3-D Scaffolds for Tissue Engineering with Control of Dendritic Cell Phenotype

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Introduction: Scaffolds in 3-dimensional (3-D) porous form for attachment, delivery, and instruction of cells in tissue engineering have been investigated using various polymeric biomaterials. Since biomaterials are used in tissue engineered combination products as scaffolds/carriers, consideration of the biomaterial-adjuvant effect should be taken into account when selecting or designing biomaterials for use in tissue engineering to minimize any enhancement of an adaptive immune response to the associated biological component. 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 enhanced expression of major histocompatibility (MHC) class I and II molecules and costimulatory molecules, with the effect of increased stimulation of T cell proliferation in an allostimulatory mixed lymphocyte reaction (MLR), and release of immunomodulatory cytokines.

Previously, we have shown adjuvant effects associated with 3-D porous poly(lactic-*co*-glycolic acid) (PLGA) scaffolds in the enhancement of the humoral immune response to associated co-delivered antigen *in vivo*.[1,2] We also have shown differential levels of DC maturation depending on the type of 2-dimensional (2-D) biomaterial films with which immature DCs were treated.[3,4]

Herein we extend our previous studies to characterize the effect of contact with 3-D porous scaffolds processed from different biomaterials on the maturation of human monocyte-derived DC using a variety of assays. An understanding of the mechanism of the biomaterial scaffold adjuvant effect is expected to suggest new selection and design criteria for biomaterial scaffolds to be used in tissue engineering.

Methods: The biomaterials used for scaffolds included porous PLGA (75:25 molar ratio) and agarose. PLGA scaffolds were prepared by salt-polymer casting particulate-leaching technique with NaCl as the leachable component with size of 90-125μm[5]. Agarose scaffolds were prepared by inverted colloidal crystal templating method using polystyrene beads as leachable component with size of 100 (±1.5%)μm in tetrahydrofuran (THF)[6]. Scaffolds were sterilized by immersing into 70% EtOH followed by exposure to UV (each for 30 min.) and subsequently placed in 6-well cell culture plate prior to contacting with immature DCs (iDCs). iDCs were generated from human peripheral blood monocytes by culturing in the media containing GM-CSF, IL-4, and 10% FBS. For scaffold treatments, 1.5×10^6 cells (iDCs) were plated in each well of a 6-well cell culture plate containing either scaffolds 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 scaffolds.

After 24 hours of scaffold treatment, effects on DC phenotype were assessed by flow cytometry for expression of maturation markers and allostimulartory capacity in MLR, as compared to iDCs and mDCs. Annexin V or propidium iodide (PI) staining of DCs treated with scaffolds was also used to examine apoptosis or necrosis of DCs, respectively, using flow cytometry. Experiments are underway to further assess DC phenotype by measuring pro-inflammatory cytokine (TNF-α and IL-6) release into supernatant using ELISAs.

Results/Discussion: DCs treated with PLGA scaffolds expressed higher levels of CD40, CD80, CD86 and HLA-DR and were allostimulatory in a MLR, as compared to iDCs, indicating DC maturation. However, DCs treated with agarose scaffolds expressed maturation markers at levels very similar to iDCs with allostimulatory ability very similar to iDCs. DCs treated with PLGA scaffolds resulted in highest average level for both Annexin V and PI compared to controls (iDCs and mDCs) or DCs treated with agarose scaffolds. Mature DCs or DCs treated with agarose scaffolds resulted in higher levels than iDCs for Annexin V but in very similar levels to iDCs for PI.

Conclusions: Differential levels of DC maturation were observed depending on the type of material used to prepare the 3-D porous scaffold. DCs treated with PLGA scaffolds support high levels of DC maturation while agarose scaffolds support moderate levels of DC maturation similar to the DC response to these respective biomaterials in the film form. Our future goals include elucidating effects of expanded surface area in 3-D porous scaffolds on DC phenotypes 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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