Available online at www.sciencedirect.com
ScienceDirect
Biomaterials 29 (2008) 834–843
Biomaterials
www.elsevier.com/locate/biomaterials

Influence of electrospun collagen on wound contraction of engineered skin substitutes

Heather M. Powell[a,*], Dorothy M. Supp[a,b], Steven T. Boyce[a,b]

[a]*Research Department, Shriners Burns Hospital, Cincinnati, OH, USA*
[b]*Department of Surgery, College of Medicine, University of Cincinnati, Cincinnati, OH, USA*

Received 20 July 2007; accepted 25 October 2007
Available online 3 December 2007

Abstract

The treatment of massive full-thickness burns with engineered skin substitutes has shown promise in clinical trials. The majority of skin substitutes are comprised of fibroblasts and/or keratinocytes on collagen scaffolds, commonly generated by freeze drying which can generate significant structural heterogeneity. Electrospinning may generate collagen scaffolds with greater homogeneity. Skin substitutes were fabricated using either freeze-dried (FD) or electrospun (ES) collagen scaffolds. Cell distribution, proliferation, organization, and maturation were assessed on each scaffold type *in vitro*, and engraftment and healing of full thickness wounds in athymic mice were tested. *In vitro* evaluation of freeze-dried collagen skin substitutes (FCSS) and electrospun collagen skin substitutes (ECSS) revealed no significant differences in cell proliferation, surface hydration, or cellular organization between the ECSS and FCSS groups. Both groups exhibited excellent stratification with a continuous layer of basal keratinocytes present at the dermal–epidermal junction. After grafting to full thickness wounds in athymic mice, both skin substitutes had high rates of engraftment: 87.5% in the FCSS group and 100% in the ECSS group. Histological evaluation of wounds revealed that bovine collagen persisted in the wound at week 8 in the FCSS group while no bovine collagen was seen in the ECSS group. At 8 weeks post-grafting, the ECSS grafts were $61.3 \pm 7.9\%$ original graft area whereas the FCSS grafts were $39.2 \pm 8.8\%$ original area ($p < 0.01$). These results indicate that ES scaffolds can be used to fabricate skin substitutes with optimal cellular organization and can potentially reduce wound contraction compared to FD scaffolds. These advantages may lead to reduced morbidity in patients treated with skin substitutes fabricated from ES collagen.

Keywords: Wound healing; Collagen; Scaffold; Wound closure

1. Introduction

In severely burned patients, the prompt closure of full thickness wounds is critical for survival. Split thickness skin grafts are commonly grafted to the wound to promote recovery [1,2]. Limited donor sites and the potential advantages of reduced numbers of surgical procedures and donor site surface area are the major impetus for development of bioengineered skin. Tissue engineering has been utilized to generate bioengineered substitutes for skin which produce greater expansion of surface area from donor skin than conventional methods [3]. In both clinical and preclinical models of skin substitutes, collagen is the most commonly used scaffolding material [4–7] due to its many advantageous properties, including low antigenicity and promotion of cell attachment and growth.

For wound management, freeze-dried (FD) collagen sponges are frequently placed onto wounds without cells [8,9], in conjunction with fibroblasts [4,10–12], or populated with keratinocytes and fibroblasts [13–15]. Previous studies have shown collagen sponges and cell-populated collagen sponges were able to promote wound healing [9,16–18]. To fabricate the collagen sponge, a suspension of collagen in acetic acid is solidified by freezing. The collagen

*Corresponding author. 3229 Burnet Avenue, Shriners Hospitals for Children, Cincinnati, OH 45229, USA. Tel.: +1 513 872 6076; fax: +1 513 872 6229.
E-mail address: hpowell@shrinenet.org (H.M. Powell).

0142-9612/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2007.10.036

is displaced by developing ice crystals, which form continuous networks of ice and collagen [19]. Subsequent sublimation of the ice crystals generates a highly porous sponge. The pore size and structure depend on the nucleation and growth rate of ice crystals during the freezing process. The formation of ice crystals is primarily controlled by the undercooling of the collagen suspension by an inverse relationship where higher nucleation rates and slower protein diffusion lead to smaller pores [20]. Rapid, quench freezing processes are commonly used in sponge fabrication and lead to variable heat transfer to the solution, generating sponges with a heterogeneous pore structure and large variation in pore diameter at different locations in the scaffold [20]. In addition, the collagen sponge is structurally different than natural extracellular matrix (ECM). Native ECM is fibrillar in structure with micron to submicron sized fibers, while collagen sponges are comprised of a reticulated network of partially denatured collagen with pore walls several microns thick. Thus, alternative methods of scaffold fabrication that are more homogeneous and biomimetic are needed.

