Tissue-Engineered Human Embryonic Extracellular Matrix For Therapeutic Device Applications

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Statement of Purpose:
A scaleable, closed hypoxic bioreactor system has been developed to support the production of human matrix, which closely resembles embryonic extracellular matrix. Embryonic or fetal extracellular matrix has been thought to play an important role in fetal wound healing, which occurs with little or no scar formation (1). This important aspect of fetal wound healing led to the development of growing neonatal fibroblasts under fetal like conditions to encourage the production of hECM and conditioned media with fetal like characteristics.

Methods:
Scalable bioreactors are used to seed newborn fibroblasts onto microcarriers and grown in suspension while being conditioned with liquid media. Within a few days, under hypoxic culture conditions that simulate the embryonic environment, cells have produced a dense embryonic-like extracellular matrix and have secreted various growth factors into the media. These culture conditions have been optimized without the need for a fetal bovine serum additive.

Results:
The hECM material has been evaluated using a variety of bioassay techniques. Using an in vitro evaluation, the hECM material was seeded with human fibroblasts and compared to a mouse ECM material equivalent. The human fibroblasts were found to preferentially migrate into the hECM material and rapidly take residence while maintaining their normal morphology. Additionally, when comparing the mouse and human ECM materials, a dose-dependant increase in cell number was associated with exposure to increasing concentrations of the hECM material as measure by the Pico Green assay.

In other studies, the chick chorioallantoic membrane (CAM) assay has been used to test the response of the hECM material on a very sensitive in vivo membrane. With the implantation of the hECM material onto the membrane in this assay, the membrane remained transparent and did not thicken. Both of these responses are indicators of a favorable in vivo response to the material. Furthermore, a microvascular response was noted within the hECM material after implantation into the CAM assay, also an indication of a biocompatible material.

Conclusions:
The scar-free wound healing characteristics of the embryonic or fetal environment offer insight to the development of future technologies in the field of tissue engineering. Specifically, wound healing in the fetal environment occurs rapidly without inflammation and scar formation where the extracellular matrix is remodeled in a very organized fashion (1, 2). In contrast, adult wound healing begins with a marked inflammatory response that stimulates cell infiltration and subsequent disorganized collagen deposition ultimately resulting in a scar (2). The unique attributes of embryonic or fetal-like extracellular matrix may offer significant advantages in the development of future medical devices or therapeutics.

This hECM material can be manufactured using a scale-up process. A variety of forms and formulations can be made to be used in applications where porcine and bovine collagen have been traditionally used but without the risk of an allergic reaction or the transmission of animal viruses. Such uses include coatings for implants to improve tissue ingrowth, vehicles for cell delivery, injectables for soft tissue augmentation, tissue regeneration patches, and use in research applications.

The development of a manufactured source of human extracellular matrix, without the presence of living cells in the final product, provides a tissue-engineered product for regenerative medicine applications. Additionally, this hECM material affords the ability to avoid previous challenges with tissue-engineered solutions that contain living cells. These historic challenges include cell retention or cell engraftment, packaging and shelf life, the regulatory approval process, and reimbursement. In this way, past learnings from the field of tissue engineering have been leveraged to deliver next generation technologies that will find their way into research applications and medical therapies.

References:

1. Adzick NS and Lorenz HP, “Cells, Matrix, Growth Factors, and the Surgeon. The Biology of Scarless Fetal Wound Repair”, Annals of Surgery 220, 10-18, 1994.

2. Clark RAF, “The Molecular and Cellular Biology of Wound Repair, Second Edition”, Plenum Press New York and London, 1996.