Technique for intraoperatory harvesting of adipose derived stem cells: towards cell treatment of recalcitrant ulcers

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Abstract

Successful wound and ulcer repair remains a major biomedical challenge in the 21st Century. Innovative and alternative treatment options have been investigated over the last decade and stem cells application has been suggested as a possible novel therapy for regenerative medicine. In particular, stem cells derived from adipose tissue have been attracting a lot of attention in recent years as an alternative to the use of cells derived from bone marrow. This technical note describes the procedure introduced by Coleman for intraoperatory harvesting of adipose derived-stem-cells and proposes a rationale for using it in difficult wound healing and recalcitrant ulcers.

Introduction

Stem cell technology is increasingly being employed to treat tissue and organs in patients. Surgeons are keen to transfer these treatment methods to other applications such as ulcer and wound healing. The use of adipose-derived-stem cells (ADSCs) appeals to surgeons due to the relative ease of locating sites for harvesting, the harvesting process and the amount of material available for use. ADSCs also offer several desirable factors including their osteogenic and chondrogenic potential, enhancement of angiogenesis, limitation of apoptosis and immunomodulatory function.[1]

Due to these advantageous characteristics, ADSCs can be explored as a promising tool to repair recalcitrant ulcers and difficult wounds in those cases where conventional treatments have failed. Although numerous promising stem cell approaches are advancing in clinical trials, intraoperative stem cell therapies using ADSCs offer more immediate benefits, by integrating an autologous cell source with a well-established surgical intervention in a single, safe procedure.

Coleman's technique

ADSCs are generally extracted from adipose tissue using a multiple stepwise procedure. Coleman's technique[2] is both the first and the most popular method for tissue regeneration:[3,4] it provides aspiration, centrifugation and the subsequent re-injection of autologous fat.

First, Kleine's solution made up of 250 mL normal saline, 20 mL of 1% carbocaine, 1 mL adrenaline and 2 ml bicarbonate is injected into the fat donor area.[5] After diffusion of the solution the harvesting procedure is undertaken, using a two-hole blunt cannula [Byron Medical (a division of Mentor Corporation), Tucson, AZ, USA] fitted directly to a 10 mL Luer Lock syringe (BD Syringe Luer-Lok tip; Becton Dickinson, Franklin Lakes, NJ, USA), which helps to reduce the pressure generated during the harvesting procedure and preserves the fat parcels.[6] Following the fat harvesting, the lipoaspirate is processed *via* centrifugation of the lipoaspirate at 3000 rpm (rotor size: 16 cm; g force: 580) for 3 min in 10 mL syringes. This separates the fat into 3 layers. The upper layer and lower layer are discarded, as they are composed of oil from destroyed fat and blood respectively, leaving the middle layer, which contains a high concentration of stem cells[7,8] and the stromal vascular fraction.

Once the middle layer has been extracted, the micro-fat graft is transferred into 1ml syringes prior to injection into the tissue to be grafted (Figure 1). A multilayer technique is used to implant the aliquots of fat, with very small amounts of fatty tissue released into the recipient area in order to optimize the successful implantation of the graft. A blunt Coleman microcannula is used to deposit the micro-fat graft *via* a number of sub dermal and hypodermal tunnels through numerous tissue planes. This technique of minimising the amount of micro-fat graft released with each introduction of the cannula increases the surface area between the grafted fat and the tissue receiving it. In this way there is a reduction in fat damage and adipocyte necrosis, with an improvement in graft vascularization and three-dimensional fat distribution. The recently grafted fat has a readily available blood supply, which facilitates its survival and reduces the possibility of fat necrosis or calcification.[9]

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Chronic venous leg ulceration and the potential role of adipose derived-stem-cells

Chronic venous leg ulceration (CVU) occurs in about 10% of cases of chronic venous disease (CVD).[10] Although mainly caused due to superficial venous insufficiency, CVU becomes more prevalent when associated with other health issues such as diabetes, malnutrition, rheumatoid arthritis, chronic anaemia or second stage peripheral arterial disease.[11,12]

CVU can be treated through surgery or through a combination of compression, wound-care and debridement. Two randomised control trials have evaluated the healing rate and risk of ulcer recurrence following superficial venous surgery. The long-term randomized study conducted by Zamboni *et al*.[13] corroborates the effectiveness of minimally invasive surgical haemodynamic correction of reflux (CHIVA) for leg ulceration secondary to superficial venous reflux. Follow-up after a 3 year period revealed a 100% healing rate at 31 days and a recurrence rate of 9% in those patients who received surgery, compared to the compression only group with a healing rate of 96% after 31 days and a recurrence rate of 38%. The ESCHAR study conducted by

Barwell *et al.*[14] also advocates simple venous surgery as a benefit to patients with chronic venous ulceration. It assessed the ulcer recurrence rate as significantly reduced in its compression and superficial venous surgery group at 12%, compared to 28% recurrence in the compression alone group.

