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CASE REPORT

Autologous mononuclear cells from different sources are seen to improve wound healing in patients with haematological malignancies

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Abstract

Introduction: Immunosuppressive state due to haematological malignancies and chemotherapy may cause disruption to wound healing despite optimum conventional treatment and standard wound dressing. Non-healing wounds are predisposed to infection whereas chemotherapy dose reductions or interruptions are associated with poor survival. *Background:* Mononuclear cells contain progenitor cells including haematopoietic and mesenchymal stem cells, endothelial progenitor cells and fibroblasts which facilitate wound healing through cytokines, growth factor secretions, cell-cell interactions and provision of extracellular matrix scaffolding. Clinical applications of autologous mononuclear cells therapy in wound healing in non-malignant patients with critical limb ischaemia have been reported with remarkable outcome. *Methods:* We report three patients with haematological malignancies undergoing chemotherapy, who received autologous mononuclear cells implantation to treat non-healing wound after optimum conventional wound care. The sources of mononuclear cells (MNC) were from bone marrow (BM), peripheral blood (PB) and mobilised PB cells (mPB-MNC) using granulocyte colony stimulating factor (G-CSF). The cells were directly implanted into wound and below epidermis. Wound sizes and adverse effects from implantation were assessed at regular intervals. *Results:* All patients achieved wound healing within three months following autologous mononuclear cells implantation. No implantation adverse effects were observed. *Conclusions:* Autologous mononuclear cells therapy is a feasible alternative to conventional wound care to promote complete healing in non-healing wounds compounded by morbid factors such as haematological malignancies, chemotherapy, diabetes mellitus (DM), infections and prolonged immobility.

Keywords: Mononuclear cells, autologous, cell therapy, wound healing, haematological malignancies

INTRODUCTION

Accelerated wound healing is desirable in cancer patients to avoid chemotherapy interruptions which may affect treatment efficacy. Due to immunosuppressive state caused by the haematological malignancies and chemotherapy, wound healing is invariably disrupted and protracted despite standard wound care. Additionally, substantial use of corticosteroids for their anti-tumour activity posed a clinical dilemma as to the timing and doses of subsequent chemotherapy.[1-3] Chemotherapy interruptions and dose reductions are known to be associated with poor survival.[4]

The use of autologous cell therapy to treat non-healing wound in peripheral vascular disease (PVD) and critical limb ischaemia (CLI) have been reported with encouraging success.[5-10] We report three patients who received autologous MNC implantation with successful wound outcome (Table 1).

Case 1

A 68-year-old male with Stage 3B IgG myeloma and concomitant immunoparesis received VCD (Bortezomib, Cyclophosphamide, Dexamethasone). After the third cycle, he developed grade 4 neutropenic sepsis, fungal pneumonia and respiratory failure requiring

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TABLE 1: Summary of patient characteristics; details of autologous MNC therapy procedure; and outcome of wound healing

Patient Details	Details of autologous MNC therapy procedure	Outcomes
68-year-old, male. Myeloma Stage 3B. Prolonged immobility On VCD chemotherapy.	Site: Non-healing, Grade 2 sacral pressure wound 8x3 cm. BM-MNC collection without G-CSF. Collection volume: 50 mL of BM aspirate. Final suspension: 10 mL of $3.7x10^6$ cells/mL. Viability: 79%.	Complete wound healing at 3 months. No adverse effects.
18- year-old, male. DLBCL Stage 4B. Emergency laparotomy. On R-CHOP chemotherapy	Site 1: Non-healing laparotomy wound 21x5 cm Site 2: Grade 2 sacral wound 3x3 cm Mobilised PB with G-CSF (mPB-MNC). First collection volume: 100 mL of PB. First final suspension: 10 mL of $19.2x10^6$ cells/mL. Viability: Not available Second collection volume: 75 mL of PB. Second final suspension: 20 mL of $12.5x10^6$ cells/mL. Viability: Not available	Complete laparotomy wound healing 8 weeks after second implantation. Remarkable sacral wound healing 22 days after first implantation. No adverse effects.
67-year-old, female. CML in chronic phase. Hydroxyurea chemotherapy.	Site: Right lateral malleolus wound 2x1cm. PB-MNC collection without G-CSF. Collection volume: 75 mL of PB Final suspension: 10 mL of $19.2x10^6$ cells/mL. Viability: 95%.	Almost complete healing 4 weeks after implantation. No adverse effects.

mechanical ventilation. Consequent to prolonged immobility, he developed Grade 2 sacral pressure measuring 8x3 cm (Fig. 1a). Tissue culture grew Methicillin resistant *Staphylococcus aureus*, *Morganella morganii* and *Acinetobacter species*. Despite adequate antibiotics, wound debridement and standard dressing for 2 months, wound healing remained poor.

