Protein Biomaterial Designs to Direct Cell & Tissue Outcomes

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Statement of Purpose: Biomaterials research continues to evolve towards more complex and biologically communicative systems for tissue regeneration. We have approached this challenge with fibrous protein matrices, particularly collagens and silks. We utilize these systems due to their versatility in terms of self-assembly, biological relevance, accessibility to genetic manipulation and their impressive mechanical properties, along with the ability to deliver appropriate topography, structure and chemistry to cells to direct their fate in vitro or in vivo. Specific strategies to regulate cell-biomaterial interactions include developing an understanding of the rates and modes of protein scaffold remolding related to protein structure, protein matrix designs for the delivery of cell signaling factors – including genetic encoding, covalent coupling or vesicle loading and scaffold designs to deliver stem cells with control of cell fate and function. In these cases, the ability to tailor the protein matrix structure, morphology and/or chemistry provides new insight into modes to control cell fate and tissue outcomes. The control of these features also mimics, to some extent, the information context found in native cell-matrix communication in normal tissues, in abnormal tissues or in tissue development.

Methods: As one example of current strategies, a flux model for collagen remodeling was developed (Abraham et al., 2007a; 2007b). Human fibroblasts (IMR-90 cells) were studied with native and denatured type I collagen, along with tissue culture plastic as a control. Radioactively labeled type I collagen and proline were used to quantify fluxes and to determine the kinetics and extent of remodeling and new matrix deposition, with consideration for mass balance. The data permitted the quantitative understanding of tissue remodeling *in vitro*, as a basis to begin to assess the influence of matrix features on rates and extent of remodeling.

Results: A mathematical model was developed based on known metabolic pathways to describe cellular collagen remodeling. Eight fluxes accompanied with mass balances provided a first approximation of the trafficking attained through the measurement of six concentrations of collagen and amino acids in the cell, media and extracellular matrix. By determining mass balances, the rate of change in concentration of collagen in the cell was determined by the fluxes of collagen and proline into and out of the cell. Phagocytosis of the denatured collagen substrate was significantly greater than that of the nondenatured collagen substrate for each time point, with the most dramatic difference at 2 days. Masses and fractions of denatured collagen substrate phagocytosed were significantly higher than those for non-denatured collagen. At all time points the total synthesis of new collagen from proline was significantly greater for cells grown on denatured collagen versus cells grown on nondenatured collagen.
Conclusions: In summary from this initial 2D flux model - collagen uptake by the cells was significantly greater when grown on denatured versus non-denatured collagen. Collagen phagocytosis was at least double than for cells grown on non-denatured collagen. Similarly, when the collagen trafficking was based on proline, cells grown on denatured collagen matrices incorporated proline into collagen at significantly greater levels than the cells grown on non-denatured collagen. The exposure of cryptic signaling sites in collagen due either to the unwinding of the triple helix or the digestion of the collagen chains, can have a direct impact on the rate and extent of remodeling of collagen. These were issues explored further in the follow on study, to track upregulation of specific MMPs.

References:

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