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Histological and mechanical evaluation of antifreeze peptide (Afp1m) cryopreserved skin grafts post transplantation in a rat model

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ABSTRACT

The objective of this study was to evaluate the use of Afp1m as a cryopreservative agent for skin by examining the transplanted skin histological architecture and mechanical properties following subzero cryopreservation. Thirty four (34) rats with an average weight of 208 ± 31 g (mean ± SD), were used. Twenty four (n = 24) rats were equally divided into four groups: (i) immediate non-cryopreserved skin autografts (onto same site), (ii) immediate non-cryopreserved skin autografts (onto different sites), (iii) skin autografts cryopreserved with glycerol for 72 h and (iv) skin autografts cryopreserved with Afp1m for 72 h at −4 °C. Rounded shaped full-thickness 1.5–2.5 cm in diameter skin was excised from backs of rats for the autograft transplantation. Non-cryopreserved or cryopreserved auto skin graft were positioned onto the wound defects and stitched. Non-transplanted cryopreserved and non-cryopreserved skin strips from other ten rats (n = 10) were allowed for comparative biomechanical test. All skin grafts were subjected to histological and mechanical examinations at the end of day 21. Histological results revealed that tissue architecture especially the epidermal integrity and dermal-epidermal junction of the Afp1m cryopreserved skin grafts exhibited better histological appearance, good preservation of tissue architecture and structural integrity than glycerolized skin. However, there was no significant difference among these groups in other histological criteria. There were no significant differences among the 4 groups in skin graft mechanical properties namely maximum load. In conclusion, Afp1m were found to be able to preserve the microstructure as well as the viability and function of the skin destined for skin transplantation when was kept at −4 °C for 72 h.

1. Introduction

Skin one of the largest organs in the body performing a principle role as a barrier against the aggressive surrounding environment. As well as, it avoids the entry of foreign chemicals and micro-organisms, excessive water loss from the aqueous interior, and also offers both stiffness and strength to overcome various mechanical loading. Further duties include sensation, temperature control and insulation. Achieving all these functions requires both stability and flexibility. Yet, the macroscopical and biomechanical properties of the skin can be vulnerable through different factors such as medical or cosmetic treatment, trauma, and diseases [43,55].

Fresh skin grafts including cadaver allograft that are used for many surgical purposes is still to be considered as the 'golden standard' [6]. Unfortunately, the use of fresh skin grafts is severely obstructed by their limited availability [5].

Short preservation of skin grafts for delayed application is still considered as a basic approach in reconstructive and plastic surgery and even in burn. The most common phenomena facing preserved skin grafts even with the presence of a rich oxygenated nutrient medium, is the ischemic necrosis that happens and continue to due to the difference in the diffusion speed between the tissue periphery and the central cells. As a result of this, the toxic metabolites cannot be removed quickly enough and the nutrients and oxygen cannot diffuse fast enough to supply the cells. Therefore, the more effective way for keeping the viability during storage of skin grafts is by reducing the tissue

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Table 1
Microscopic criteria for assessment skin graft performance.

Microscopic parameters	Scores		
	0	1	2
Epidermal integrity	Destroyed	Partial	Normal
Epidermal-dermal junction	Discontinued	Partial	Normal
Collagen organization	Amorphic	Disturbed	Normal
Fibroblast presence	< 10	10–20	> 20
Graft adherence (%)	< 25	25–75	> 75
Leukocytes infiltration	Intense	Similar to control	No leukocytes

temperature thus reducing the various nutritional needs, the cellular metabolic rate and metabolite production [24].

Temporary storing tissue at freezing or refrigerated temperatures is a very common short time technique that are used for preserving tissues before using them in a clinical or experimental setting. This kind of storage could have some effects on the mechanical properties of the stored tissues [15].

It is generally accepted that a primary mode of injury experienced by the tissues that are subjected to subzero temperatures is associated with the transformation of intracellular water from the liquid to the solid crystalline state, *i.e.* intracellular ice formation [34]. All approaches for cryopreservation aim to overcome the biological, chemical, mechanical and thermal stresses of ice crystal formation and recrystallization associated with subzero cryopreservation which affect deeply on the viability and efficiency of tissues used for transplantation and grafting.

To keep good tissue viability, tissue banks around the world have their own techniques for storage and preservation of skin grafts, but the most useful procedures that are commonly in use include: refrigeration at 4 °C, glycerolpreservation with high concentration of glycerol, and cryopreservation at subzero temperatures [4,22,23].