Methods: Collection, isolation and implantation of BM-MNC

Under local anaesthesia, 50 mL of BM was aspirated from posterior superior iliac spine. BM-MNC were isolated using density gradient centrifugation technique and diluted with Dulbecco's phosphate-buffered saline [DPBS, (Ca-, Mg-)] (Gibco; Grand Island, New York, USA) at 1:1 ratio. Diluted cell suspension was layered on top with Lymphoprep™ (Stemcell Technologies, Canada) and centrifuged at 2500 rpm for 30 minutes (Kubota 6200). MNC in the interphase layer were extracted and washed twice with DPBS by centrifuging at 1000 rpm for 10 minutes. Pellet of cells were suspended in 3 mL of ammonium chloride solution (Stemcell Technologies, Canada) for 5-10 minutes to lyse contaminating red cells. Wash steps were repeated as previous. The cell pellets were suspended in 10 mL of normal saline and cell viability were counted by haematocytometer and Trypan Blue Staining. The final product was 10 mL suspension containing $3.7x10^6$ cells/mL with 79% viability. Under local anaesthesia, BM-MNC were implanted into the wound edges, base and below epidermis with a 16-gauge needle. Using grid map, cell suspensions of 1 mL each were implanted into every 2 cm distance point.

Results

Sacral wound began to contract after five days (Fig. 1b) and completely healed less than three months (Fig. 1c) after BM-MNC implantation. No implantation adverse effects were observed.

The full blood counts pre-implantation showed white cells $6.2x10^6$/L, haemoglobin 9.7 g/dL, and platelets $354x10^6$/L. Three months post-implantation, the counts were $14.2x10^6$/L, 9.8g/dL and $367x10^6$/L respectively. The IgM level improved from 8.74 mg/dL to 35.3 mg/L, which may also reflect response to chemotherapy.

FIG. 1: Case 1: **(a)** Non-healing, infected sacral wound 8x3 cm prior to BM-MNC implantation. **(b)** Sacral wound 7.9x1.8 cm five days after BM-MNC implantation. **(c)** Complete healing three months after BM-MNC implantation.

The respiratory function and oxygenation status returned to normal. No post-implantation biopsy and immunohistochemistry was performed to demonstrate presence/persistence of progenitor cells.

Case 2

An 18-year-old male with Stage 4B diffuse large B cell lymphoma (DLBCL) underwent emergency laparotomy following perforated lymphomatous small bowel. A few months earlier he received Bevacizumab and mediastinal radiotherapy for presumed thymoma in another institution. He had stormy complications post-operatively including candida peritonitis. Upon histopathological confirmation of DLBCL, he received R-CHOP (Rituximab, Cyclophosphamide, Adriamycin, Vincristine, Prednisolone). Despite standard dressing for two months, the 21x5 cm laparotomy wound was slow to heal (Fig. 2a) and he also developed Grade 1 sacral wound measuring 3x3 cm (Fig. 2b).

Methods: Collection, isolation and implantation of mPB-MNC

The patient received 10 μg/kg G-CSF for 3 consecutive days. 75 mL blood was collected via venepuncture and processed as described in Case 1. The final suspension contained 20 mL of $12.5x10^6$ cells/mL. Under local anaesthesia, mPB-MNC were implanted into laparotomy and sacral wound bases, edges and below epidermis with a 16-gauge needle. Using grid map, cell suspensions of 1 mL each were implanted into every 2 cm distance point. The observed improvement in laparotomy wound was minimal (Fig. 2c), therefore a second mPB-MNC collection and implantation was performed 22 days later with 10 mL suspension containing $19.2x10^6$ cells/ml.

