Biomaterials for Tissue Engineering with Control of Dendritic Cell Phenotype

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Introduction: Biomaterials are used as scaffolds for cell attachment and delivery in tissue engineered constructs. Furthermore, many of the same biomaterials are used as polymeric carriers for vaccines. Since biomaterials are used as scaffolds/carriers in such combination products questions are raised concerning the role that the biomaterial component of these devices plays in any potential immune responses towards the biological component due to a biomaterial adjuvant effect. Clearly from a tissue engineering point of view, immune responses are to be minimized or all together avoided while DNA or protein-based vaccines seek to initiate and enhance a protective immune response.

Adjuvants function in enhancing an immune response by interacting with antigen presenting cells, most notably, dendritic cells (DCs) during an innate immune response, to induce their maturation such that they become efficient at presenting antigen for effective stimulation of T cells for an adaptive immune response. Functional changes associated with DC maturation include acquiring a cell morphology with extensive cellular processes, enhanced expression of major histocompatibility (MHC) class I and II molecules and costimulatory molecules, with the effect of more effective stimulation of T cell proliferation in an allostimulatory mixed lymphocyte reaction (MLR), release of immunomodulatory cytokines and activation of the transcription factor NF-κB.

Previously, we have shown adjuvant effects associated with PLGA in the enhancement of the humoral immune response to associated antigen[1,2] can be attributed to a maturation of DCs upon *in vitro* culture with poly(lactic-*co*-glycolic acid) (PLGA) microparticles or films[3]. We also have shown differential levels of DC maturation depending on the biomaterial on which immature DCs were cultured[4].

Herein we extend our previous studies to further characterize the effect of different biomaterials on the maturation of human monocyte-derived DC using a variety of assays. An understanding of the mechanism of this adjuvant effect is expected to suggest new selection and design criteria for biomaterials to be used in tissue engineering.

Methods: The biomaterials tested included alginate, agarose, chitosan, hyaluronic acid (HA), and 75:25 PLGA and were prepared as cross-linked films using chemistry as appropriate, on the Teflon dishes. All biomaterial films were exposed to UV for 30 minutes for each surface and subsequently placed in 6-well cell culture plate prior to contacting with immature DCs (iDCs). Endotoxin contents of biomaterial films processed were measured using a chromogenic substrate and were at least 10 times less than the FDA limit of 0.5 EU/ml.

iDCs were generated from human peripheral blood monocytes by culturing in the media containing GM-CSF, IL-4, and 10% FBS. For biomaterial treatments, 1.5×10^6 cells (iDCs) were plated in each well of a 6-well cell culture plate containing either biomaterials or controls. iDCs were left untreated for the negative control or treated with the maturation stimulus, 1μg/ml Lipopolysaccharide (LPS), for the positive control of mature DCs (mDCs) in wells without biomaterial films.

After 24 hours of biomaterial treatment, effects on DC phenotype were assessed by examining morphology by phase contrast microscopy and cytospins, proinflammatory cytokine (TNF-α and IL-6) release into supernatant using ELISAs, flow cytometry for expression of maturation markers, and allostimulartory capacity in MLR, as compared to iDCs and mDCs. To assess the effect of time on activation of DC NF-κB transcription factor, p50, this was measured after 5 hours and 24 hours of biomaterial treatment, as compared to controls.

Results/Discussion: DCs treated with PLGA or chitosan films exhibited dendritic processes, expressed higher levels of CD86 and HLA-DQ, were allostimulatory in a MLR and released higher levels of proinflammatory cytokines (TNF-α and IL-6), as compared to iDCs, indicating DC maturation. DCs treated with alginate or agarose films demonstrated a similar phenotype to iDCs. DCs treated with HA film demonstrated lower levels of maturation markers, inhibited allostimulatory T cell proliferation and released proinflammatory cytokines to a lower extent than iDCs. Interestingly, DCs treated with alginate film showed a significantly higher level in TNF-α release compared to DCs treated with PLGA film. No significant difference was observed in levels of NFκB transcription factor subunit, p50 for DCs treated with different biomaterials films except for DCs treated with HA films which showed non-detectable value of p50.

Conclusions: In this study, differential levels of DC maturation were observed depending on the type of biomaterial film used to treat cells. DCs treated with PLGA or chitosan films support high levels of DC maturation, alginate or agarose films support moderate levels of DC maturation and while hyaluronic acid films inhibited DC maturation. Our future goals include elucidating the physiochemical biomaterial properties which influence DC maturation and correlating these *in vitro* effects on DC maturation to their *in vivo* adjuvant effect in supporting an immune response to biomaterial-associated antigen.

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References:

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