To generate scaffolds with greater homogeneity and homology to natural ECM, electrospinning has been utilized to produce a fibrous matrix consisting of submicron to nanometric sized fibers. Electrospinning is an inexpensive process that has been used to fabricate nonwoven fibrous scaffolds out of a wide array of materials, including collagen [21–25]. In electrospinning, polymer solution is pumped through an aperture (i.e. syringe needle) that is electrically charged. A charge is induced on the liquid droplet at the tip of the needle by the electric potential between the needle and a grounded collection plate. When the electric field reaches a threshold, the repulsive electric force within the liquid overcomes the surface tension of the solution, causing a charged jet of solution to be ejected from the droplet of polymer solution [22]. The polymer jet is then accelerated toward the target, which is oppositely charged or grounded. This process generates nonwoven meshes composed of nanometric to micron-sized fibers. Fiber diameter and morphology of the electrospun (ES) scaffold are largely controlled by concentration and molecular weight of the polymer [26–29]. Many other factors, including flow rate, quality of the solvent, and surface tension result in variation of fiber diameter and morphology [22]. By altering these factors, a nonwoven mesh with a fibrous structure similar to native ECM can be generated with narrow tolerances.

This study evaluates the use of ES collagen scaffolds for the preparation and transplantation of human skin substitutes. Skin substitutes were fabricated utilizing either ES collagen or FD collagen scaffolds. Cell distribution, proliferation, organization, and maturation on each scaffold type were evaluated and the ability of the resultant skin substitutes to engraft and heal full thickness wounds athymic mice was assessed.

2. Materials and methods

2.1. Collagen scaffolds

FD and ES collagen scaffolds were prepared from comminuted bovine hide collagen (Kensey Nash; Exton, PA). For collagen sponges, fibrous bovine collagen powder (SEMED F; 0.60% wt./vol.) was homogenized in 0.5 M acetic acid, cast into sheets, frozen, and lyophilized as previously described [30] but without lamination. ES collagen scaffolds were fabricated using a 10% wt./vol. solution of acid-soluble collagen (SEMED S) in hexafluoropropanol (HFP; Sigma, St. Louis, MO). Matrices were spun at a potential of 30 kV onto an 8.5 cm^2 grounding plate that was positioned perpendicular to the tip of the needle. The ES and FD scaffolds were physically crosslinked by vacuum dehydration at 140 °C for 24 h [31], then chemically cross-linked in a solution of 5 mM 1-ethyl-3-3-dimethylaminopropylcarbodiimide hydrochloride (EDC; Sigma, St. Louis, MO) in 100% ethanol for 24 h. The scaffolds were then disinfected in 70% ethanol for 24 h and rinsed thoroughly following a procedure previously described [7] in preparation for cell inoculation.

2.2. Scanning electron microscopy

The morphology of the FD and ES scaffolds was examined by scanning electron microscopy (Hitachi S-3000). Punch biopsies from dry scaffolds were collected from six distinct regions within a scaffold and mounted onto aluminum stubs, sputter coated with gold-palladium and imaged in secondary electron mode with a 5 kV accelerating voltage. Images were collected; homogeneity of the scaffolds was assessed qualitatively and fiber diameter of ES scaffolds was assessed quantitatively.

2.3. Preparation of skin substitutes

Skin substitutes were prepared from FD and ES collagen scaffolds populated with human dermal fibroblasts (HF) and epidermal keratinocytes (HK) isolated from surgical discard tissue with Institutional Review Board approval. HF were inoculated onto ES or FD scaffolds, cut into 6.5 × 6.5 cm^2, at a density of 5.0×10^5 cells/cm^2 and incubated at 37 °C and 5% CO_2 in UCMC 160 medium [32] for 2 days. Scaffolds were then inoculated with HK at a density of 1.0×10^6 cells/cm^2. One day following HK inoculation (skin substitute incubation day 1), the cell-scaffold constructs were placed onto a perforated stainless steel platform covered by a cotton pad to establish an air–liquid interface and incubated up to 21 days with the skin substitute culture media replaced daily. All experiments were conducted utilizing a total of three different donor cell strains.

2.4. Fluorescein diacetate (FdA) staining

FdA has been previously used in a preclinical model to determine the viability and distribution of cells within cultured skin substitutes [33]. FdA penetrates the cell membrane and remains colorless until the acetate moieties in FdA are cleaved nonspecifically by esterases that convert the nonfluorescent FdA to fluorescein [34]. Thus, the viable cells can be visualized when the intracellular FdA is exposed to ultraviolet light (366 nm) [35,36] and intensity of fluorescence is proportional to the number of viable cells. To visualize the distribution of cells on the ES and FD scaffolds, skin substitutes fabricated using these two scaffolds were stained with FdA 1 day following HK inoculation. A freshly diluted solution of FdA (0.04 mg/ml) in phosphate buffered saline (PBS) was prepared, vacuum filtered, and warmed at the time of the assay. The skin substitutes were immersed in the FdA solution for 20 min, then samples were transferred to an empty 150 mm culture dish (Corning Inc.; Corning, NY) for photography. A UV lightbox (Fisher BioTech.; Pittsburgh, PA) illuminated the skin substitutes and the resultant fluorescence was captured on Polaroid black and white film type 667 (Sigma; St. Louis, MO). A photograph of each skin substitute was scanned and analyzed using Metamorph software program (Molecular Devices Corporation;