Three Dimensional Constructs Composed from Extracellular Matrix Particles

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Statement of Purpose: The extracellular matrix (ECM) is a naturally occurring scaffold material that supports *in-vitro* cell growth and *in-vivo* tissue reconstruction. Commercially available forms of ECM, such as small intestinal submucosa (SIS) and urinary bladder matrix (UBM), are typically limited to a two-dimensional sheet form. A three dimensional structure is desirable for many tissue engineering applications. The purpose of this study was to fabricate a 3D construct from UBM-ECM powder while maintaining the characteristic ultrastructure and biocompatibility of ECM scaffolds.

Method: Source of Particulate Material: Bladders were harvested from market weight pigs and the tunica serosa, tunica muscularis externa, tunica submucosa, and muscularis mucosa were removed by mechanical manipulation. The remaining basement membrane and tunica propria layers that make up UBM-ECM were decellularized and disinfected with 0.1% peracetic acid solution and phosphate-buffered saline (PBS) and deionized water washes. The sheets were then lyophilized to remove residual water, snap frozen in liquid nitrogen and pulverized with a Waring blender until the liquid nitrogen evaporated. The pieces of lyophilized UBM-ECM were then ground into particles with a Wiley Mini Mill using a 60-mesh filter (250μm)[1].

Fabrication of 3D Constructs: UBM-ECM powder was saturated in a Newtonian pH balanced liquid like PBS to a slurry consistency, about 3.5ml of liquid for each 1.0 gram of powder. The hydrated powder was then packed in plaster of Paris molds (2cm x 2cm x 0.7cm) and stored in a humid environment in a 37ºC oven. The constructs and molds were then progressively dehydrated over a period of two days until completely dry. After complete drying, the constructs were soaked in PBS for 30 minutes and machined for a smoother finish by scraping the surface with a scalpel. The constructs were then returned to the 37ºC oven until completely dry.

Scanning Electron Microscopy (SEM) of 3D Constructs: Scanning electron imaging was performed on 3D constructs sterilized with 2MRad gamma irradiation. These constructs were fixed in 2.5% glutaraldehyde in 0.1M PBS overnight and progressively dehydrated in graded series of 30% to 100% ethanol washes, critical point dried, mounted on studs and sputter-coated prior to imaging with JSM6330F SEM.

Cell Seeding of Construct Surface: The constructs were sterilized using 2MRad gamma irradiation and soaked in appropriate cell culture media overnight prior to cell seeding. NIH3T3 fibroblasts and human microvascular endothelial cells (HMECs) were seeded on the surface of separate 3D ECM constructs at a concentration of $1x10^6/cm^2$ and maintained in culture for 10 days (DMEM supplemented with NaHCO3, 1% penicillin/streptomycin, and 10% fetal calf serum for NIH3T3 fibroblasts; MCDB131 supplemented with 1% hydrocortisone, 1% L-glutamine, 1% penicillin/streptomycin, and 10% fetal calf serum for HMECs). UBM hydrated sheets are excellent substrates for cell growth[2], and thus served as the positive control. After 10 days in culture, the constructs were harvested and placed in 10% neutral buffered formalin and processed using histological methods. Hematoxylin and Eosin (H&E) and Masson's Trichrome stains were performed for each construct and analyzed using a Nikon e600 microscope.

Results/Discussion: The fabrication method resulted in constructs that shrunk uniformly and possessed no structural defects. The final size of the constructs was approximately 1.5 cm x 1.5cm x 0.3cm, a 76% volume decrease from the size of the hydrated powder. The SEM images show the morphological characteristics of the particles as they were compacted in the 3D form (Figure 1). The fibrous nature and irregular contour of the ECM ultrastructure was maintained. The surface showed a large surface area for cell attachment. After 10 days in cell culture, the constructs were embedded and sectioned for histological processing. The UBM hydrated sheets were a suitable cell substrate for NIH3T3 fibroblasts and HMECs, characterized by a monolayer on the luminal surface and cell invasion into the abluminal surface. For the 3D constructs, HMECs migrated in between the ECM particles of the 3D constructs and formed a confluent layer on the surface. NIH3T3 fibroblasts also migrated in between the ECM particles and formed a confluent layer on the surface that was several cells thick (Figure 2).

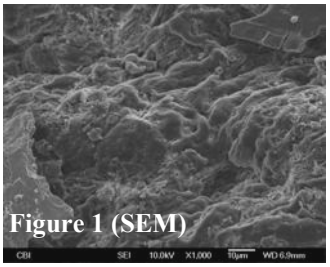

Figure 1 (SEM)

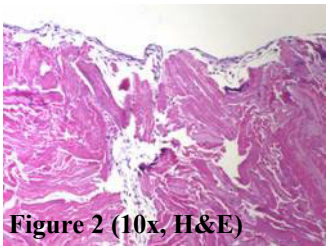
Figure 2 (10x, H&E)

Conclusions: A fabrication method that employs slow dehydration to make 3D constructs from UBM-ECM powder results in a 3-dimensional bioscaffold that conserves the ultrastructure and cell-compatible components of the native ECM. The utility of this scaffold configuration will expand the possible applications of naturally derived ECM scaffolds that are now limited to a 2-dimensional sheet form.

References:

1) Gilbert TW, Biomaterials, 2005;26:1431-1435.
2) Hodde JP, Tissue Eng., 2002;8-2:225-234.