Published in final edited form as:

Biomaterials. 2007 December ; 28(34): 5114–5120.

Phenotypic Dichotomies in the Foreign Body Reaction

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Abstract

To better understand the relationship between macrophage/foreign body giant cell adhesion and activation on surface-modified biomaterials, quantitative assessment of adherent cell density (cells per mm²) and cytokine production (pgs per mL) were determined by ELISA. Further analysis to identify cellular activation was carried out by normalizing the cytokine concentration data to provide a measure of cellular activation. This method of analysis demonstrated that hydrophobic surfaces provided statistically significantly greater adherent cell densities than hydrophilic/neutral surfaces. However, when cell activation parameters were determined by normalization to the adherent cell density, the hydrophilic/neutral surfaces demonstrated statistically significantly greater levels of activation and production of IL-10, IL-1 β , IL-6, IL-8, and MIP-1 β . With increasing time, production of the anti-inflammatory cytokine IL-10 increased, whereas IL-1 β , IL-6, and IL-8 decreased and MIP-1 β was relatively constant over the culture time period. This observed dichotomy or disparity between adhesion and activation may be related to surface-induced adherent cell apoptosis. Further evaluation of macrophage activation on biomaterial surfaces indicated that an apparent phenotypic switch in macrophage phenotype occurred over the course of the in vitro culture. Analysis of cytokine/chemokine profiles with surface-modified biomaterials revealed similarities between the classically activated macrophages and the biomaterial-adherent macrophages early (day 3) in culture, while at later timepoints the biomaterial-adherent macrophages produced profiles similar to alternatively activated macrophages. Classically activated macrophages are those commonly activated by LPS (lipopolysaccharide) or IFN- γ (interferon- γ) and alternatively activated macrophages are those activated by IL-4/IL-13 or IL-10. Surface modification of biomaterials offers an opportunity to control cellular activation and cytokine profiles in the phenotypic switch, and may provide a means by which macrophages can be induced to regulate particular secretory proteins that direct inflammation, the foreign body reaction, wound healing, and ultimately biocompatibility.

Keywords

macrophages; adhesion; activation; hydrophilic, hydrophobic, and ionic surface chemistry; foreign body reaction

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Introduction

The effects of surface chemistry on adherent cellular behavior have been a key area of research for years. This research is driven by the notion that defining these relationships will aid in establishing criteria for designing biomaterials utilized in future applications. Complexities in defining these relationships arise in the not fully understood mechanisms by which adherent cells interact with the surface involving protein adsorption, integrin expression, ligand-integrin binding, cell signaling and the subsequent effects these interactions have on the resulting cellular behavior. The concept that minimizing cellular adhesion minimizes cellular activity on a biomaterial surface has been an accepted tenet prompting numerous studies to investigate ways to minimize cellular adhesion. Recent research has focused on understanding how biomaterial surface chemistry directs adherent macrophage activity and behavior including cytokine and chemokine production as a means to direct subsequent juxtacrine and paracrine biological responses (i.e. inflammation and wound healing) to implanted biomaterials.

Our recent studies have demonstrated that hydrophobic surfaces support macrophage adhesion and fusion, while hydrophilic/neutral surfaces markedly inhibit macrophage adhesion and fusion.[1,2] Monocyte/macrophage adhesion and fusion was seen on PET surfaces coated with hydrophobic poly(styrene-co-benzyl N, N-dimethyldithiocarbamate) (BDETDC) as shown in Figure 1. On surfaces in which the PET was rendered hydrophilic using a photografted acrylamide modification (PAAm), macrophage adhesion and fusion was significantly inhibited to values equal to or less than a third of the values seen on BDETDC. These findings were supported by previous research in our laboratory by Brodbeck et.al. and confirmed with the increase in macrophage fusion seen with the incorporation of hydrophobic silicone modifications to polyurethanes.[3,4] Interestingly, when the PET surface was modified with a hydrophilic/anionic (PAAmNa) or hydrophilic/cationic modification (DMAAPAAmMel), cellular adhesion levels were markedly greater than the hydrophilic/neutral surfaces (2 to 45 fold greater) and were comparable to values observed on hydrophobic surfaces (Figure 2).

Inverse Relationship of Cellular Adhesion and Activation

Generally speaking, monocytes adhere to a biomaterial, differentiate into macrophages that become activated, and then fuse to form multinucleated giant cells. Naturally, the term cellular “activation” is very broad and may involve numerous responses. A given cell can be “activated” to varying degrees and produce varying responses. Nevertheless, macrophage activation has been investigated in-depth for well over 40 years and researchers have differentiated inactive cells from active cells based upon the up- or down-regulation of gene expression, protein production, biological surface molecules (i.e. receptors, integrins, and protein markers), and reactive oxygen species secretion in addition to the resulting behaviors (i.e. phagocytosis or fusion).[5,6] Some of these components (i.e. integrin activity and cytokines) that mark a cell as “active” can also up- or down-regulate intracellular processes, in turn, activating these cells through apocrine, juxtacrine, and paracrine interactions.

