EDC Crosslinking Increases Cultured Skin Substitute Stability and Strength

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Introduction: The morbidity rate in critically burned patients with burns covering greater than 40% of their total body surface area is closely related to the limited availability of donor sites[1]. Bioengineering of skin generates greater amounts of grafts from a smaller donor site than conventional methods[2]. Collagen is commonly used as a scaffold material for skin regeneration due to its low immunogenicity and high biocompatibility. However, poor mechanical properties and rapid degradation rates can cause graft instability and difficult handling. Cross-linking collagen scaffolds with 1-ethyl-3-3-dimethylaminopropylcarbodiimide hydrochloride (EDC) has been shown to decrease degradation rates and increase the mechanical properties of collagen scaffolds [3]. Collagen scaffolds crosslinked with EDC have been investigated for use in dermal substitutes, but the effects of EDC cross-linking on full thickness skin substitutes have not been reported previously. In this study, the effect of EDC cross-linking on both the structural and physical properties of full thickness cultured skin substitutes (CSS) was investigated.

Materials and Methods: Freeze-dried collagen-GAG scaffolds were chemically crosslinked for 6 hrs with a solution of 50 mM MES hydrate in 40% ethanol-water (pH 5.5) with increasing concentrations (0, 1, 5, 10, or 50 mM) of EDC and N-hydroxysuccinimide (NHS) at a ratio of 1:1. Human fibroblasts and keratinocytes were inoculated on the crosslinked and control scaffolds at densities of 0.5 X 10^6/cm^2 and 1 X 10^6/cm^2 respectively, and cultured up to 21 days at 37ºC and 5% CO_2 with the media changed daily. Graft quality was assessed via area measurements, surface electrical capacitance (SEC), cell viability (MTT), and histolological evaluations at 7, 14 and 21 days with tensile testing performed prior to inoculation, and at culture day 14.

Results: Comparison of graft area after culture for one day reveals that crosslinking reduces graft contraction. Control grafts (0 mM) have a 35.9 ± 7.2% reduction in area, while crosslinking at 1 mM reduces area loss to 14.0 ± 5.1%. Histological results indicate that EDC crosslinking at 1and 5 mM produces no inhibition of cellular organization with fibroblasts penetrating into the collagen sponge forming a dense cellular layer upon

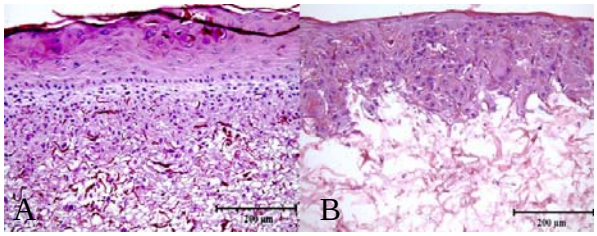

Figure 1. A) Control and B) 50 mM EDC crosslinked CSS at day 7. Control grafts have well organized and stratified epidermal and dermal layers. Fibroblast density in the 50 mM graft is low with a poorly formed epidermis.

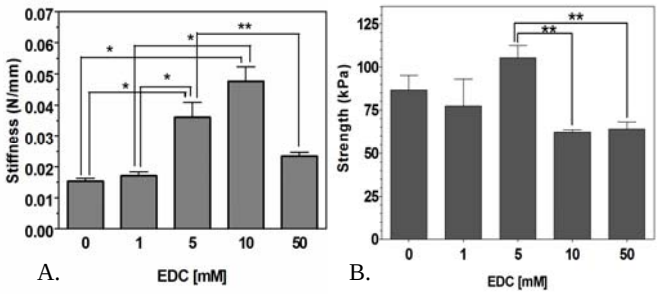

Figure 2. A) Stiffness of acellular grafts and B) ultimate tensile strength (UTS) of cell-polymer grafts versus EDC concentration. * = $p < 0.001$ ** = $p < 0.05$

which the keratinocytes have attached and produced a well stratified and keratinized epidermis with a continuous layer of basal keratinocytes at the dermal-epidermal junction (Fig 1A). At both 10 and 50 mM EDC, CSS are not well organized with no clear stratification (Fig 1B). This anatomical disorganization coincides with a failure to form to an epidermal barrier (a key functional component of skin) and prevents the surface of the graft from drying. SEC measurements indicate the surfaces of the 10 and 50 mM grafts are significantly wetter than the 0, 1 and 5 mM grafts at day 14 ($p<0.01$) and day 21. Cell viability is also reduced by higher concentrations (>5 mM) of EDC with MTT values of the 10 and 50 mM grafts less than half the absorbance of the 0, 1, and 5 mM grafts at day 14. Tensile testing of acellular grafts shows an increase in stiffness and a reduction in strain at break from 63.7 ± 12.9% elongation in control grafts to 31.3 ± 6.9% elongation in 50 mM grafts. While increasing EDC concentration up to 10 mM generates stiffer scaffolds (Fig 2A), a similar trend is not seen in cultured grafts. The lack of cell viability and poor cellular organization in the 10 and 50 mM grafts decreases the strength of the bioengineered skin compared to the 0, 1, and 5 mM grafts (Fig 2B).

Conclusions: The results above indicate that low concentrations (≤ 5 mM) of EDC cross-linking can be used to prevent premature degradation and contraction of the grafts. While increasing EDC concentrations >5 mM generates positive effects on mechanical properties of acellular scaffolds, the negative effects on cell viability and organization prevent the formation of a skin substitute that would be effective for wound healing. In this study, 5 mM EDC was an optimal crosslinking concentration to generate a beneficial combination of physical and biological properties in this model of tissue-engineered skin.

References

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