Antifreeze proteins (AFPs) have the ability to protect many organisms from freezing in subzero temperature environments. Since their discovery 45 years ago [56], there has been a growing interest in their various cryoprotective ability as a result of their well-known to sustain the super-cooled state of body fluids through preventing growth of ice. Secondly, they possess capability of inhibiting recrystallization, and thirdly, antifreeze proteins can assist as plasma membrane protections when temperature is low [11,26,54]. Whereas, Anti-freeze peptides are kind of antifreeze molecules that are usually derived from native AFPs and used in various scientific applications. They consist of small number of amino acids not less than 25 comparable to other types of AFPs that are frequently contain increased numbers of amino acids. This kind of antifreeze agents can act as useful antifreeze tool to inhibit ice crystals formation, decrease recrystallization, and reduce thermal hysteresis (TH) value in various scientific applications. The benefits of these peptides over large proteins are their simpler structure, the relatively easy synthesis procedure, reduction of the TH value, enhancement and sustain cold tolerance for a short period of time [38,49].

Afp1m is peptide fragments derived from the parent AFP1 of Antarctic yeast known as Glaciozyma Antarctica (G. Antarctica). This yeast has eight different genes that express various types of AFPs; only one AFP gene has been completely characterized (UniProtKB accession code D0EKL2). The predicted secondary structure of G. Antarctica AFP consisting of four α-helices and three β-strands. The α-helical region of the native AFP has been suggested to be responsible for the inhibition of

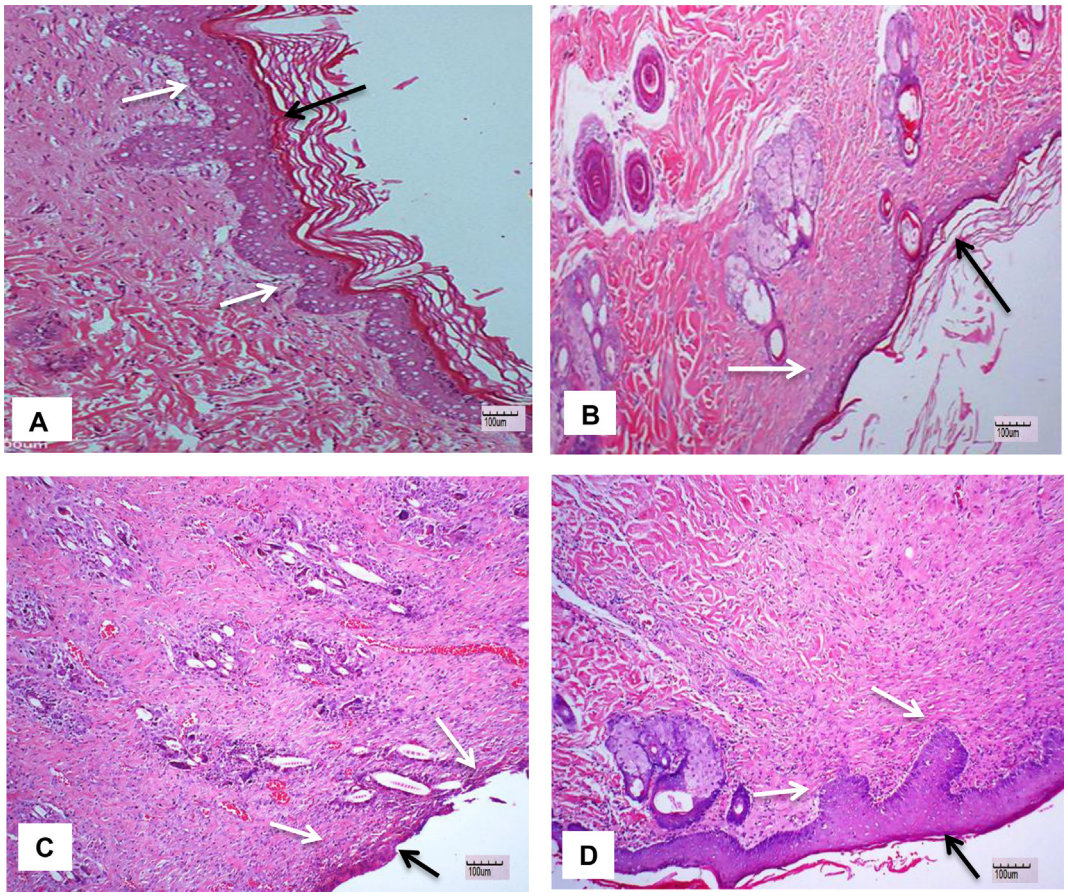

Fig. 1. Photomicrographs showing the skin architecture particularly the epidermal integrity (black arrow) and dermal-epidermal junction (white arrow) of skin graft in group 1, fresh skin autograft from the same site (A); group 2, fresh skin autograft from different site (B); group 3, skin autograft cryopreserved with glycerol for 72 h (C); group 4, skin autograft cryopreserved with Afp1m for 72 h (D) (H&E).