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Short communication

The biomechanical and histological sequelae of common skin banking methods

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ABSTRACT

Human skin allografts are used worldwide as an adjunct for the healing of burns when autograft skin is not available or not indicated. Allograft skin comes from human cadaveric donors, and so must be preserved until use. This study forms the first investigation to compare the mechanical and histological integrity of human split-thickness skin grafts preserved by either glycerolisation or cryopreservation (with or without the cryoprotectant DMSO). Stress relaxation was used to assess mechanical properties, whilst histological analysis allowed for evaluation of structural integrity.

Preservation of tissue, whether by freezing or glycerolisation, altered the relaxation behaviours of skin. Young's modulus upon initial loading significantly decreased for skin frozen without cryoprotectant, but remained unchanged for skin frozen with cryoprotectant and skin preserved with glycerol. After 1.5 h of stress relaxation, both fresh skin and skin frozen without DMSO displayed similar relaxation rates. Samples frozen with DMSO or preserved with glycerol had increased relaxation rate and had not reached load equilibrium within this time.

To understand the structural basis for the biomechanical changes, samples were histologically assessed. All preservation protocols resulted in a similar degree of visible damage, but cryopreservation appeared particularly damaging to the extracellular matrix, whereas glycerolisation caused dramatic separation of the epidermis from the underlying dermis.

The mechanical property alterations reveal that preservation results in laxity, which clinically could hinder contact dependent healing properties, but alternatively may increase capacity for coverage. The structural changes confirm that preservation techniques do not conserve grafts in an *in vivo* state.

1. Introduction

Cadaveric allograft skin is most commonly used as a 'biological bandage' in partial-thickness and full-thickness major burns (Vloemans et al., 2002). Skin grafts provide a physical barrier to the environment, promote re-epithelisation of the wound (Vloemans et al., 2003), and encourage neovascularisation (Burd et al., 2002). Moreoever, burns treated with allograft skin have been shown to heal with a lower incidence of hypertrophic scarring (Horch et al., 2005). As donor skin is a rare and valuable resource, an effective preservation system is necessary to gain maximal utility from each donation. Many countries now have, or are in the process of setting up, their own skin procurement and preservation infrastructure (Gore and De, 2010). The most common allograft skin preservation techniques are glycerolisation and cryopreservation.

Preservation through glycerolisation involves placing the tissue in increasing concentrations of glycerol solution in order to fix the free water in both the intra and extracellular spaces (Mackie, 1997). However, glycerolisation has been found to cause both mechanical and structural changes to various connective tissues. For example, increased stiffness of human sclera with no visible change to the connective tissue matrix (Schirmbeck and Cruz, 2007), reduced stiffness of sheep aortic valve with a grossly deformed cellular structure (Aidulis et al., 2002) and no mechanical changes in rat aorta despite complete endothelial destruction and tunica cell death with extracellular matrix sparing (Fahner et al., 2004) have all been reported. Although no previous studies have identified glycerolisation-induced alterations of human skin allografts, the effects of this treatment on other connective tissues suggest that skin may also be affected.

Successful cryopreservation protocols used by many skin banks employ a combination of penetrative cryoprotectants, such as dimethylsulphoxide (DMSO), and staged cooling or slow

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programmable freezing to minimise cellular damage during freezing (Castagnoli et al., 2003). However, extracellular ice formation is a certainty with all protocols; this causes damage to the tissue's extracellular matrix (ECM), creating the potential for altered mechanical properties (Fuller, 2004). The mechanical consequences of cryopreservation on skin allografts have not yet been investigated; however, goat aortic valves cryopreserved in DMSO demonstrated no significant difference from fresh samples for elastic modulus, extensibility or stress-relaxation behaviour (Aidulis et al., 2002), whereas aortic valves snap frozen without DMSO (and therefore subject to severe intracellular and extracellular ice nucleation), were fractured. Similarly, human tendons cryopreserved without a cryoprotectant, showed a lower tissue yield point (Farrant, 1965).

A good biological bandage should be as skin-like as possible, both in structure and function (Sheridan, 2011). The aim of this study was to elucidate the biomechanical and histological outcomes of glycerolisation and cryopreservation on human split-thickness skin allografts.

2. Methods

2.1. Skin procurement and sample preparation

Surplus skin was obtained from consenting patients undergoing plastic surgery. This work was ethically approved by the National Research Ethics Service (UK) and was institutionally sponsored. Immediately following removal, skin was stored in RPMI medium (Sigma-Aldrich, UK) at 4 °C for a maximum of 48 h before testing or preservation.

Seven patients with a total of nine operations contributed skin to this study (2 axilla, 3 abdomen, 3 breast, 1 thigh). Samples were prepared by manually dissecting off the adipose tissue and a thin layer of deep dermis. The resulting sheet of split-thickness skin was then divided into multiple samples (1 cm × 6 cm), which were distributed amongst the treatment groups. Eighty total samples were analysed (19–21/treatment group for biomechanics; 4–5/treatment group for histology). Fresh samples were tested immediately, whilst treatment groups were tested after 2 weeks of preservation.

