Comparison of Histological Changes of Recipient Wound Bed by Platelet Rich Plasma and Adipose Derived Stem Cell in Dogs

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Abstract: The aim of wound healing is to promote rapid wound closure and prevent excess scar formation. Open wound management, until the wound is considered suitable for reconstruction or until it has healed by second intention, has been the treatment of choice for centuries. Frequent and painful dressing changes over prolonged periods intensifies overall case management, and may impact treatment costs. Wound healing stimulated and optimum microenvironment for successful reconstruction that can be created by employing modern methods such as Platelet Rich Plasma and Stem Cell therapy. When skin was damaged, not only epithelial cells were destroyed, but also a large quantity of collagen was also lost. Currently, there is a growing interest in the use of autologous platelet concentrates (PC), also known as platelet rich plasma (PRP) for stimulating the healing process and promoting regeneration through growth factors instead of reparation (Mazzucco et al., 2008). Stem cells and progenitor cells offer a potential answer to this dilemma (Kim et al., 2012). Adipose derived stem cells (ADSCs) are an attractive and abundant stem cell source with therapeutic applicability in diverse fields for the repair and regeneration of acute and chronically damaged tissues. Several wound characteristics were considered that determined the level of histopathological changes e.g. depth and length of healed wound, epithelial stratification, incorporation of the dermal substitute, degree of neutrophil, macrophage, fibroblast, foreign body giant (FBG) cell infiltration and extent of elastin formation. Wounded tissue stained with Masson’s trichrome showed a clear view of collagen fibres deposition and reorganisation compared to Hematoxylin and Eosin staining. The present study was undertaken to compare and evaluate the wound healing between Platelet Rich Plasma and Adipose Derived Stem Cells.

Keywords: Wound healing- Recipient wound bed –Platelet Rich Plasma – adipose derived stem cell-Mason’s trichrome staining-hisopathological evaluation.

1. Introduction

Full-thickness skin loss occurred commonly in dogs. These wounds were often managed with dressings and bandages until healing occurred by granulation tissue formation, contraction, and epithelisation (second intention healing). The duration of open wound management contribute significantly to therapeutic challenges, patient morbidity, and increased costs of treatment. Adequate blood supply and improved oxygen tension in the wound was essential for optimal fibroplasia and collagen production.

There is a growing interest in usage of autologous platelet concentrates (PC), also known as platelet rich plasma (PRP) that stimulate the healing process and promote regeneration instead of reparation. The author also added that platelet alpha granules contained at least seven growth factors that were directly involved in the healing process. Of those proteins, transforming growth factor beta isoform 1 (TGF-β1) was of pivotal importance for its actions on cell proliferation, angiogenesis and extra cellular matrix deposition.

The field of tissue engineering proposes to repair and regenerate damaged organs using a combination of cells, biomaterials, and cytokines. The availability of cells capable of differentiation along multiple lineage pathways has limited the progress and development of these modalities. Stem cells and progenitor cells offer a potential answer to this dilemma (Kim et al., 2012).

Adipose derived stem cells (ADSCs) are an attractive and abundant stem cell source with therapeutic applicability in diverse fields for the repair and regeneration of acute and chronically damaged tissues. ADSCs display properties similar to that observed in BMSCs and, upon induction, undergo osteogenic, chondrogenic, adipogenic and neurogenic differentiation in - vitro (Kilroy et al., 2007).

Wounded tissue stained with Masson’s trichrome showed a clear view of collagen fibres deposition and reorganisation. The Abramov’s histological scoring system (modified Greenhalgh’s scoring system) was used for scoring epithelisation, fibrosis, angiogenesis, and collagen level; the number of macrophages under this system was modified. (Abramov et al., 2007).

2. Materials and Methods

The platelet rich plasma was prepared by the Double centrifugation tube method as per the standard protocol of Perazzi et al., (2013). About 8 ml of whole blood was collectedatraumatically through a single cephalic vein puncture. The blood was centrifuged for 20 minutes at 2,800 rpm to achieve separation of cell layers. The final solution, obtained by mixing different buffy coats in a sterile 15 ml Falcon tube, was centrifuged without applying a brakeat1300rpmfor 15 minutes for good separation of platelet pellets in the supernatant layer (Plate 12). The platelet poor plasma was drawn off so that the platelet rich plasma remained in the tube after resuspending the platelet pellet within the remaining volume of plasma in an
A 3.5 mm punch biopsy instrument was used to take skin specimens from the recipient wound of each animal on 3rd, 7th, and 14th day, with the dogs under sedation. Punch biopsy was used in dogs as a method for skin healing investigation (Abramov et al., 2009). The canines adipose cells, thus obtained not exceeding the second passage was used for topical administration with appropriate substrate on the recipient wound bed.