Results

The laparotomy wound showed marked healing with healthy granulation tissue five days after the second implantation (Fig. 2d), and complete healing eight weeks later (Fig. 2e). The sacral

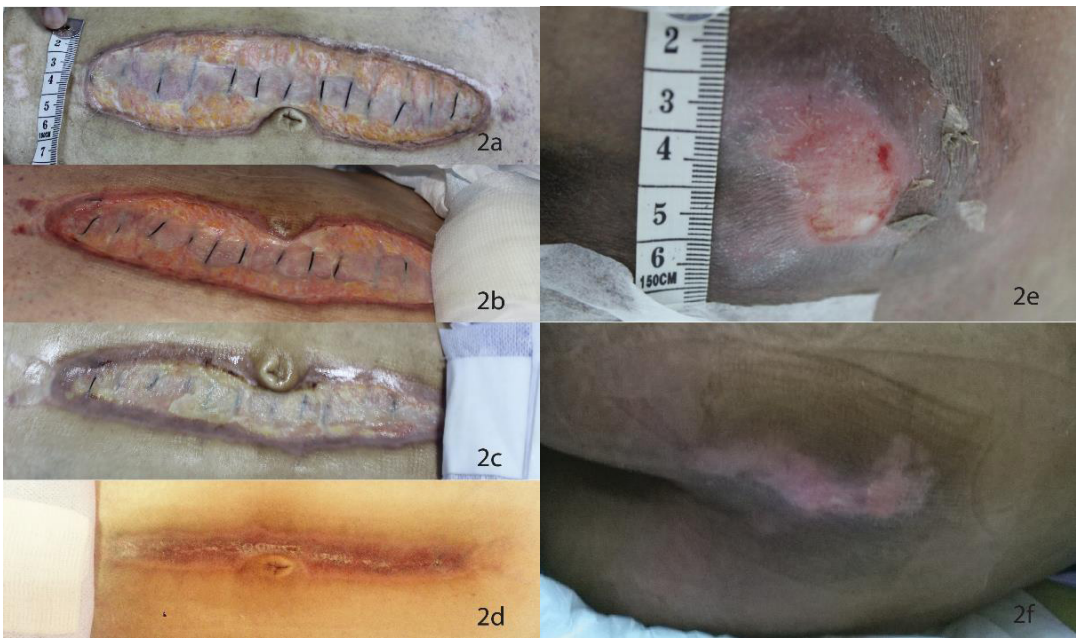

FIG 2: Case 2: **(a)** Non-healing laparotomy wound measuring 21x5 cm. **(b)** Minimal improvement five days after the first mPB-MNC implantation. **(c)** Granulation tissue growth five days after the second mPB-MNC implantation. **(d)** Complete healing eight weeks after the second mPB-MNC implantation. **(e)** Non-healing sacral wound measuring 3x3 cm prior to mPB-MNC implantation. **(f)** Remarkable improvement 22 days after mPB-MNC implantation.

wound showed remarkable improvement within 22 days after the first implantation (Fig. 2f). No implantation adverse effects were observed.

The full blood counts pre-implantation showed white cells 25.2x10^6/L, haemoglobin 8.5 g/dL, and platelets 74x10^6/L. Two months post-implantation, the counts were 27.2x10^6/L, 10.0g/dL and 86x10^6/L respectively. No immunoglobulins levels were available. The peripheral oxygen saturation oxygenation remained normal. No post-implantation biopsy and immunohistochemistry was performed to demonstrate presence/persistence of progenitor cells.

Case 3

A 67-year-old female with Type 2 DM presented with one week history of right ankle abscess, requiring incision & drainage and received Augmentin. No improvement was observed after one week and she later received Ciprofloxacin. Full blood count revealed leukocytosis 21.1x10^6/L and thrombocytosis 900x10^6/L. Later, she was diagnosed with chronic myeloid leukemia in chronic phase and received Hydroxyurea. The right lateral malleolus wound was tender and erythematous, measuring 2x1 cm (Fig. 3a). Despite eight weeks of standard dressing, the wound remained poorly healed.

