Chick Chorioallantoic Membrane Assay for Evaluation of Fibrous Encapsulation of Decorin-Coated Implants

Marisa L. Sylvester, Buddy D. Ratner.
University of Washington.

Statement of Purpose: Encapsulation of medical devices after implantation has led to reduced functionality and device failure of long term implants. To improve healing and allow long-term implant functionality, the prevention of fibrous encapsulation is necessary. Key players in the development of fibrosis have been identified as well as natural inhibitors to these molecules. One of these key players is the pro-fibrotic cytokine transforming growth factor beta (TGF-β) whose activity can be neutralized by the proteoglycan decorin, a natural inhibitor of fibrosis. In this work, a novel decorin surface coating was created and assessed for its ability to reduce fibrous encapsulation. A chick chorioallantoic membrane (CAM) model was evaluated as a simplified *in vivo* method of measuring fibrous encapsulation of decorin-coated and control samples. This membrane is used in a well-established angiogenesis model[1] and has been reported to produce a fibrous capsule around implants[2-4]. Use of this model could allow for more rapid, cost effective and simpler screening of treatments to prevent fibrous encapsulation as compared to established rodent implantation models.

Methods: Decorin was attached to the surface of a polymer implant via a type I collagen affinity coating. This decorin coating was characterized to ensure expected surface composition via electron spectroscopy for chemical analysis (ESCA). Decorin attachment to the surface was quantified by radiolabeling. To verify biological activity of the decorin coating, binding of TGF-β to the surfaces was quantified by incubating TGF-β solutions with decorin-coated and control samples. A Quantikine human TGF-β1 solid-phase ELISA (R&D Systems) was used to measure the amount of TGF-β which remained in solution after exposure to samples. A CAM model[2] was evaluated as an *in vivo* screening method for treatments to reduce fibrous encapsulation. Fertilized chicken eggs (Hyline Farms, Puyallup, WA) were placed horizontally in an egg incubator for 3 days at 37 C. On day 4, each egg was cleaned and a 1 cm x 1 cm window was cut into the shell. Clear plastic was taped over the window to prevent contamination and allow visualization. At day 7 of incubation, decorin-coated and control samples were gently placed on top of the CAM approximately 0.5 cm from the embryo. Samples were allowed to incorporate for 11 days post-implant and then fixed *in situ* with 10% neutral buffered formalin. Samples were then explanted along with the surrounding tissue, paraffin embedded, cut into 5 μm thick sections, and stained with Masson's Trichrome to allow visualization of fibrous capsules. Images were obtained at 4x and 10x on a Nikon upright microscope.

Results: ESCA data confirmed expected elemental composition of decorin surface coating. Radiolabeling data showed that increases in decorin solution concentrations corresponded to increases in decorin surface quantity. At the highest solution concentration evaluated, 150 μg/ml, the quantity of decorin bound to the surface was approximately 300 ng/cm^2. The corresponding quantity of TGF-β bound to these surfaces was approximately 88 pg/cm^2 using a 2 ng/ml solution concentration. Following 11 day implantation in CAM models, decorin-coated and uncoated control samples had similarly low rates of incorporation into the membrane. This poor incorporation resulted in difficulty explanting samples along with surrounding tissues for histological examination. Frequently samples would separate from surrounding tissue during explantation. Of the 48 samples which were initially implanted, only 27 remained incorporated in and attached to surrounding tissue through explantation and were able to be processed and evaluated. Analysis of Masson's trichrome stained sections resulted in decorin-coated and uncoated samples whose fibrous capsules were undetectable or immeasurable in most cases. Fibrous capsule formation was unable to be compared between groups. Though published work on the CAM assay has reported development of a fibrous capsule, these studies did not report any measurements of capsule thickness[2-4].

Conclusions: Surface characterization techniques confirmed successful creation of a decorin surface coating and surface quantities of decorin were consistent with a monolayer of the proteoglycan. Quantification of TGF-β binding to the surface further confirmed biological activity of the collagen-bound decorin. Fibrous capsule formation around decorin-coated samples as compared to uncoated controls was unable to be assessed after implantation in the CAM model. It was determined that the CAM assay is not a suitable method for screening treatments to prevent fibrous encapsulation. This model had significant draw for reduced cost, availability and accessibility of fertile eggs and required equipment and production of a capsule in a shorter time frame than established rodent encapsulation models. Due to the challenges of sample incorporation as well as the inability to accurately measure capsule thicknesses and compare between treatment groups, we do not recommend continued evaluation of this model for work on fibrous encapsulation. In early stages of the fibrotic response, however, this assay has reported potential for evaluating biosensors[4]. In order to determine a significant reduction in fibrous encapsulation surrounding implants, a more robust fibrous capsule is preferred. More specifically, a larger fibrous capsule such as that found in rodent models would allow detection of measurable differences between treatment and control groups. Further *in vivo* evaluation of decorin coatings and uncoated controls is being carried out in a rodent subcutaneous implant model.

References:

1. (Leibovich SJ. *Nature.* 1987;329:630-632.)
2. (Valdes TI. *J Biomed Mater Res.* 2002;62:273-282.)
3. (Klueh U. *J Biomed Mater Res A.* 2003;67:838-843.)
4. (Valdes TI. *J Biomed Mater Res A.* 2003;67:215-223.)