Effects of substratum elasticity on dendritic cell maturation

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Statement of Purpose: In the human body, dendritic cells (DCs) constantly scan peripheral tissue areas for foreign pathogens. Upon contact, these pathogens are internalized by DCs leading to cell maturation and migration.[1] DCs transmigrate to the lymph nodes and interact with T cells, resulting in defined immunological responses regulated by the degree of DC maturation, phenotype, and soluble mediators.

DCs potentially have a mechano-sensing ability to perceive and respond to their surrounding environment, for example, to detect whether there is tissue damage or self-damage causing a softer or stiffer cell structure. As a result, immature DCs or DC precursors progress to different phenotypes or maturation stages. This study examines how DCs are affected by the change of stiffness of synthetic tissue substrata. Cells were grown on various stiffness of polyacrylamide (PA) gels coated with adhesion molecules to ascertain if substratum rigidity can be sensed by the DCs and cause alterations in cell specificity upon maturation. PA gels were chosen because of their reproducibility, optical quality, and because these gels do not interact directly with cells.[2] Additionally, the crosslinking of an adhesion molecule to these non-adhesive substrates created a physically manipulative but chemically stable environment for these plated cells. [2,3]

Methods: Various stiffness of polyacrylamide (PA) gels were made by increasing the percentage of bis-acrylamide added to the polyacrylamide monomer solution. Gels were photoactivated to allow laminin to covalently attach to the gel. A Micro BCA protein assay was used to assess the level of un-reacted protein to ensure equivalent amounts of protein on the gel of varying stiffness. (SpectarMax M5, Molecular Devices) DCs were plated at a concentration of $2x10^5$ cells on each gel. Morphology of cells was examined by a 20x objective lens on a fluorescence microscope (Eclipse TE2000-U, Nikon) at various durations. Cells were subsequently stained with FITC - CD80, PE - MHC class II, PE - MHC class I, PeCy5 – CD86, and 7-AAD and analyzed by flow cytometry with the FACSCAN2 (BD Biosciences). IL-6 secreted by cells was assessed by Enzyme-Linked Immunosorbent Assay (ELISA).

Results: The morphology, motility, maturation, and phenotype of DCs grown on gels of varying stiffness were evaluated. Deformation of cells occurs during many cellular events including response to physical environments.[4] Cytoskeleton rearrangement upon deformation initiates signaling cascades and changes in gene regulation.[5] Almost all the cells on the softer gels were rounded while cells were spread out or rounded with more protruding dendrites on stiffer gels.

Motility is important for DCs because of the necessity to traverse epithelial barriers and migrate into lymphatic areas. DCs use actin-rich compartments called podosomes to translocate across extracellular matrices.[6] Rounded morphology and formation of aggregates, resulted from a reduced level of podosomes and subsequential lack of cell adhesion, are commonly exhibited by either an immature or late maturation state.[6] Formation of cell aggregates was observed on gels of all stiffness, but at varying rates of formation. Podosomes of cells on the softer gels more rapidly diminished than cells on stiffer gels and resulted in earlier formation of cell aggregations.

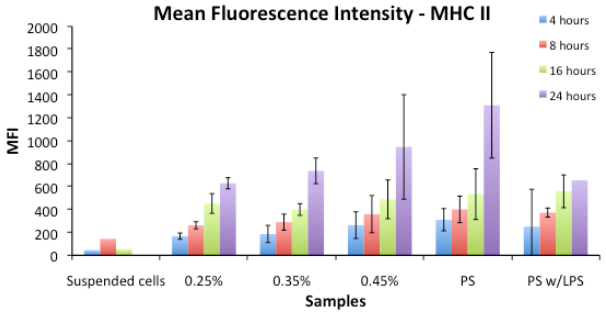

Figure 1. Expression of MHCII on DCs cultured on gels of varying stiffness at different timepoints.

Subsequently, the degree of maturation of DCs was assessed by examining the level of various surface markers including MHC II, CD80, and CD86. MHC II is mainly located intracellularly in immature DCs and translocates to the cell surface upon maturation. CD80 and CD86 are co-stimulatory molecules that assist in the formation of the immunological synapse between DCs and T cells. In this study all three molecules were up-regulated as the substratum stiffness increased, showing that a rigid environment may facilitate the maturation.

DCs can exhibit a tolerogenic as opposed to an immunogenic phenotype. Mechanics-induced maturation leads to a more tolerogenic maturation in contrast with DCs matured by microbial stimuli.[7] Tolerogenic DCs secreted a reduced level of pro-inflammatory cytokines, such as IL-6. We observed that DCs on stiffer gels produced a lower level of IL-6 compared to those on softer gels, which demonstrated that DCs on stiffer gels adopted a more tolerogenic phenotypes.

Conclusions: DCs grown on substrata of increasing stiffness resulted in enhanced levels of maturation. Cell motility was shown to decrease as the substratum stiffness increased. Cells on softer gels obtained a rounded morphology, while on cells on stiffer substrata adopted a rounded as well as an elongated morphology. DCs grown on stiffer substrata possessed a tolerogenic phenotype indicated by decreased generation of IL-6 compared to DCs on softer substrata.

References:

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