Methods: Collection, isolation and implantation of PB-MNC

Seventy-five mL of PB were collected via venepuncture. PB-MNC were isolated using technique as described in Case 1. The final suspension contained 10 mL of 19.2x10^6 cells/mL with 95% viability. Under local anaesthesia, PB-MNC were implanted into wound base, edges and below epidermis with a 16-gauge needle. Cell suspensions of 1 mL each were implanted into every 2 cm distance point.

Results

After five days, the wound contracted down to 1x1 cm with resolution of erythema (Fig. 3b) and almost completely healed by four weeks (Fig. 3c). No implantation adverse effects were observed.

The full blood counts taken during Hydroxyurea therapy, pre-implantation showed white cells 6.4x10^6/L, haemoglobin 11.3 g/dL, platelets 897x10^6/L. Four weeks post-implantation, the counts were 17.5x10^6/L, 8.9 g/dL and 260x10^6/L respectively. No immunoglobulins levels were available. The peripheral oxygen saturation remained normal, however specifically no

transcutaneous oxygen level of right lower limb was measured. No post-implantation biopsy and immunohistochemistry was performed to demonstrate presence/persistence of progenitor cells.

DISCUSSION

Complex interactions between cells, extracellular matrix (ECM), growth factors and cytokines lead to tissue inflammation, matrix foundation, collagen production, epithelialisation, angiogenesis and wound closure.[11] The risk factors for poor healing in our patients were immunosuppression, infection, DM and immobility. Chemotherapy such as Adriamycin, Bevacizumab and corticosteroids interrupt macrophage functions, angiogenesis and wound contraction.[1-2,12] Radiotherapy damages fibroblasts and causes reduced tensile strength, capillary telangiectasia and skin necrosis.[13] Standard wound therapy was insufficient in achieving timely healing for chemotherapy to be resumed. Unfortunately, delaying chemotherapy decreases its efficacy and results in poorer outcome.[4]

Cell therapy to restore perfusion and promote wound healing has been extensively studied in non-healing ulcers of the lower limbs in PAD and CLI. A direct comparative trial of BM-MNC versus mPB-MNC in PAD showed no significant differences between the two cell types in wound healing, although mPB-MNC improved rest pain and ankle-brachial index (ABI) significantly.[5] In contrary, other studies reported that BM-MNC significantly improved ulcer size, ABI and rest pain compared to PB-MNC.[6-7] Recent review reported that autologous cell therapy improved wound healing by 59% but when comparing different cell sources, only BM-MNC significantly improved wound healing.[8] Nevertheless, data from randomised controlled trials (RCTs) reviewed suggested that direct comparative trials do not consistently show superiority of one cell type over another.[8]

MNC contains lymphocytes, monocytes, granulocytes and progenitor cells e.g. endothelial progenitor cell (EPC), mesenchymal stem cells (MSC) and fibroblasts.[14] Our MNCs were sourced from BM, PB and mobilized PB, and none underwent *in vitro* manipulation e.g. culture or expansion. Different MNC sources may possess different biological and functional activities, however flow cytometry were not performed to enumerate the proportion of each cellular components. Although BM is the commonest source for cell therapy[15], mPB-MNC is now the preferred source due to ease of collection in a large quantity, avoidance of general anaesthesia and faster haematopoietic engraftment.[16] G-CSF mobilised cells also possess differentiation capabilities into EPC.[17]

Recent preclinical data suggest optimum blood vessel formation require paracrine and structural contributions from multiple progenitor cell lineages.[9] Fibroblasts allow the formation of ECM scaffolding and activate endothelial cells (EC) to secrete stimulatory cytokines for angiogenesis.[18] Myeloid haematopoietic progenitor cells produce angiocrine signals to stimulate angiogenesis, while EPC intertwine vessel walls during vasculogenesis. MSC differentiate into pericytes to stabilise vessels and produce chemokines e.g. transforming growth factor beta (TGF-β), prostaglandin E2 (PGE2) and vascular endothelial growth factor (VEGF) to attract macrophages during arteriogenesis.[9,19] In DM, defects in EPC function and VEGF level contributed to impaired angiogenesis.[20]

To the best of our knowledge, this is the first report on autologous MNC implantation to treat non-healing wounds in patients with haematological malignancies. Despite concurrent chemotherapy, our patients underwent MNC collections and implantation procedures with impressive results. It may be partly associated with the quiescent, non-dividing G0 state of adult stem cells are predominantly in.[21] Only a small number of adult haematopoietic stem cells enter the cell cycle, while the majority remain quiescent under steady state, a feature that is closely associated with protection from myelotoxic insults.[22] MNC from mobilised PB allow ease of collection with higher number of cell doses accrued compared to BM.

