PRODUCTION OF FULL-THICKNESS HUMAN SKIN SUBSTITUTE TISSUES WITHOUT ANIMAL-DERIVED COLLAGEN

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Introduction: Currently-available full-thickness skin substitutes contain a stratified epidermal layer grown on a dermal equivalent (DE) composed of human dermal fibroblasts (NHDFs) in an animal-derived (often bovine) collagen gel. Animal-derived collagen gels fail to recreate the normal composition, structure, and function of the human dermis, and despite rigorous screening and quality control, introduce variability and the potential for the transmission of adventitious agents, such as bovine spongiform encephalopathy. In contrast, long-term cultures of NHDFs can form *de novo* tissues, composed entirely of cells and their secreted extracellular matrix (ECM). The objective of this study was to use NHDF-derived DEs to create skin substitute tissues composed entirely of human cells and their secreted extracellular matrix.

Methods: NHDFs were seeded into porous tissue culture inserts and allowed to secrete and assemble ECM components into a cohesive DE. NIKS keratinocytes were then seeded onto the DEs and allowed to grow to confluence. The tissues were then cultured at the media/air interface to promote formation of a fully-stratified epidermal layer. DEs and mature skin substitutes were analyzed for thickness, histological appearance, collagen content (Sirius Red assay), cell density and viability (MTT), barrier function (surface electrical capacitance), and tensile properties. Tissue properties were compared to skin substitutes generated using collagen-gel based DEs.

Results: NHDF cultures deposited significant quantities of new extracellular matrix (collagen density >100 μg/cm^2 of tissue surface), forming DEs >50 μm in thickness, with substantial mechanical strength (>1 MPa). In contrast, collagen-gel DEs remained composed primarily of the input collagen and had negligible mechanical properties. NHDF-derived DEs supported formation of mature skin substitutes containing fully-stratified epidermal layers with viability, mechanical properties, and barrier function comparable to tissues produced with gelled collagen DEs.

Conclusions: NHDF-derived DEs have properties closer to those of normal human skin and support the production of full-thickness skin substitutes composed solely of human cells and cell-derived ECM. Use of these DEs has the potential to significantly improve the consistency and durability of skin substitutes.

Applicability of Research to Practice: Elimination of animal-derived collagen from tissue-engineered skin substitutes improves product safety by removing a potential source of adventitious agents. The use of skin substitutes more closely resembling normal skin may also reduce immunological responses to the tissue and allow grafts to remain on the patient for longer periods.

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