Using the notion that an active cell produces greater amounts of given cytokines and/or chemokines, which is common in the study of biomaterial/cellular interactions, a cell can be defined as being in a more activated state than at previous timepoints or in comparison to other cells. The production of these proteins by activated cells may influence the behaviors of other cells advancing a biological response (i.e. inflammation, the foreign body reaction, wound healing, and apoptosis). Adherent macrophages were investigated in our study for a material-dependency in the production of cytokines, chemokines, matrix metalloproteinases (MMPs), and tissue inhibitors of MMPs (TIMPs), which were then utilized to draw conclusions about the activation state of these cells.

Previously, cellular activation was considered to correlate with cellular adhesion. The fewer number of cells that adhere to a particular surface, the less the overall activation of these cells. This has been shown to be true in some instances in which decreasing cellular adhesion decreased the resulting effects of these cells (i.e. reactive oxygen species production, production of a particular cytokine, and fusion) concluding that these adherent cell populations are less active.

Our recent studies suggest that macrophage adhesion does not correlate with or indicate a level of macrophage activation.[1,2] As discussed above, macrophage populations on the hydrophilic/neutral PAAM surfaces were significantly fewer in number than the macrophage populations on the hydrophobic BDEDTC surfaces (Figure 3.A). One would expect the concentrations of cytokines/chemokines to be greater on the hydrophobic BDEDTC surface in comparison to the hydrophilic/neutral PAAM surface following the above train of thought. This was not the case. As shown in Figure 3.B, the IL-10 cytokine concentrations on the hydrophilic/neutral PAAM surfaces were equal to the hydrophobic BDEDTC surfaces. To further analyze this relationship, the concentration of cytokine production was normalized to the adherent cell density to provide the quantity of each cytokine/chemokine produced by an adherent cell (Figure 3.C). Analysis of the concentrations for IL-1 β , IL-6, IL-8, and MIP-1 β on the hydrophilic/neutral PAAM surfaces revealed that these concentrations often times were also equal to or greater than on the hydrophobic BDEDTC surfaces (shown in the left column of Figure 4), prompting a similar normalization analysis (shown in the right column of this figure). For every protein analyzed, the amount of cytokine/chemokine produced by a cell adherent to PAAM was greater than on BDEDTC by a 2 to 77 fold increase (average increase of 20 fold). Analysis of the hydrophilic/neutral PAAM surface compared to the hydrophilic/ionic surfaces, PAANa and DMPAAMel, revealed the same inverse relationship between cellular adhesion and activation. One explanation could be that the BDEDTC population contained FBGCs that may not be as active or produce as great an amount of cytokines/chemokines as adherent macrophages. Distinct surface molecules (i.e. mannose receptors and integrin) are present in FBGCs and macrophages.[7–9] However, the concentrations were usually comparable at later timepoints when the FBGC population increased in number on BDEDTC. Another mechanism that may direct this disparate protein production could result from the increased release of certain cytokines/chemokines in cells undergoing apoptosis. Bzowska et.al. demonstrated that changes in the cytokine production profiles of monocyte cultures occurred as monocyte apoptosis in these cultures increased.[10] Specifically, IL-10 levels increased with apoptosis while IL-1 β and TNF- α levels were unaffected. We know from previous research that there is a greater percentage of apoptotic cells on the PAAM surfaces compared to the BDEDTC surface, which provide a mechanism by which adherent cells are reduced on these surfaces.[3]

Collectively, this study redefines the relationship between cell adhesion and activation. In addition, it implicates material surface chemistry in the production of cytokines and chemokines, and on cellular activation via multiple mechanisms, prompting further in-depth research into this area.

Biomaterials and Cellular Activation

In immunological or biological studies of macrophage behavior, macrophage activation has been classified based upon specific stimuli that have similar resulting biological events (i.e. cytokine/chemokine production, integrin and surface molecule expression). “Classical” activation was initially seen *in vivo* by Mackaness et.al., when the antimicrobial activities of macrophages were enhanced in mice infected with *Mycobacterium bovis bacillus Calmette-Guerin* (BCG). These cells currently are classified as active macrophages stimulated by lipopolysaccharide (LPS) or interferon gamma (IFN- γ) that up-regulate pro-inflammatory

cytokines (i.e., IL-6, tumor necrosis factor (TNF) and IL-1), inhibit anti-inflammatory cytokines (i.e., IL-10), variably up- or down-regulate chemokines, produce nitric oxide, and down-regulate mannose receptors and arginase production.[5,6,11–14] Macrophages were also found to be activated by IL-4 and IL-13 producing a distinctly different set of biological results including the inhibition of pro-inflammatory cytokines (i.e., IL-6, TNF, and IL-1), promotion of IL-10 and IL-1ra (receptor antagonist) cytokine production, opposing regulation of chemokines, up-regulation of mannose receptors, and production of arginase.[5,11,12,14–17] This class of macrophages was coined “alternatively” activated macrophages. Additional classes stimulated by IL-10 and other factors have since been identified and also are referred to as alternatively activated macrophages, but are usually denoted to be distinct from IL-4 and IL-13 alternatively activated macrophages when discussed.[5,18] The production profiles of numerous cytokines and chemokines by classically, IL-4/IL-13 alternatively, and IL-10 alternatively activated macrophages are shown in Table 1.

