# Decellularized Extracellular Matrix Microparticles Support Fibroblast and Mesenchymal Stem Cell Growth and are a Vehicle for Cellular Delivery in a Model of Anastomosis Healing

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**Statement of Purpose:** Extracellular matrix (ECM) materials for animal or human sources have become an important material in soft tissue repair. Mechanical dissociation of these materials into small fragments produces microparticle bits[1] of these materials that can deliver a very large number of cells grown on the bits in a very small overall volume. Cells delivered to a site of a wound or an anastomosis may promote rapid healing by a paracrine effect of cytokine secretion and also direct migration of the cells across the site with mechanical support by collagen deposition. In this study, migration of cells off these bits into a carrier gel was quantified. Utilization of seeded bits as a carrier for cell delivery for potentially increasing the cellularity and strength of a surgical anastomotic site was investigated.

**Methods:** Decellularized porcine mesothelial ECM sheet material[2] (DSM Biomedical, Exton, PA) was mechanically dissociated into ~200 µm diameter bits. Primary human dermal fibroblasts were seeded on 10 mg of ECM bits. The seeded bits were cultured in a rotating bioreactor for 3 days. The seeded bits were suspended (n=4 each) in a collagen gel 2 mg/ml or 4 mg/ml, a fibrin gel 20 mg/ml fibrinogen and 20 units/ml or 10 units/ml of thrombin, or finally on tissue culture plastic with no gel carrier. Migration of the fibroblasts off the bits into the gel was quantified. Seeded bits with M-cherry red labeled fibroblasts were deposited on anastomosis model of overlapping sheets of decellularized porcine mesothelial ECM. The bits were covered with a thin layer of 2 mg/ml collagen gel with controls of cells only with a covering gel, collagen gel without bits or cells and just the rehydrated ECM sheets only model (n=6 each).

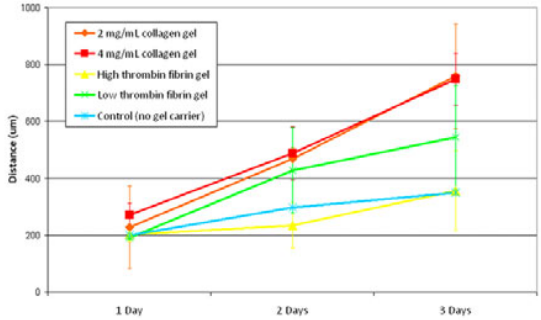


Figure 1. Migration distance of fibroblasts off ECM bits

The seeded bits were cultured on the anastomosis model (n=5) for 14 days then the anastomosis model was subjected to tensile testing. Primary human bone marrow mesenchymal stem cells were seeded on the ECM bits as a potential cell source for a clinical therapy.

**Results:** Primary human dermal fibroblasts and primary human bone marrow derived mesenchymal stem cells attached to the ECM bits with seeding efficiency of greater than 95% cellular attachment in less than an hour seeding time. Cells proliferate to confluence on the bits in seven days. Migration of fibroblasts off the bits in a carrier gel begins in less than 24 hours and is most rapid in 2 and 4 mg/ml collagen gels, Fig 1. Labeled fibroblasts migrated off the bits and across the anastomosis junction over two weeks and substantiated the tensile strength by 0.45 ±0.13 N, Fig 2. The cell with gel controls and gel only controls added no tensile strength compared to just the rehydrated ECM sheets only model.

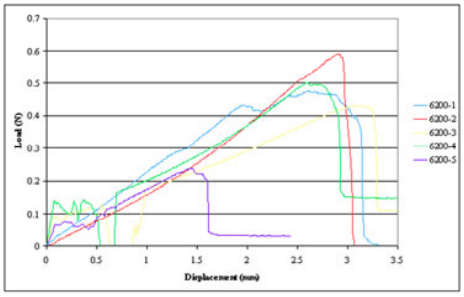


Figure 2. Tensile strength of anastomosis model supported by fibroblasts from seeded ECM bits

**Conclusion:** This study demonstrates the feasibility of fibroblast and MSC seeding and culture on microparticle bits of decellularized ECM derived from porcine mesothelium. Fibroblasts robustly migrate off the bits within carrier gels and can transfer to an adjacent anastomosis model. The fibroblasts when delivered in sufficient quantity from the bits can strengthen the anastomotic model. This approach of delivering cells to a surgical site on bits for direct mechanical and paracrine benefit may be a potential gateway to innovative therapies for a wide variety of surgical treatments.

**References:**

[1]Gilbert TM. Biomaterials 2005;26:1431–1435
[2]Hoganson DM. Biomaterials. 2010;31:6934-6940