2.2. Glycerolisation

Based on the accepted glycerolisation procedure (De Backere, 1994), individual samples were placed in a 5 ml solution of glycerol and RPMI, and continuously agitated at 33 °C in a water bath. Samples were incubated in 50%, 70%, and finally 85% glycerol solution for 2 h each, then moved to conical polypropylene tubes and stored at 4 °C for 2 weeks.

During deglycerolisation, samples were lightly dabbed with paper towel to remove excess glycerol, then placed in 5 ml of pre-warmed phosphate-buffered saline (PBS) at 33 °C and continuously agitated for 10 min before the first PBS change. Subsequent PBS changes occurred every 10 min for a total of 90 min.

2.3. Freezing and thawing treatment

Tissue was cryopreserved in RPMI ± 10% DMSO using an accepted staged freeze-thaw protocol (Castagnoli et al., 2003). Prior to freezing, tissue was incubated in 5 ml of preservation medium for 20 min at 4 °C whilst continuously agitated. Samples were then placed in double-layered heat sealed 8 × 6 cm^2 plastic packets with 10 ml preservation medium and placed at −20 °C for 60 min. They were then moved to −80 °C for 24 h, and finally placed in the liquid phase of liquid nitrogen for 2 weeks.

To thaw, samples were removed from liquid nitrogen and immediately placed in a 37 °C water bath and agitated for 4 min, then placed in 5 ml of 20% sucrose in RPMI and continuously agitated for 20 min at room temperature. This process was repeated with an additional 20% sucrose wash, followed by two washes in RPMI, for a total wash time of 80 min.

2.4. Biomechanical testing

After removal of a 1 × 1 cm^2 sample for histology, the thickness of the 1 × 5 cm^2 samples was measured using digital Vernier callipers (Table 1) to ensure consistency between groups.

Skin samples were tested in uniaxial tension using a Mach-1 materials testing machine (Biomomentum, Canada). Each sample was randomly oriented and immobilised between two clamps, one affixed to a 10 kg load cell, and the other to an immovable base plate. The resulting area between the clamps tested in uniaxial tension was 1 cm by 4 cm. Each sample was loaded to 3000 g at 1 mm/s. After the 3000 g load was reached, the tissue was allowed to relax for 1.5 h (a time-point sufficient for control skin to reach stress equilibrium). Young's modulus and stress relaxation property calculations for fresh skin are shown in Fig. 1. The mechanical properties investigated represent the average properties of the split-thickness skin constituents (epidermis and dermis). During experimentation petroleum jelly was applied to the skin to prevent desiccation.

Table 1
Sample thickness measured immediately prior to biomechanical testing.

	Fresh	Glycerol	Frozen w/o cryoprotectant	Frozen in 10% DMSO
Number of samples	20	19	20	21
Mean thickness and standard deviation (mm)	0.92 ± 0.29	0.87 ± 0.44	0.91 ± 0.38	0.88 ± 0.35
Range (mm)	0.72–1.50	0.53–1.50	0.60–1.54	0.52–1.6

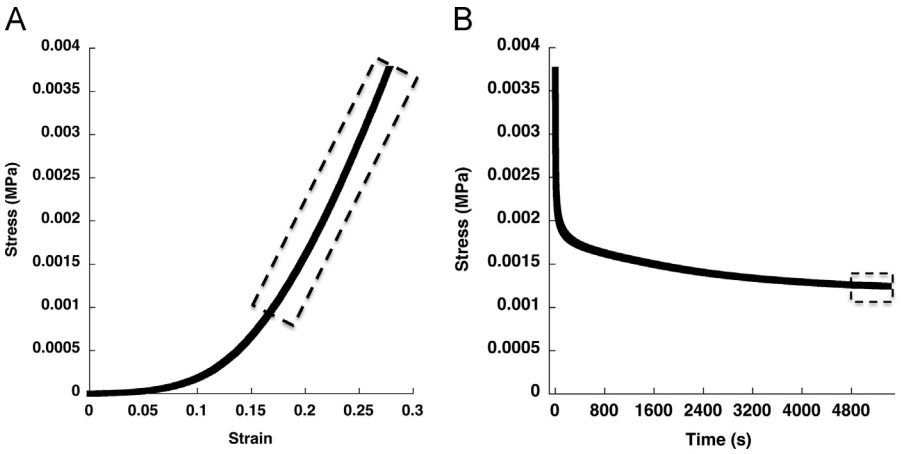

Fig. 1. Loading data of a representative untreated skin sample. Analysis of initial load resistance data allowed the investigation of the tissue's Young's modulus (A). Measuring the rate of stress relaxation over the last 200 s allowed the investigation of a final rate of relaxation (B), and measuring the stress level at the end of the 1.5 h relaxation period allowed the investigation of the final absolute relaxation (last data point on B).