



## 2009 Research Grant Program Winning Abstract

### Macrophage Diversity

By **Scott Rapoport**

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Biomaterials have enjoyed widespread use in the clinic. Recent studies have suggested that implanted biomaterials can activate innate immunity which in turn can influence engraftment. Therefore the proposed research contained in this abstract seeks to utilize BD's OptEIA ELISA kits to assess the activation state of immune cells (specifically macrophages) interacting with various biomaterial candidates in vitro and to characterize in vitro cytokine cascades from innate immune cell populations pre-treated to elicit functional phenotypes. In a broad sense, this project will enable the selection and/or tuning of biomaterial candidates for a desired immune cell activation state of benefit to regenerative medicine applications.

The interstitial populations of macrophages found in most tissues are likely some of the first innate immune cells, aside those found in the blood, to encounter an implanted biomaterial. Depending on the activation state of these first-responders, the progression of inflammation, healing, and regeneration, are all strongly decided by complex cellular cytokine cascades. Specifically, macrophages have been shown to possess at least four functional phenotypes characterized by two major categories. Current research in mice shows that inflammatory monocytes can be activated to either a M1 (classically activated) or an M2 (alternatively activated) macrophage phenotype. M1 macrophages tend to be proinflammatory, leading to persistent inflammation, fibrosis, and engraftment failure. M2 macrophages can be further subdivided into three distinct polarizations: M2a, M2b, and M2c. M2 macrophages tend to initiate type II inflammation (M2a), immunoregulate/ immunosuppress (M2b), and participate in matrix modeling and tissue repair (M2c). Each macrophage polarization possesses a distinct activating cytokine regime, unique surface markers, and cytokine secretion profile. For example, in solely considering interleukins (IL), M1 macrophages strongly express IL-1,-6,-12,-15,-18,-23 while M2 macrophages strongly express IL-1,-1 $\beta$ ,-6,-10,-12. Further differences can be found among the M2 subtypes that allows for their identification in a similar fashion. Thus, BD's IL-specific ELISA kits can be utilized to ascertain which polarization is present.

Prior work in our group has focused on the culturing of human Thp-1 monocytes (ATTC) in a transwell environment with biomaterials and tissue-specific cell isolates. Thp-1 monocytes can with activated to a macrophage phenotype through the use of phorbol ester. In particular study, the Thp-1 cells never directly contact the biomaterials or the tissue-specific cell isolates. So, the questions addressed here were whether or not cytokine profiles from macrophages are capable of altering tissue-specific cell isolates and their behavior on biomaterials. However, to accurately answer these questions, knowledge of the macrophage activation state and corresponding cytokine secretion profile is required as determined by costly ELISA assays.

Therefore, the proposed work pending the success of this funding opportunity is as follows:



- I. Assess macrophage phenotype modulation on the iterative design of biomaterial scaffolds.
- II. Assess the cytokine dosing regimes required for specific polarization of macrophages.
- III. Assess the stability of polarized macrophages in culture.
- IV. If resources remain Assess polarity of interstitial macrophage populations found in whole tissue cell isolates.

In (I.), focus is on the development of biomaterials scaffolds for regenerative medicine applications that will facilitate an environment in which macrophages are stimulated towards an M2 (M2c) phenotype in the absence of other cell lines. This will include functionalizing scaffolds of interest with potent cytokine combinations. In (2.), ex vivo programming of macrophages using focused cytokine assemblages has already been demonstrated. However, in our model system, Thp-1 cells would require assessment along the same pathway. Further, (III.) addresses the potential for phenotypic plasticity in polarized macrophage cells. In other words, once converted, how long and how stable are these polarizations in vitro? Lastly, many regenerative medicine companies employ autologous cell populations to populate scaffolds prior to implantation. Since many of the cell isolates are derived from whole tissues, the potential exists for there to be a large contribution from interstitial macrophages that have been entrained in the isolation process. Therefore, it becomes an important question to ascertain the activation state of these macrophages that wind up an integral part of a construct.

In conclusion, it is apparent that BD's funding opportunity would greatly facilitate some basic questions about macrophage activation in our regenerative medicine platform. Specifically aiding in the rational design of biomaterial scaffolds as well as in characterization of cell therapeutics utilized in construct formation.

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