In Vitro Evaluation of a Novel Biosynthetic Hydrogel

Marisha L. Godek, Kevin Lavigne, Nathaniel Mast, Walter Skalla, Angela Throm, Jane Mayotte, Rebecca Bushnell and Steven Bennett

Covidien Surgical Devices, North Haven, CT 06473 U.S.A.

Statement of Purpose: A need exists for an adhesive material that joins tissue planes without inhibiting wound healing, a requirement currently marginally fulfilled by fibrin glues.[1] Maintaining tissue apposition throughout wound healing sequelae without acting as a barrier to cellular ingrowth requires a delicate balance between attracting and promoting cellular attachment and infiltration, and biomaterial persistence. Potential clinical indications include abdominoplasties, mastectomies and bowel sealing. Biosynthetic materials exploit the properties of biological components to facilitate wound healing, while synthetic components offer the ability to tailor characteristics such as strength and persistence. This study examined the properties of and cellular response to novel biosynthetic hydrogel materials formed by crosslinking serum albumin with a reactive poly(ethylene glycol). To promote cellular ingrowth across the hydrogel, commercially available dextran beads were mixed into the novel material. Monocyte-macrophage and fibroblast cells, significant to wound healing processes, were used to evaluate the materials.

Methods: The hydrogel was created via standard *N*-hydroxysuccinimide crosslinking chemistry; a 4-arm 2,000 M.W. poly(ethylene glycol) succinimide succinate (JenKem Technology USA, Inc.) was reacted with bovine serum albumin (sterile filtered, SeraCare Life Sciences) solution, 25% w/v in 0.01 M sodium phosphate, pH 6.0. Commercially available DEAE Sephadex® A-25 microspheres ("beads") were converted into a hydrolytically unstable product via oxidation with sodium periodate (both Sigma Aldrich). The bead slurry was subjected to a series of washes (alternating 5 M NaCl and water).[2] The final product was added to the hydrogel solution in a 10 mg "beads" per 1 mL hydrogel solution ratio. Gel time, percent swelling and persistence *in vitro* (in sterile PBS at 37°C) were assessed; hydrogels were further characterized via environmental SEM (EVO-SEM, Zeiss, Figure 1). Cellular response and cytotoxicity (via LIVE/DEAD® kit, Invitrogen) were assessed using murine fibroblast L929 and NIH 3T3 and murine monocyte-macrophage J774A.1 cell lines (ATCC). Cells were (sub) cultured per ATCC recommendations. For all experiments cells were seeded at a density of 10,000 cells/ 1.91 cm^2/well, visualized using a Zeiss Axiovert 200 M microscope and photographed with a Zeiss camera. All images were representative of multiple fields and samples (minimum n value of 3).

Results: *Material characterization.* Gel time, percent swelling and persistence were comparable for the novel hydrogel constructs. Average gel times were 99 and 113 seconds, percent swelling was 22% and 20% and persistence time was 23 and 20 days for the hydrogels with and without DEAE beads, respectively (all tests n = 3). Mechanical testing indicated an average elastic modulus of 488 kPa ± 93 for the hydrogel with beads, versus a value of 315 kPa ± 55 for the hydrogel alone (n = 6, standard deviations reported). *Cellular response.* Cytotoxicity, cell adhesion and proliferation on control and novel biosynthetic hydrogels were comparable for all cell types. Viability testing indicated that the majority of cells on all materials were live (data not shown). Typically, monocyte-macrophage morphologies were more compact than either fibroblast cell line, with the NIH 3T3 exhibiting the most spread morphologies. Ultimately each cell line tested was able to adhere to, proliferate on and efficiently colonize the control and test surfaces. Cultures were terminated on Day 31. For materials containing DEAE beads, cells adhered to both materials but preferred the beads and migrated through the gel via the bead surfaces.

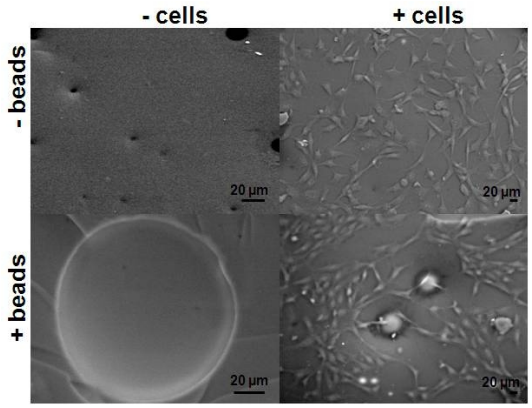

Figure 1. SEM of novel hydrogel constructs with/without DEAE beads, and with/without NIH 3T3 fibroblasts, as indicated.

Conclusions: The novel hydrogel materials comprising serum albumin and poly(ethylene glycol) with or without DEAE beads exhibited similar mechanical and physical properties. Significant to wound healing events *in vivo*, these materials supported colonization by monocyte-macrophage or fibroblast cells. The degradation products of the material did not adversely affect cell viability over the 31 day time period. Interestingly, the materials which contained DEAE beads supported cellular infiltration through the full thickness of the gel. These materials may be suitable for applications which require tissue plane apposition and tissue ingrowth where a degradable material is desired.

References:

1) Saltz, R. Aesth.Plast. Surg.1998; 22: 439-443.
2) Tawil, NJ. Wound Rep. Regen. 1999; 7: 389-399.