

A Simple High Throughput Approach to Study the Effects of Biomaterials on Dendritic Cell Phenotype

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Introduction: Biomaterials are widely used as the carriers of biologics in combination products for tissue regeneration or vaccine delivery. Success of such engineered products relies on their ability to minimize or maximize host immune response, respectively. Dendritic cells (DCs), the most potent antigen presenting cells, are critical in bridging innate to adaptive immunity in the response to a foreign entity by stimulating naïve T cells. In the immature state, DCs can be stimulated by adjuvants and become mature DCs, upregulating their expression of costimulatory and major histocompatibility class I and II molecules. To achieve the desired host response, biomaterials are an excellent tool to modulate the DC phenotype and thereby control the immunological outcome.

A differential biomaterial effect has been observed on DC phenotype depending on the biomaterial used. For example, DC maturation was induced by poly(lactic-co-glycolic acid) (PLGA) or chitosan films, not induced by agarose or alginate films, and inhibited by hyaluronic acid films^{1,2}. However, it was unclear which material properties contributed to such differential effects. Hence, a simple high throughput methodology has been developed to allow for the screening of DC phenotype upon treatment with biomaterial surfaces with graded variations in properties. The challenge was that since DCs are loosely adherent in culture, traditional cell-based ELISAs could not be used due to expected cell loss, hence, a 96-well filter plate was used to retain the cells during washes. Here we present results that validate the high throughput method, which will be useful to study DC phenotype upon culture on combinatorial arrays of biomaterials.

Methods: PLGA (75:25 molar ratio) films were prepared by solvent casting and cut to fit into wells. The films were exposed to UV for 30 min on each side immediately prior to the experiment. Agarose film was cast directly into wells. Endotoxin content (QCL-1000 LAL assay, Cambrex) of films and the filter membrane of the 96-well black filter plate were below 0.1 EU/ml.

DCs, derived from human peripheral blood mononuclear cells during a 5 day culture in the presence of inducing cytokines², were treated with biomaterials for 24 hours in 96-well or 6 well (conventional culture control²) tissue-culture plates. The extent of DC maturation was compared to controls: untreated immature DCs (iDCs) and lipopolysaccharide-treated mature DCs. Treated DCs were transferred to a black 96-well filter plate and double-stained with anti-CD86-PE (maturation marker) and anti-DC-SIGN-FITC (both maturation marker and normalization factor). Here we define a new parameter for DC maturation and normalization: CD86-PE/DC-SIGN-FITC. DCs, treated with biomaterials in

the 6-well format were also analyzed for CD86 and DC-SIGN using flow cytometry (FC).

Hence, to validate our approach, results of CD86/DC-SIGN expression for DCs treated with biomaterials in the 6-well format were compared by FC versus fluorescent plate reads. Furthermore, results of CD86/DC-SIGN expression for DCs treated with biomaterials in the 6-well format were compared to the 96-well format, using a fluorescent plate read.

Results/Discussions: The extent of biomaterial-induced DC maturation, whether in a 6-well plate format (the conventional culture method) or a 96-well plate format, were similar, hence the use of a 96-well format is appropriate (Figure 1). In addition, DCs could be analyzed by the filter plate method or by the standard FC method to yield similar trends in DC maturation, further confirming previous trends wherein PLGA films induced DC maturation but agarose films did not (Figure 1). In this way, measurement of DC maturation using a filter plate read was appropriate. Collectively, this study validated the filter plate approach for the assessment of DC maturation upon biomaterial contact.

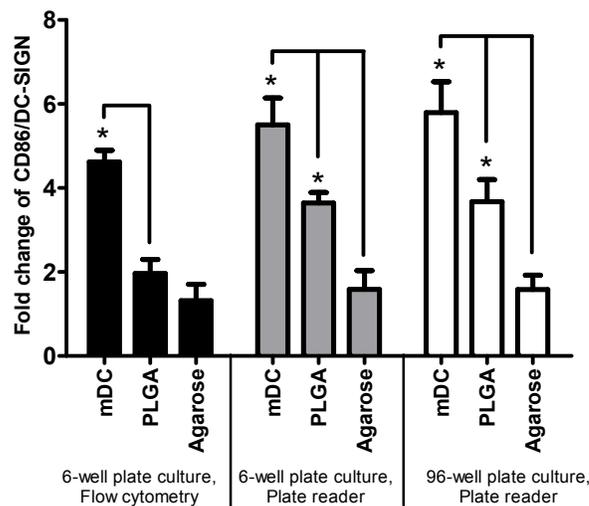


Figure 1: Comparison of fold change of CD86/DC-SIGN expression for DCs treated with biomaterials in the 6-well format by FC (black bars), 6-well format by fluorescent plate read (grey bars), and 96-well format by fluorescent plate read (white bars), as compared to iDCs. *: $p < 0.05$ compared to iDC. Brackets: $p < 0.05$ between samples.

Conclusions: This high throughput methodology will be used to allow for assessment of DC phenotype upon treatment with arrays of biomaterials.

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References: 1. Babensee JE, Paranjpe A, J Biomed Mater Res, 2005, 74A: 503-510. 2. Yoshida M, Babensee JE, J Biomed Mater Res, 2004, 71A: 45-54.