Characterization of a Model System to Study Synovial Membrane Transport Properties

Robert Stefani, BS, Andrea R. Tan, PhD, Adam B. Nover, MS, J Chloe Bulinski, PhD, Gerard Ateshian, PhD, Clark Hung, PhD.
Columbia University, New York, NY, USA.


Introduction: The native synovium is mainly composed of macrophages and fibroblast-like synoviocytes, which are contained in a dense extracellular matrix. The synovium lines the articular surfaces of the joints, secreting lubricating and inflammatory molecules into the joint space. In order to fully understand the interplay of cytokines and other molecules in the synovial fluid in contact with cartilage, we must also understand the semi-permeable nature of the synovium. Studies have evaluated the transport of large lubricant molecules such as HA and lubricin across a model synovial monolayer, however the movement of smaller inflammatory cytokines has been ignored [2]. As such, the aim of this study aimed to characterize the transport properties of synovial cell monolayers on a transwell filter system, a system that has been well established for making such impedance measurements of epithelial and endothelial cell monolayers.

Methods: Juvenile bovine synovial tissue was harvested and digested in collagenase type IV. After filtration, synovial cells were expanded in alpha-minimum essential medium containing 10% FBS, antibiotics, and 5 ng/ml FGF-2 at a seeding density of 1760 cells/cm2. To create an in vitro synovial layer, passage 2 synovial cells were seeded at 100,000 cells/cm2 on 12 mm transwells (3 μm, polyester membrane). Sheets were grown for four days in growth medium consisting of DMEM with 10% FBS and 50 ug/ml ascorbic acid-2-phosphate, which has been shown to support the growth of a membrane layer [1]. Following two days of serum starvation (DMEM with 0.5% FBS), sheets were treated with 10 ng/ml IL-1α for three days. After IL-1 treatment, growth media was reintroduced for a three day recovery period.

Cell sheet integrity and solute permeability were assessed throughout the study. An Endohm 12 chamber was used to measure the transmembrane resistance values both pre- and post- IL-1 treatment and after the recovery phase. Transmembrane resistance (commonly referred to as TEER) is a common measure of endothelial monolayer integrity. To model the passive diffusion characteristics of a small molecule, 10 kDa FITC-labeled dextran was added to the top chamber of the transwell and samples were collected from the bottom well at 1, 4, 8 and 24 hour time points. Dextran was quantified by measuring fluorescence signal on a plate reader (565 nm). 10 kDa dextran was chosen as a molecule of comparable size to inflammatory cytokines in the joint, such as IL-1 and TNF-α and 70 kDa dextran was used to model the approximate size of TGF-beta. The general morphology and viability of the cell sheets was assessed using a LIVE/DEAD calcein/ethidium bromide stain. Immunohistochemistry was conducted by fixing transwells in 4% paraformaldehyde and staining for lubricin (rabbit polyclonal primary antibody, FITC-conjugated goat anti-rabbit secondary antibody). Statistics were performed using a paired student’s t-test and error bars are expressed as standard error.

Results: The engineered synovium exhibited characteristics of native synovium, and had the expected solute transport properties (Figure 1). Live/dead staining of a synovial cell sheet grown on a transwell
filter showed high viability and morphology similar to native synovium. Immunohistochemistry staining of lubricin showed moderate staining in the control group and intense staining in IL-1 treatment group. Flux of 10 kDa dextran was significantly greater than the flux of 70 kDa dextran after 24 hours. In the interleukin-treatment study, flux across interleukin-treated cell sheets was significantly greater than the flux across control cell sheets at one hour (Figure 2). After one hour, approximately 16% and 20% of the total dextran had diffused through the control and IL-1 treated sheets, respectively. Transmembrane resistance normalized to pre-treatment values showed that the control group significantly decreased post-treatment and IL-1 did not.

**Discussion:** We have described a tissue engineered cell sheet that is similar in morphology and lubricin content to native synovium. The transport of small (and large) molecules across this sheet can be readily quantified using established techniques, and we have identified temporal differences due to inflammatory cytokines. In the current study, we established our ability to grow a multilayered synovial cell membrane atop a transwell filter. Measures of solute transport across this synovial membrane demonstrated anticipated increases in solute diffusivity with decreasing solute molecular weight. The synovial membranes were able to respond with increased production of lubricin when exposed to interleukin, in support of findings by Blewis, et al. who showed increased lubricin release into the media of groups treated by IL-1 [2]. Additionally, measures of transmembrane resistance showed modulation of baseline impedance. These studies will help to establish a model system that can contribute fundamental information regarding the transport across synovial membranes, providing a tool for research as well as development of tissue engineering strategies for engineering the synovium [3].

**Significance:** These studies will help to establish a model system that can contribute fundamental information regarding the transport across synovial membranes, providing a tool for research as well as development of tissue engineering strategies for engineering the synovium [3].
Figure 2: Left: At one hour time point, flux of interleukin-treated cell sheets was significantly greater than the flux of control cell sheets. Right: Transmembrane resistance normalized to pre-treatment values showed that control group significantly decreased post-treatment and IL-1 did not.

ORS 2015 Annual Meeting

Poster No: 0425