Extracellular Matrix Molecules Enhance Allograft Skin Viability

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Statement of Purpose: Cadaveric allograft skin is currentl y the ideal clinical choice for temporary wound coverage for burn victims.[1] Cadaveric skin is also used as a screening method for delivery of pharmaceuticals, and as a source of cells used in bioengineering models. However, the viability of the skin is compromised when cryopreserved or cultured more than 96 hours. Extension of the shelf life of cadaveric skin would increase the availability of cold-stored tissue. Depletion of extracellular matrix components during storage is believed to play a role in the rapid loss of viability.[2] A novel method was developed to prolong the viability of the skin by supplementing currently used storage media with hyaluronan (HA) and chondroitin sulfate (CS).

Methods: Split-thickness human cadaveric skin was obtained and placed in RPMI-1640 (Mediatech, Inc. Herndon, VA) at 4°C. Within 24 hours, the skin was cut into 6 mm pieces and immediately placed in storage media. The control medium was RPMI-1640 with 0.05 mg/L gentamicin. The supplemented media consisted of the control media with 0.05 mg/mL each HA (1700 kDA) and CS (shark) combined. The samples were stored at 4°C. An *in vitro* viability assessment was conducted using the Orion Dissolved Oxygen Probe (Thermo Orion Beverly, MA).[3] For each test, a sample of cultured skin was placed in media, contained in a Teflon® cartridge, epidermal side down. The probe was inserted into the cartridge, with dissolved O_2 (mg/L) readings taken continuously every ten seconds for 2000 seconds (33.3 minutes). A lower concentration in the media indicates the skin requires and is consuming more O_2 and is more metabolically active. To assess *in vivo* function, athymic nude female mice (*nu/nu*) were given a split thickness cadaveric skin xenograft, 6 mm in diameter. The skin was stored for 7 days prior to transplantation in either control or supplemented media. The transplanted grafts were secured with Tegaderm™ (3M St. Paul, MN) and bandaged. After 1 week, the graft area was excised for histological assessment.

Results / Discussion:

Net oxygen consumption values were calculated by taking the difference between the medium alone and medium with skin at each time point; these values were averaged for the duration of the experiment. Significant differences were determined at $p<0.05$ (Figure 1). The decrease in O_2 consumed in the control groups from day 21 to day 28 suggests greatly decreased viability ($p<0.01$). However, the dissolved oxygen concentrations of the supplemented media remain relatively constant, with the HA/CS solution closest to that of the newly harvested skin (fresh group).

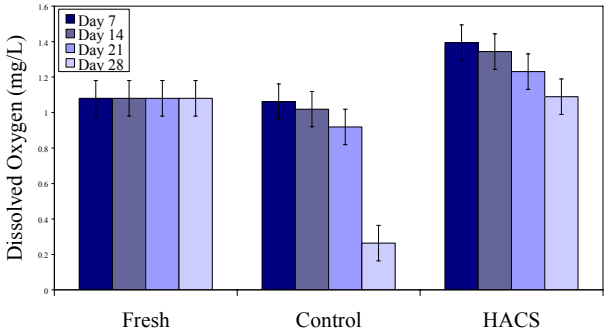

Figure 1. Amount of O_2 consumed by stored human cadaveric skin.

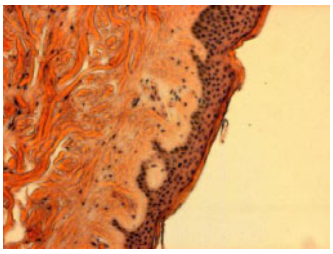

Figure 2. Histology of human skin xenograft of skin stored in supplemented media for 7 days. Graft excised after 1 week.

Histological assessment of the grafted skin showed better engraftment with the supplemented skin at day 7 than the control. Controls showed separation of keratinized cell layer, indicative of early stages of degeneration (not shown). The dermal/epidermal junction was conserved through the graft width in the supplemented grafts, with well-defined interdigitation of the papillary layer. Presence of blood vessels indicated graft vascularization, a positive sign of healing. Migration of host cells into the grafted tissue at the graft/host interface was evident and signs of inflammatory response, suggesting healing. The grafted human tissue was in good condition, well maintained and accepted.

Conclusions: Supplementing storage media with HA and CS preserved cadaveric skin viability to 28 days post-harvest and improved skin engraftment. It is believed that supplementing the media with HA and CS imparts their biological functions onto cadaveric skin, thereby improving the viability and healing properties.[4,5] This study illustrates new techniques used to ensure cell and tissue viability and function. Such studies will aid the development of improved biomaterials which contain or interact with living cells and tissues.

References:

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