CONCLUSION

Despite initial non-healing wound after standard therapy, we observed healthy granulation tissue formation and epithelialisation after autologous MNC implantation. Cell therapy provides a promising alternative to conventional wound treatment to facilitate healing especially in patients receiving chemotherapy. A well-designed comparative randomised study involving a larger number of patients are warranted to determine the optimum cell source, dose and implantation regimen for the treatment of non-healing wound in patients undergoing chemotherapy to further understand the mechanism of wound healing and tissue regeneration at cellular level.

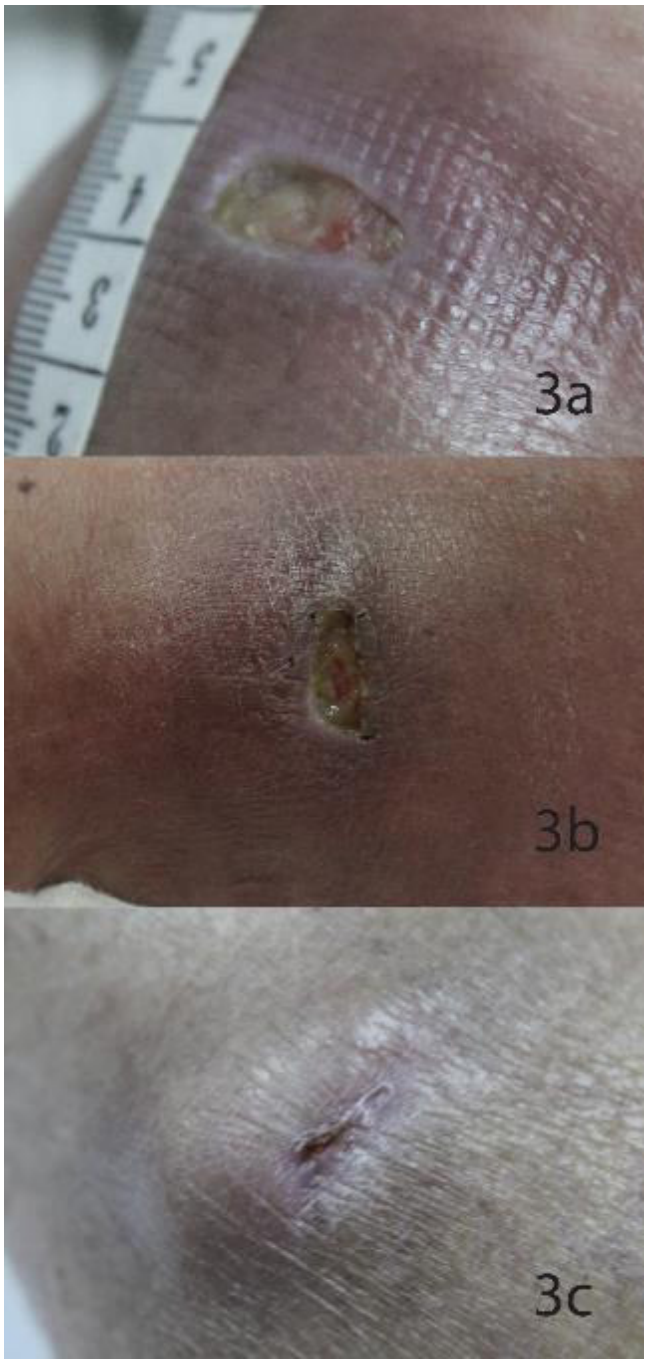

FIG. 3: Case 3: **(a)** Non-healing right lateral malleolus wound with pus discharge measuring 2x1 cm prior to PB-MNC implantation. **(b)** Wound began to dry with tissue contraction five days after PB-MNC implantation. **(c)** Wound almost completely healed four weeks after PB-MNC implantation.

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