[1]. One of the goals of biomaterial engineers is to reduce or eliminate this response. There are several surface modifications that attempt to complete this goal[2]. However, none of these methods are completely ideal. This research focuses on a novel polymerizable lipid system, bis-SorbPC (BSPC) (Figure 1), which has, yet to be tested as a non-activating surface towards macrophages. It is hypothesized that the biomimetic nature of this bilayer will potentially minimize macrophage activation. A negative control study has been conducted with silicon substrates modified with BSPC as a proof of concept that this system can limit macrophage activation. The murine derived macrophages utilized in this study show a morphological change when activated[3]. The difference between an activated (A) and non-activated cell (NA) is shown in Figure 1. This change is analyzed to determine the extent of macrophage activation of the lipid treated substrates.

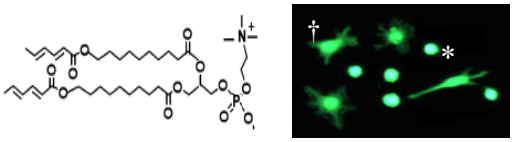

Figure 1. Structure of BSPC (left). Comparative examples of NA (*) and A (†) macrophages (right).

Methods: Silicon wafers with a 100 nm oxide layer were purchased from University Wafer. Lissamine (DHPE) was purchased from Invitrogen. BSPC synthesis was conducted as referenced[4]. Potassium persulfate and sodium bisulfite (Fisher) were utilized as redox initiators for polymerization. The silicon wafers were cleaned with acetone, ethanol, and water before being treated with piranha solution directly prior to use. Lipid vesicles composed of 1.5 mol% Lissamine DHPE and 98.5 mol% BSPC were prepared as referenced[4] and deposited on the cleaned wafers. The bilayer was polymerized by either UV exposure or redox initiation. A bare wafer and a wafer with deposited lipids that were left unpolymerized and dried were used as negative controls. Ellipsometry, contact angle, and fluorescent microscopy were utilized to further characterize the dried bilayers. RAW 264.7 macrophages were cultured on tissue culture plastic and passed at 70-80% confluency. Macrophages were seeded at a density of 100,000 cells/cm^2. The cells were cultured for 24 hours and then a live/dead staining kit (Invitrogen) was applied to fluorescently analyze the vitality of the cells. ImageJ^{TM}, provided by NIH, was utilized to analyze the fluorescent images. Activity of cells was determined based on average size and circularity. The fluorescent analysis was able to provide indirect evidence of macrophage activation.

Results: The ellipsometric and contact angle data compares with published data[4]. The thickness of the unpolymerized samples was around 1 nm, while the UV and redox polymerized samples were from 4-5 nm. The contact angle showed an increase in hydrophilicity compared to the bare substrate in both the redox and UV polymerized samples. The unpolymerized sample was more hydrophobic than the control. This is due to the exposure of alkyl end group of BSPC. The bilayers were analyzed with fluorescent microscopy to determine uniform coverage. The redox polymerized samples had the most uniform coverage, while the unpolymerized had the least. For the cell study, it was found that the total number of cells on each of the sample was fairly uniform. However, as alluded to earlier, the amount of A and NA cells differed greatly for certain samples. Figure 2 displays the ratio of NA/A cells, as determined by ImageJ^{TM} analysis. The higher ratio indicates a non-activating substrate.

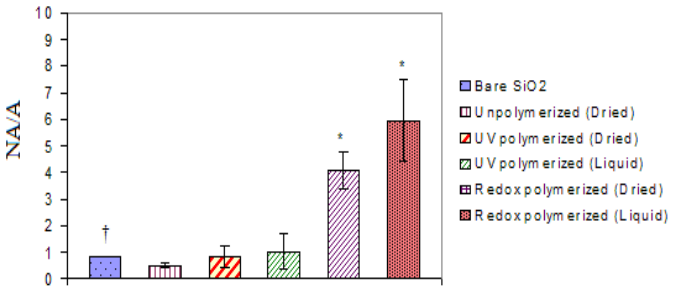

Figure 2. Activation analysis of RAW 264.7 macrophages on substrates with and without polymerized BSPC (n=3). * signifies statistical difference from the bare, unpolymerized and UV polymerized substrates, respectively, $p < 0.05$. † signifies statistical difference from the unpolymerized samples only, $p < 0.05$.

Conclusions: The ellipsometric, contact angle, and fluorescent data proved the formation of stable lipid bilayers, for the redox polymerized samples, even after drying. The preliminary results indicate that redox polymerized bilayers show the most promise for future analysis as a non-activating surface towards macrophages. In future work, immunohistological study of the RAW 264.7 macrophages will be applied to further quantify the current findings. Also, various cell types such as microglia will be analyzed with redox polymerized samples to better comprehend the cellular response.

References:

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