Prolonged Controlled Release of a Gene Inducer as a Means to Spatially-Control Gene Expression in a Cellularized Scaffold

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Statement of Purpose: While differentially controlling cell behavior within a scaffold is a challenge, such control might ultimately allow generation of more complex tissue constructs from, for instance, precursor cells. A potential method of exerting such control would be through local stimulation of desired gene expression. Inducer molecules that provide control over the timing and level of target gene expression have been utilized in a variety of experimental systems.[1] One system uses a synthetic, non-steriodal analog of 20-hydroxyecdysone called RheoSwitch Ligand 1 (RSL1, 382 Da, Intrexon Corp) to induce expression of a gene of interest in previously transfected cells.

While gene inducer systems provide for systemic temporal control of the degree of gene expression, local spatial control remains a challenge. We have thus incorporated RSL1 into a biodegradable elastomer to investigate its extended controlled release, the ability of this released RSL1 to "turn on" a target marker gene, and the ability of an RSL1 differentially loaded scaffold to differentially induce marker gene expression.

Methods: A B16 murine melanoma cell line was stably transfected with the RheoSwitch gene expression system coupled to a green fluorescent protein (GFP) reporter construct (Intrexon Corp). Poly(ester urethane)urea (PEUU) was synthesized from poly(caprolactone diol) and butyl diisocyanate, with chain extension by putrescine.[2] PEUU was dissolved in dimethyl sulfoxide and cast into films or fabricated into porous scaffolds using thermally induced phase separation (TIPS). To spatially control GFP expression TIPS scaffolds were made such that an outer circumference of a cylindrically-shaped scaffold was free of RSL1 while the interior core of the cylinder contained RSL1. Films and 3D scaffolds were variably loaded with RSL1 (0-150 uM) and cultured with B16 cells. Gene expression was assessed by fluorescence visualization. Films were cultured with B16 cells in static culture and B16 cell-seeded TIPS scaffolds were cultured in a spinner flask system to allow nutrient diffusion throughout the scaffold.

For *in vitro* release studies RSL1-loaded films were placed in 90% v/v phosphate buffered saline and 10% v/v acetonitrile to mimic sink conditions for the hydrophobic RSL1 molecule. RSL1 release was quantified by absorbance at 250 nm. Released RSL1 bioactivity was assessed by recovery of RSL1 by evaporation and addition to support media for B16 cells.

Results: RSL1 was released at near zero-order kinetics (**Fig. 1**) for approximately one year *in vitro* and remains bioactive through this period as shown by induced GFP expression levels (**Fig. 2**). Lower loading doses of RSL1 in PEUU films lead to lower gene expression levels by both contact with degradation solutions and for cells grown directly on RSL1-loaded films. Additionally, cells within TIPS scaffolds expressed GFP in interior regions loaded with RSL1 and did not fluoresce in peripheral regions not loaded with RSL1.

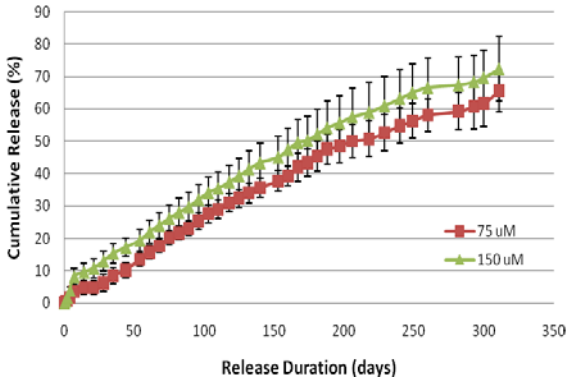

Fig. 1 RSL1 *in vitro* release from PEUU films loaded at 75 and 150 uM.

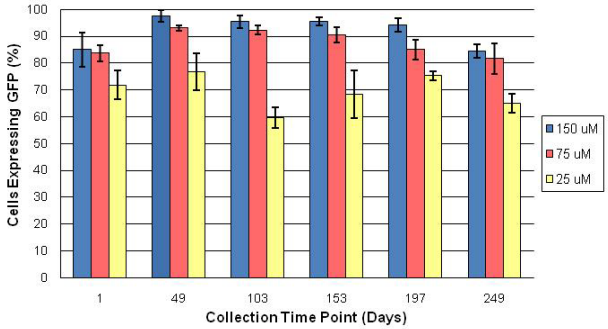

Fig. 2 Bioactivity of RSL1 released from PEUU films

Conclusions: The ability to provide controlled release of a bioactive inducer molecule over a period exceeding 10 months and the ability to spatially control gene expression behavior within a scaffold were demonstrated. This ability to spatially control gene expression for extended periods of time within a cell seeded scaffold may open opportunities to differentially drive cell behavior toward the generation of more complex tissue constructs.

References:

1. Alexander HK. Arch. Immunol. Ther. Exp. 2007;55: 139–149.
2. Guan J. Biomaterials. 2005;26:3961–3971.