The cells were subjected to trypsinization when they reached a confluence of 70%, using 0.025% trypsin and then plated at a density of 5000 cells per cm². Later, the cultures were passaged repeatedly after achieving a density of 70 to 80% (Kim et al., 2013).

(Plate 26). The Endotoxin content was performed by the Limulus amebocytelysate (LAL) test and the adequacy was 2.5 EU/ml (Wagers and Wisseman, 2004). The canine adipose tissue was then enzymatically digested using collagenase and then subjected to dissociation with 0.075% collagenase for a period of 30 to 45 minutes. The enzyme activity was neutralized by washing the cells with DMEM-HG (Dulbecco's modified Eagle Medium – High Glucose) containing 10% FBS (Fetal bovine serum). The resultant content was centrifuged at 1200g for 5 to 10 minutes to pellet the cells. (Plate 23, 24) The cells were seeded in tissue culture plates at a density of 3000 to 3500 cells per cm² in DMEM-HG with 10% FBS containing antibiotics.

The dermal region on 7th day showed thin incomplete, immature amount of collagen fibres. The inflammatory cells were few and mainly consisted of lymphocytes, plasma cells and macrophages. Moderate neovascularisation with immature collagen were observed in animals treated with adipose derived stem cells when compared to platelet rich plasma.

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In the present study on 14th day, the scar surface got reduced due to wound contraction and hyperplasia of the epidermis rapidly in the dermis. The mean ± S.E. values for collagen proliferation, epithelisation and angiogenesis was 2.20 ± 0.63, 2.30 ± 0.51, 2.35 ± 0.71 and 1.72 ± 0.80, 2.40 ± 0.63, 2.97 ± 0.03 and 2.67 ± 0.71, 1.88 ± 0.59 and 1.50 ± 0.22 respectively on 3rd, 7th and 14th day for animals treated with platelet rich plasma and the mean ± S.E. values for collagen proliferation, epithelisation and angiogenesis was 2.47 ± 0.63, 2.60 ± 0.72, 2.40 ± 0.62 and 1.80 ± 0.78, 2.47 ± 0.62, 2.99 ± 0.00 and 2.50 ± 0.91, 2.23 ± 0.63 and 1.57 ± 0.12 respectively on 3rd, 7th and 14th day for animals treated with platelet rich plasma. The mean ± S.E. values for collagen proliferation, epithelisation and angiogenesis was 2.28 ± 0.83, 2.38 ± 0.72, 2.40 ± 0.62 and 1.80 ± 0.78, 2.47 ± 0.62, 2.99 ± 0.00 and 2.50 ± 0.91, 2.23 ± 0.63 and 1.57 ± 0.12 respectively on 3rd, 7th and 14th day for animals treated with adipose derived stem cell therapy.

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4. Discussion

Platelet growth factors of the platelet rich plasma are peptides that promoted cell proliferation, differentiation, chemotaxis, and induced the migrations of various cells preferably macrophages and myofibroblast. Raul et al. (2007) showed that a single application of PRP used in incisional wounds amplified the inflammatory response with an increased wound influx of neutrophils and macrophages as observed in the present study also.

Damage of the skin inevitably induces local inflammation and recruits an inflammatory cell which acts as a source of cytokines that affect the wound healing process. Previous studies by Beldon (2010) demonstrated that adipose derived stem cells download pro-inflammatory cytokines and studies by Beldon (2010) demonstrated that adipose derived cytokines that affect the wound healing process. Previous and recruits an inflammatory cell which acts as a source of cytokines that affect the wound healing process. Previous and recruits an inflammatory cell which acts as a source of cytokines that affect the wound healing process.

On 7th day granulation tissue with fibrin crusts, fibroblast and neovascularisation was observed in all the cases. However increased amount of immature collagen fibres were observed in Masson trichrome staining in platelet rich plasma group followed by ADSC group as per Abramov’s histological scoring system (modified Greenhalgh’s scoring system). Dermis cellularity increased mainly due to fibroblasts proliferation and new matrix deposition as opined by Hallet al. (2010) also.