However, a significant proportion of recalcitrant ulcers respond to treatment but do not heal.[15-18] In these patients, advanced dressings and skin grafts are generally used to achieve healing[21] and new regenerative approaches including the use of ADSCs have been proposed.[19]

The positive effect of ADSCs is due to an increase in vascularization, which is fundamental to the healing process.[20,21] ADSCs are able to release angiogenic factors, and have shown increased angiogenesis in wound healing when injected or delivered *via* a scaffold.[22] Treatment is customised for each patient and requires a clean and healthy wound bed with no devitalised tissue and carefully managed protease levels.[23]

Debridement and application of adipose derived-stem-cells in the wound bed

There are few reports about the treatment of venous leg ulcers (VLUs) using ADSCs. However, ADSCs have shown a positive impact on wounds healing in preclinical and clinical studies. Recent ADSCs applications *in vitro* and *in vivo* have demonstrated that they are attracted to the wound site and affect regeneration processes by means of paracrine mechanisms in addition to fusion and differentiation, for instance, into keratinocytes or dermal fibrobasts.[24-27] Promising applications in wounds and ulcers healing have been reported, although thus far these are small studies with a total of only 98 patients.[28-32] Bartsich and Morrison[33] discussed the long-term treatment of chronic sickle cell ulcers and the possible use of a skin graft and fat grafting to achieve healing. Current treatment using skin grafting and local wound care is often unsuccessful long-term, as wounds that have healed break down again. So, treatment involving permanent alteration of the wound bed, with recruitment of a new cell population and subsequent fat grafting, was applied.

Our group was attracted by first reports on application of cell therapy in unhealed ulcers, and performed preliminary application in selected cases. In our procedure, we first perform a detailed wound bed preparation following this sequence. Prior to the