Previous studies have shown antibody arrays and ELISAs were utilized to confirm the presence of numerous cytokines, chemokines, MMPs, and TIMPs and to determine any increases or decreases in concentration between materials and over time as shown in Table 1. Analysis of cytokine/chemokine profiles in our studies revealed similarities between the classically activated macrophages and the biomaterial-adherent macrophages at day 3, while at later timepoints the biomaterial-adherent macrophages produced profiles similar to IL-4/IL-13 alternatively activated macrophages. Based upon these activation state classes, these findings suggest that biomaterial adherent macrophages undergo a phenotypic switch over time from an activation state similar to classically activated macrophages into one that is comparable to an alternatively activated macrophage. Material surface chemistry did not appear to be a factor in this switch; however, it is possible that the interaction of the macrophage with the biomaterial surface initiated this phenotypic switch.

Conclusions

This research is pivotal in that it provides evidence that macrophage activation does not proportionally correlate to macrophage adhesion as previous dogma has indicated. In this case, the amount of a given protein measured in the surrounding milieu was not always distinct between materials. Factors controlling the adherent cell density (i.e., surface chemistry and cytokine stimuli) and the potential mechanisms involved (i.e., apoptosis, fusion, cytokine binding and intracellular signaling) would elucidate how these activation levels could be directed. Additional research needs to be conducted to examine other key cytokines and chemokines that potentially could be modulated in this same manner. Controlling this cellular activation and the potential phenotypic switch it could create provides an additional means by which macrophages can be induced to regulate particular secretory proteins that direct inflammation, the foreign body reaction, wound healing, and ultimately biocompatibility.

Acknowledgements

Contract Grant Sponsor: National Institute of Health, National Institute of Biomedical Imaging and Bioengineering

Contract Grant Number: EB-000275

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Figure 1. Disparate Effects of Hydrophobic and Hydrophilic Surfaces on Macrophage Adhesion (A) and Fusion (B)

Numerical notations are the ratio of the data for PAAm surfaces to the data for BDEDTC surfaces. Mean $\pm$ SEM, n=3. “*” indicates a statistical difference between values ($p < 0.05$).

Figure 2. Disparate Effects of Hydrophilic Non-Ionic and Ionic Surfaces on Macrophage Adhesion (A) and Fusion (B)

Numerical notations are the ratio of the data for PAAm surfaces to the data for either the PAANa or DMAPAAmMeI surfaces. Mean $\pm$ SEM, n=3. “*” indicates a statistical difference between values ($p < 0.05$).

Figure 3. Direct Comparison of Cellular Adhesion (A), Cytokine Concentration (B), and Cellular Activation (C) on Hydrophobic and Hydrophilic/Neutral Surfaces

Cellular activation equals cytokine concentration (pg/mL) measured in 1mL of media normalized to the total number of adherent cells (cells/mm²). Mean $\pm$ SEM, n=3,4. “*” indicates that a statistical difference between values ($p < 0.05$).

Figure 4. Cytokine/Chemokine Concentration (A–D) and Cellular Activation (E–H) on Hydrophobic and Hydrophilic/Neutral Surfaces

Cellular activation equals cytokine concentration (pg/mL) measured in 1mL of media normalized to the total number of adherent cells (cells/mm²). Mean $\pm$ SEM, n=3,4. “*” indicates that a statistical difference between values ($p < 0.05$).

Table 1
A Comparison of Alternatively Activated and Classically Activated Macrophages

Parameter	Alternative [^]		Biomaterial Effects (Day 3 / Day 10) [^]		
	Classical [^]	IL-10	Hydrophobic	Hydrophilic & Neutral	Hydrophilic & Anionic
Cytokines[*]					
IL-1	↑5,6,12	↓5,11	↓12,17,19	↑/↓	↑/↓
IL-6	↑5,6	↓5,11	↓19	↑/↓	↑/↓
IL-10	↓11	↑5,6,11		↑/↓	↑/↓
IL-12	↑5,6,11	↓11		↔	↔
TNF	↑5,6,11,20	↓5,11	↓19,20	ND	ND
Chemokines[*]					
IL-8	↑11		↓19	↑/↓	↑/↓
MIP-1β	↑11	↓11	↓21	↑/↓	↑/↓
MDC	↓5,11,14	↑5,14		↑/↓	↑/↓
TARC	↓5,11	↑5		↔	↔
Mig	↑11,14	↓11,14	↓11,14	↔	↔
RAANTES	↑11	↓11	↓11,21	↑/↓	↑/↓
IP-10	↑6,11,14	↓11,14	↓11,14	↔	↔
ENA-78	↑11			↑/↔	↑/↔
MCP-1	↑11	↓11	↓21	↑/↓	↑/↓
MIP-1α	↑6,11			ND	ND
Eotaxin	↑11			↔	↔
Eotaxin-2	↓11,14	↑11,14		↑/↓	↑/↓
GRO	↑11			↔	↔

^{*} Bold: measured by ELISA and cytokine array; otherwise: only by cytokine array

[^] “↑”/“↓”/“↔”: level of production increased or decreased.