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Allogeneic mesenchymal stem cells, but not culture modified monocytes, improve burn wound healing

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ABSTRACT

The use of cell therapy to improve burn wound healing is limited as a validated cell source is not rapidly available after injury. Progenitor cells have shown potential to drive the intrinsic wound regeneration.

Two sources of cells, allogeneic mesenchymal stem cells (MSC) and autologous culture modified monocytes (CMM), were assessed for their ability to influence burn wound healing. Both could be widely available shortly after injury. Cells were delivered in a fibrin matrix following contact burns in a porcine burns model.

Application of MSC significantly decreased the area of unhealed burn compared to CMM or delivery matrix alone (6% MSC, 27% CMM, 24% Matrix, $p < 0.001$). MSC treated wounds showed histological evidence of improved wound healing with increased collagen content (MSC 49%, CMM 42%, $p < 0.01$), increased epidermal area (MSC 8.8%, CMM 6.1%, $p < 0.01$) and dermal thickness (MSC 1108 μm, CMM 1007 μm, $p < 0.01$) compared to CMM treated wounds. Labelled MSC and CMM were identified in the wounds after 2 weeks by immunohistochemistry and FACS.

A single application of allogeneic MSC improves the rate of burn wound healing and improves the histological appearance of the burn wound. These cells show potential as a cell therapy that is rapidly available following burn.

1. Introduction

Rapid burn wound closure is well correlated with survival [1], improved aesthetic appearance, a decreased chance of adverse or hypertrophic scarring [2] and a decreased risk of infection [3]. The gold standard treatment for deep burns remains autologous skin grafting despite many years of research into alternatives. Current research focusing on cell based therapies using progenitor cells is a new therapeutic paradigm aimed at improving wound healing biologically, improving time to healing and reducing scarring [4–6]. The concept of using cell based therapies for the treatment of burns has evolved over the last 30 years since the initial cultivation of human dermal keratinocytes [7]. Constructing tissue from stem cells has the potential advantage that the component parts can be simplified at the initial stage and hence a greater proportion of the regenerative process can be

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driven by the intrinsic bioengineering capacity of the cells [5].

One of the major limitations in exploiting cell therapy in burns is identifying the right pool of progenitor cells and the right delivery system for these cells. The specific problem in the treatment of burns is finding a source of cells that is rapidly available after burn; any strategy employing autologous cells needs to circumvent the time delay involved in expanding sufficient cell numbers in culture. The alternatives are to use allogeneic cells or identify a source of rapidly available autologous cells. Allogeneic cells can be pre expanded and characterised to be ready for use in the initial stages of burn treatment [8].

Pluripotent cells are mobilised into the peripheral blood after burn [9,10] contributing to burn wound healing [11]. Bone marrow derived stem cells have been successfully used to treat burns experimentally [6,12] and in human subjects [13]. Mesenchymal stem cells (MSC) derived from the bone marrow act through a combination of paracrine cell signalling and cell trans-differentiation, enhancing wound regeneration and improving angiogenesis [14]. The potential use of autologous MSC as a therapy for burns is hampered by the delay of several weeks required for cell isolation and expansion in culture before sufficient numbers are present for use therapeutically. Bone marrow depletion after burn has also been reported and may further limit the number of cells that can be isolated [5,15]. The efficacy of an allogeneic source of MSC in burns wound healing has yet to be properly evaluated.

Another potential source of cells is monocytes. The mononuclear phagocytic system consisting of circulating monocytes as well as their macrophage progeny is central to wound repair and is an attractive area for investigation of an autologous source of cells that could be rapidly available for use as a cell therapy within a few days of injury. The mononuclear fraction of peripheral blood is a source of circulating stem cells [16,17], CD34+ mononuclear progenitor cells are mobilised into the circulation after burns, both in pigs [9] and human subjects [10]. CD34+ cells isolated from the Mononuclear Cell (MNC) MNC population accelerated the neovascularisation and epidermal healing in a model of chronic full-thickness skin wounds in diabetic mice [18]. CD14+ mononuclear cells also contain a mixture of other precursors that can differentiate into non haematopoietic cells, including epithelial cells, smooth muscle cells and endothelial cells, under certain permissive conditions. These cells appear to act predominantly by paracrine secretion and although there is some evidence of differentiation [16,17,19]. Culture modification of mononuclear cells for a few days induces a more progenitor cell like profile and behaviour [20]. The effect on burn wound healing of culture modified monocytes (CMM) is currently unknown. However they represent a potentially large, rapidly available pool of autologous cells (as these cells highly express MHCII they are not suitable for allogeneic delivery) that would be available shortly after injury.

Cells need to be delivered in a scaffold that can facilitate growth, attachment and differentiation. Such a scaffold leads to better incorporation, organisation and tissue formation. Fibrin based hydrogels have been used to improve burn healing by giving both a source of cells and a temporary extracellular matrix [21,22], improving cell survival and differentiation [23] as well as angiogenic factor secretion [24]. Fibrin based hydrogels in current clinical use and various other applications are currently commercially available.

The objective of this research was to determine the optimal cell type to induce rapid wound healing in a burn wound model. Both MSC and CMM have shown some promise in mouse models of wound healing and this study was conducted to evaluate which strategy was more efficacious in a porcine model of burn wound healing, a more representative model of wound healing.

We hypothesised that burn wound healing could be positively influenced by either autologous CMM or by allogeneic MSC. We aimed to evaluate the effect of each cell type on the rate of healing compared to the delivery vehicle alone or no treatment.

Primary outcome measures were time to wound healing. Secondary outcome measurements included further morphometric analysis of the healing wound to determine contraction and epithelialisation, as well as histological analysis of the healing wound for collagen content, dermal and epidermal thickness.

2. Methods

2.1. *Induction of burn and application of cells*

The study was approved by Animal Experimental Ethics Committee, University College Cork, Ireland and performed under a licence from The Department of Health and Children, Ireland.

Three Female Landrace pigs weighing between 25 and 30 kg were used in this study. They were housed individually and received standard porcine diet once daily and water ad libertum. Anaesthesia was induced with Xylazine (2.0 mg/kg)/ketamine (15 mg/kg), and maintained with 2% Isofluorane. Contact burns were induced by application of a pre-heated (to 80 °C) brass block for 30 s, inducing a deep partial thickness burn. 12 burns (6 on each side) were induced in each pig at a spacing of 5 cm between each burn, each with an area of 4.5 cm^2, affecting in total approximately 5% total body surface area. Wounds underwent superficial debridement of the burn eschar to reveal a wound bed with fresh punctate bleeding, before immediate application of cells. Each pig had 3 replicates of each of the 4 experimental groups.

The Four experimental groups compared in this study were; allogeneic Mesenchymal Stem Cells (MSC), autologous Culture Modified Monocytes (CMM), the fibrin delivery matrix only (Fibrin) and a dressing only group (Dressing) to control for any influence of the fibrin matrix on wound healing. Cells were delivered in a fibrin matrix at a concentration of $1 \times 10^6/cm^2$ 4.5×10^6 cells in total per wound (6.25 mg/ml fibrin, 12.5 μ/ml thrombin 1 ml total (Tisseel™, Baxter Healthcare, Ireland) [23]. Cells were secured in situ with an occlusive adhesive dressing (Tegaderm™, 3 M Healthcare), followed by a layered non slip dressing composed of gauze and melonin, secured with an adhesive tape, double tubigrip and further tape. Images were taken of the wound and evaluated for wound contraction and re-epithelialisation.