Engineering a human skin equivalent using electrospun poly(DTE carbonate) matrix as dermal scaffold

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Introduction: Human skin Equivalents (HSEs) are being designed and constructed to serve a variety of basic and applied research needs, and for clinical applications. Current approaches in making HSEs include use of synthetic polymers such as PLGA [1], PEGT/PBT [2], and polystyrene [3] as dermal scaffolds. HSEs containing polymeric dermal scaffolds are particularly useful in *ex-vivo* applications, such as transdermal drug delivery, percutenaous absorption studies, testing of pharmaceutical and toxic compounds etc. They also offer improved mechanical properties, control over composition and customization for specific applications. This study introduces a novel amino-acid derived synthetic polymer poly(DTE carbonate) for skin tissue engineering. This L-tyrosine derived polymer incorporates functional groups that could be modified to tailor the properties of the polymer, and hence the dermal scaffold for skin equivalent engineering. The suitability of an electrospun 3D scaffold for construction of a human skin equivalent is being investigated, along with basic studies of cell-material interactions in scaffolds with reference to an engineered skin.

Methods: The synthesis of poly(DTE carbonate) has been reported before [4]. A 26% (w/v) solution of poly(DTE carbonate) in 85/15 THF/DMF was electrospun at a steady electric potential of 21 kv and the polymer fibers were collected on a rotating aluminum mandrel to generate a 3D scaffold containing randomly oriented fibers. Scanning electron microscopy (SEM) was used to determine the ultrastructure of the scaffold at submicron-resolution. Attachment and cytoskeleton organization of neonatal human dermal fibroblasts (HDF) was investigated using F-actin staining with Alexa fluor phalloidin 594 (Invitrogen, Carlsbad, CA) and fluorescence microscopy. Cell viability and proliferation in the scaffold was measured using the MTS assay [5]. A dermal equivalent (dermal layer of skin equivalent) has been constructed by applying a mixture of purified collagen and HDF to the 3D polymer scaffold using a centrifugal seeding technique [6].

Results/Discussion: The electrospun poly(DTE carbonate) scaffold has an average fiber diameter of 2.9 μm and a variety of pore sizes ranging from 30-60 μm, as determined by SEM analysis (Fig 1). Human dermal fibroblasts (HDF) attach and spread well on this polymer, as indicated by actin cytoskeleton organization and presence of lamellipodia in many cells. HDF growing in this scaffold were elongated and had cellular and cytoskeletal outgrowths attaching to scaffold fibers, indicating that the scaffold supported cellular infiltration and integration (Fig 2). There was a 65% seeding efficiency of HDF on this scaffold and the cell growth was exponential over the evaluation period of 8 days. The presence of the poly(DTE carbonate) scaffold in the dermal equivalent reduced the contraction seen in keratinocyte seeded collagen only dermis. Preliminary analysis of the dermal equivalent showed successful cell seeding and collagen polymerization. Detailed analysis is now being carried out using high-resolution microscopy, histological and staining techniques.

Fig 1. SEM of electrospun. poly(DTE carbonate) scaffold prior to cell seeding. Scale bar 10 μm.

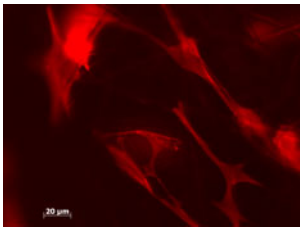

Fig 2. HDFs growing within the scaffold at day 3. Red fluorescence is F-actin staining. Scale bar: 20 μm

Conclusion: Poly(DTE carbonate) has been used for the first time to improve the mechanical strength of an engineering human skin equivalent. Cellular interaction studies indicate that skin cells interact favorably with the electrospun 3D scaffold, supporting its use in the design of full skin equivalents. The methods developed in our laboratory result in a skin equivalent, especially useful as a test bed for transdermal drug delivery studies and skin penetration testing of pharmaceutical compounds.

References:

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