Clinical and Histological Evaluation of The Healing of Meshed Skin Grafts of Different Meshing Sizes Covered by Fresh Amniotic Membrane Dressings

Protocol of thesis submitted in partial fulfillment of MD degree in plastic, burn and maxillofacial surgery

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AM	Amniotic membrane
H&E	Hematoxylin and eosin
STSG	Split thickness skin graft
FTSG	Full thickness skin graft
SSEA-3 and -4	Stage specific embryonic antigen 3 and 4
Oct-4	Octamer-binding transcription factor 4
HIV	Human immuno-deficiency virus
KGy	Kilogray
HLA	Human leukocyte antigen
α-SMA	Alpha smooth muscle actin
EGF	Epidermal growth factor
Bfgf	Basic fibroblast growth factor
KGF	keratinocyte growth factor
TFG	Transforming growth factor
NGF	Nerve growth factor
PDEF	Pigment derived epithelium factor
TNF	Tumor necrosis factor
IL	Interleukin
HGF	Hepatocyte growth factor
MEM	Minimum Essential Medium
DMEM	Dulbecco's Modified Eagle's medium
DMSO	Dimethyl sulphoxide
PBS	Phosphate buffered saline
RPMI	Roswell Park Memorial Institute
EDTA	Ethylene diamine tetraacetic acid

PDGF	Platelet-derived growth
VEGF	Vascular endothelial growth factor
EGFR	Epidermal growth factor receptor
POSAS	Patient and observer scar assessment scale
OSAS	Observer scar assessment scale
PSAS	Patient scar assessment scale
TBSA	Total body surface area
E&G.	Excision and grafting
REC	Research ethics committee

Introduction

Skin grafts can be used either as sheet grafts or as fenestrated grafts by passing them through a mesher, a mechanical meshing device. Sheet grafts are traditionally seen as milestone for resurfacing post burn raw areas of up to 20% total burnt surface area (TBSA) as they avoid the potentially poor cosmoses of the fenestrated graft. **(Archer et al, 1998 and Nikkhah et al, 2014)**

Meshed split thickness skin autograft, especially when needed to be widely expanded, don't obtain immediate biological coverage. In cases of patients with burned large total body surface areas, this leaves patients at risk of exposure to multiple metabolic problems and life threatening infections. In a trial to control these risks in the open skin autografts interstices, a sandwich technique with an allograft overlay has been introduced. **(Alexander et al, 1981) (Smith et al, 2000)**

Meshed skin grafts interstices (gaps) heal by the process of epithelization. This consists of 3 cellular functions; keratinocyte proliferation, migration and differentiation. **(Robnson., 1996)**

Each gap on the meshed graft could be considered an individual wound and has to heal by secondary intention. Therefore, the wider is the mesh the more it is liable to heal by secondary intention and scarring. **(Nikkhah et al, 2015)**

The interstices of the widely expanded meshed skin grafts suffers from infection during the healing process and the formation and persistence of granulation tissue results in delayed healing, and consequent severe wound contraction***(Chu et al ,2000)***

Human amniotic membrane (HAM) consists the inner layer of the fetal membranes (the outer layer being formed by the chorion) and has been reported as a valuable biomaterial in reconstructive surgery and wound-healing researches since its initial description as a transplantable material by J Davis in 1910 **(Parolini et al., 2009)**

Amniotic membrane (AM) with its natural tissue planes, anti-inflammatory and antibacterial properties make it a natural choice for potential wound management, which was confirmed with the application of natural membrane in various clinical situations. **(John, 2003 and Sheikh et al, 2014)**

The most important features of fresh amniotic membrane(AM) can be divided to 4 categories: rapid adherence to the wound bed, increased angiogenesis, inhibition of protease activity and PMN infiltration and rapid re-epithelialization and promotion of wound healing **(Mohammadi .,2011)**

AM, in burn management, has the efficiency of decreasing the bacterial counts in wound bed. It also has ability to decrease loss of protein, electrolytes and fluids, reducing pain, accelerating wound healing and subsequently reducing scar tissue formation. **(Niknejad et al, 2008, Halim et al, 2010 and Sheikh et al, 2014)**

In developing countries that consume a lot of medical resources, it is important to find a suitable material for the dressing of burns which improves healing and is readily available, easily applicable and cost-effective. It should also be protective against infection and desiccation. These purposes were served by amniotic membranes. **(Mohammadi et al, 2015)**

The disadvantage of the use of amniotic membrane is that there is some risk of viral infection transmission **(Anatassov et al., 1994)**. This has been overcome by collection of placentae from screened donors.

No previous in vivo studies have determined the rate of epithelization and healing in meshed grafts covered by fresh amniotic membranes, neither examined the healed gaps of the meshed grafts histologically.

Aim of the work

The aim of this study is to determine the rate of in vivo healing of the meshed skin autograft with different meshing sizes applied to post burn raw areas when covered by fresh amniotic membranes. Histological examination will determine the features of the tissues that filled the gaps (interstices) of meshed skin autografts.