Platelet rich plasma and adipose derived stem cells stimulated the synthesis and maturation of collagen, thus resulting in an increased tensile strength of the healed skin. Tensile strength has commonly been associated with the organization, content, and physical properties of the collagen fibril network and was one of the necessary parameters for determining the pharmacological effects of potential wound healing agents as opined by Pol et al. (2000). Angiogenesis and Fibroplasia was higher in platelet rich plasma cell treated animals when compared with other groupon the recipient wound bed which indicated platelet rich plasma and adipose derived stem cells accelerated the inflammatory reaction and initiated the healing process in the early phases of healing of recipient wound bed more rapidly.

Neovascularisation was progressively higher in PRP treated animals in comparison with the ADSC treated animals on the recipient wound bed. During the treatment with PRP all wounds reacted with a clear increase in redness due to augmented blood supply. Therefore it was proposed that platelet rich plasma on skin lesion enhanced capillary growth increased overall blood supply to the injured area, a feature regarded as a milestone for successful wound repair (Sartim et al., 1998).

The platelet concentration in most of the cases were relatively four times the baseline intravascular platelet value which was considered as the minimal concentration for accelerating epithelisation, angiogenesis, growth, vascular fibroblast proliferation and extracellular collagen matrix synthesis and deposition as also observed by Arguelles et al. (2006).

Similarly adipose derived stem cells also promoted fibroblastic proliferation resulting in intensified granulation tissue formation. The accumulation of large collagen fibres likely increased the wound strength as opined by Kim et al. (2013) also. Another hypothesis was that adipose derived stem cells exerted paracrine effects on wound healing such as promoting fibroblast proliferation and exaggerating angiogenesis as opined by Lee et al. (2011). MSC released significant amounts of growth factors such as epidermal growth factor and transforming growth factor in a hypoxic environment in vitro Chen et al. (2009) which could also be the reason for the beneficial effects on wound in group of animals treated with adipose derived stem cell.

References


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**Plate 1:** Group I– 3rd Day
Wound Bed - Moderate Angiogenesis (arrow)
H&E Stain = 50µm

**Plate 2:** Group I-3rd Day
Wound bed - Granulating tissue with moderate immature collagen fibres with Neovascularisation
Masson’s Trichrome Stain = 50µm

**Plate 3:** Group I– 7th Day
Wound Bed - Moderate Epithelisation and Neutrophilic Infiltration
H&E Stain = 50µm

**Plate 4:** Group I- 7th Day
Wound Bed - Moderate Pulpy immature collagen fibres with Epidermal Peg
Masson’s Trichrome Stain = 20µm

**Plate 5:** Group I -14th Day
Wound Bed - Hyperplastic, Complete epithelisation
H&E Stain = 100µm

**Plate 6:** Group I -14th Day
Wound Bed - Mature collagen Fibres with Moderate Neovascularisation
Masson’s Trichrome Stain = 20µm
Plate 6: Group II – 3rd Day
Wound Bed - Moderate Angiogenesis with Neutrophilic and Macrophage infiltration
H&E Stain = 50µm

Plate 7: Group II- 3rd Day
Wound Bed - Epidermal peg with Moderate Neovascularisation
Masson’s Trichrome Stain = 20 µm

Plate 8: Group II – 7th Day
Wound Bed - Moderate Epithelisation and Neutrophilic Infiltration
H&E Stain = 50µm

Plate 9: Group II- 7th Day
Wound Bed - Moderate immature collagen fibres with Epidermal Peg
Masson’s Trichrome Stain = 50µm

Plate 10: Group II- 14th Day
Wound Bed - Hyperplastic Hyperkeratinised Epidermis and Dermis
H&E Stain = 100µm
Plate 11: Group II- 14th Day
Wound Bed - Mature collagen fibres with Moderate formed Epidermal peg
Masson’s Trichrome Stain = 20 μm

Table: Histopathological findings collagen, epithelisation and angiogenesis of recipient wound bed

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Treatment Days (Recipient Wound Bed)</th>
<th>Mean ± S.E.</th>
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<td>3rd Day</td>
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<tr>
<td>Collagen</td>
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<td>2.28 ± 0.83</td>
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<td>Epithelisation</td>
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<td></td>
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