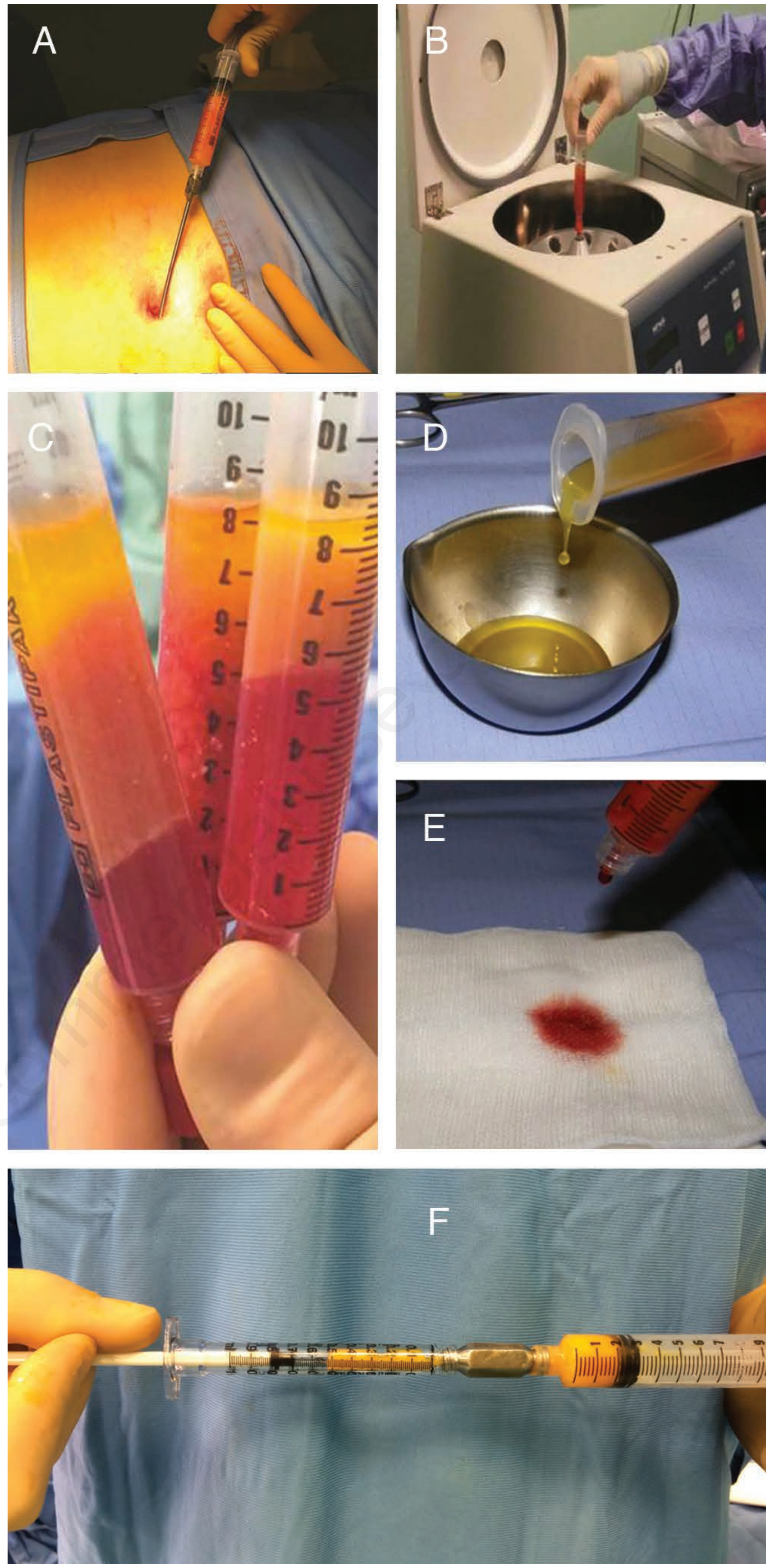

Figure 1. Coleman's technique for harvesting adipose derived-stem-cells (ADSCs). In this particular case the harvesting is performed in the periumbilical area after tumescent local anaesthesia infiltration (A); the adipose tissue is centrifuged in order to separate the different components (B); the samples are separated into: an upper layer of yellow oil, a middle layer composed of adipose tissue with high concentration of stem cells and a bottom layer of blood (C); after oil (D) and blood (E) elimination the ADSCs are aspirated by Luer-Lock syringe technique (F).

procedure, the wound area is sanitized using Chlorexidine, then debridement of ulcers is performed by the means of Versajet device (VERSAJET™, Versajet Hydrosurgery System, Smith and Nephew, Hull, UK), a debridement tool able to cut and remove necrotic tissue and fibrin based on a high pressure jet of water which produces the Venturi effect.[34] We emphasize here that wound bed preparation needs to be meticulous before implantation: the microenvironment for wound regeneration mainly depends on interactions between stem cell progenitors and their niche, and it provides proliferation and differentiation of the cells.[35] After this stringent preparation of the wound bed, it is possible to apply Coleman's Technique, as described above. The suspension of ADSCs is injected into the wound bed and the wound margins; a scaffold of commercial hyaluronic acid is positioned on the wound bed in order to cover the implanted cells. Finally, advanced foam dressing protects the healing area before the application of compression.

Centrifugation according to Coleman's procedure creates a graded density of fat with varying characteristics that influence lipoaspirate persistence, properties, and quality.[36] Compared with other fat-non processing methods, centrifugation clears the fat from most blood remnants and shows the highest concentration of mesenchymal stem cells.[37] It is safe and feasible, does not impair cell viability and can augment ADSCs content.[38] The inoculation of these non manipulated cells present in the lower centrifuged high-density layer (HDL) could accelerate the healing of recalcitrant venous and mixed leg ulcers and could be used for the regeneration of large defects: the HDL displays the highest expression of mesenchymal stem cells and endothelial markers, such as vascular endothelial growth factor A (VEGFA), indicating the larger vascular potential of the cells in this layer (Figures 2 and 3).[36] Moreover, in presence of specific growth factors, stem cells present in the HDL can differentiate into several lineages, including osteogenic, adipogenic, condrogenic, neuronal, glial, and endothelial lineages.

Figure 2. Pre-operative Martorell's ulcer (A); intraoperative injection of adipose derived-stem-cells (B) post-operative leg ulcer after 4 weeks of treatment (C) and after 8 weeks of treatment (D).

Figure 3. Pre-operative venous leg ulcer over the medial malleolus (A) and over the lateral malleolus (B); post-operative leg ulcer after 14 weeks of treatment (C-D).

Conclusions

As a treatment which is based on a widely recognised, safe procedure, intraoperative ADSCs therapy using Coleman's technique provides a new alternative and potentially regenerative approach to the clinical challenges of recalcitrant ulcers and difficult wound healing. This surgical intervention in one single, safe procedure could become an alternative treatment in selected patients, with promising results and future potential. However, at present, there are neither standardised protocols for the clinical application of ADSCs in VLUs nor a consensus on the number of cells (present in the HDL) necessary for different therapeutic options. Therefore, standardised protocols and larger randomised controlled trials are indispensable to ensure the safety and effectiveness of ADSCs in VLUs application.

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