Does the surface on which a macrophage differentiates determine its subsequent form and degradative function?

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Statement of Purpose: If an in vitro cell model system is to be used to accurately predict in vivo performance, it is crucial that it be physiologically relevant. For instance, while many workers have investigated macrophage responses to biomaterial surfaces, they have used macrophages *after* full differentiation on another substrate. This approach is discordant with the in vivo situation since, there, macrophages are first recruited to the site of a biomaterial as monocytes, and then differentiate in response to local conditions and substrate. It is thus important to understand whether the differentiation of the monocyte to a macrophage is altered by the surface to which it initially attaches. One recent study has shown that monocytes differentiated on different polymer surfaces have altered functional parameters after 14 days; in that study, though, the macrophage responses were assessed only *after* these macrophages had been fully differentiated on the surface and then removed[1]. In the present study we have investigated the influence of the biomaterial surface on monocyte-derived-macrophage (MDM) morphology and function *during* a 14-day differentiation period. The ultimate target biomaterials for our work are decellularized xenograft tissues: substrates used as heart valve substitutes, yet with poorly characterized biocompatibility. An accurate in vitro representation of the in vivo environment will be a critical tool toward understanding the premature inflammatory failures plaguing such valves.

Methods: Monocytes were isolated from the blood of human volunteers and cultured for 14 days, as previously described, onto three surfaces: polydimethylsiloxane (PDMS), tissue culture polystyrene (PS) and decellularized bovine pericardium (DBP)[1]. The bovine pericardium was decellularized utilizing a process modified from that of Courtman et al.[2] DNA was quantified after 3, 7 and 14 days of differentiation. At 14 days, cells were fixed for scanning electron microscopy (SEM) and stained for actin and DNA for visualization using confocal microscopy. Cell lysates at the three time points were analyzed using immunoblotting for the expression of monocyte-specific esterase (MSE) and CD68, and quantified using chemiluminescence. Monocyte lysates were also analyzed for mRNA expression.

Results/Discussion: DNA content, representative of cell attachment, was not significantly different between the three surfaces, at any time point. This implies that equal numbers of cells were able to initially attach to—and subsequently differentiate on—each surface. Morphologically, however, there were distinct differences between the MDMs responses on the three surfaces. SEM images demonstrated that the cells differentiated on PDMS and PS spread onto the flat polymer surfaces; however cells cultured on decellularized pericardium were not spread and were more likely to display an elongated, possibly motile morphology. Representative images of the different morphologies are shown in Figure 1 below. Using confocal microscopy, actin staining was consistent with SEM: i.e. cells cultured on DBP were smaller and less spread than were the cells on either of the two polymers. Preliminary studies of MSE expression over the three time points demonstrated that expression of the 57 kDa MSE isoform increased with differentiation time, with cells cultured on PS demonstrating higher MSE expression after the 14-day differentiation period than was seen with cells on either DBP or PDMS. Investigation of additional isoforms of MSE with increasing differentiation time showed a triplet after 14 days on PDMS and PS—a feature absent on DBP. This may imply that the differentiation surfaces signaled differences in internal protein synthesis and processing. At the end of the 14 day differentiation period, cells cultured on DBP expressed less CD68 than did cells cultured on PS, perhaps indicating that the native collagen structure of the DBP surface is not a sufficient stimulus to fully differentiate monocytes.

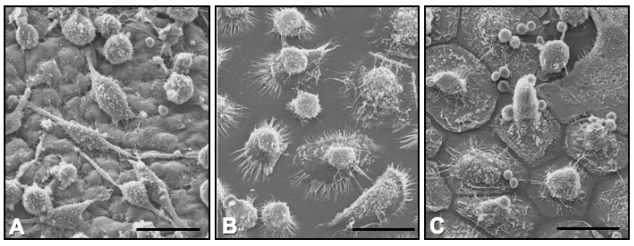

Figure 1. SEM images of monocyte-derived macrophages cultured for 14 days on (A) DBP, (B) PDMS and (C) PS. Scale bar represents 30 μm.

Conclusions: This is the first study to examine the differentiation of monocytes on decellularized extracellular matrix and to compare the response with that on two control polymers. These results demonstrate that the differentiation surface affects the macrophages' subsequent phenotype. The results have significant implications for in vitro analysis of macrophage response and function: the cues that the macrophage receives during its differentiation period may direct its phenotype and therefore presage its subsequent function.

References: [1]Labow RS et al. (2005) Biomaterials 26: 7357. [2]Courtman D.W.et al. (1994) J Biomed Mater Res 28:655

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