Two, easy-to-handle homologous tissues enhance diabetic ulcer healing in arteriopathic patients: a case series
Homologous "waste" tissues support diabetic ulcer treatment

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Summary
Aims: We describe our experience with the combined use of homologous products and standard medical and surgical care in treating chronic ulcers in arteriopathic diabetic patients.
Material and Methods: Of the 200 arteriopathic diabetic patients with deep ulcers who underwent lower limb revascularisation surgery at our hospital between January 2012 and August 2014, Texas Wound Classification grades 2 and 3 cases were offered either amniotic membrane or fat grafting as a complementary treatment. 17 patients accepted. Data concerning wound healing and healing time, revascularisation patency, and number of amputations were recorded.
Results: 16 out of the 17 patients achieved ulcer healing following the intervention, with an average healing time of 25.3 days. Pervious revascularisation occurred in 10 of these cases. Vessel re-occlusion occurred in 8 patients but did not subsequently relapse. Outcomes were stable at the end of 20-month follow-up. There were no major complications.
Conclusions: As part of a multidisciplinary approach to deep ulcers in patients with concomitant diabetes and peripheral artery disease, amniotic membrane and fat grafting after revascularisation

appear to promote timely, effective and stable healing, and should therefore be considered before resorting to amputation.
Key words: amniotic membrane, diabetic ulcers, fat graft, mesenchymal stem cells

Acronym list:
MSC: mesenchymal stem cells
ADSC: adipose-derived stem cells
VAC: vacuum-assisted closure
NPWT: negative-pressure wound therapy
SVF: stromal vascular fraction
PRP: platelet-rich plasma
DFU: diabetic foot ulcer
TWC: Texas Wound Classification
FDA: US Food and Drug Administration
EMA/CAT: European Medicines Agency/Committee for Advanced Therapies and Medical Devices
PAD: peripheral artery disease
QoL: quality of life
ECM: extracellular matrix
MMPs: metalloproteinases
VEGF: vascular endothelial growth factor
PDGF: platelet-delivered growth factor
AM: amniotic membrane
GM-CSF: Granulocyte macrophage colony stimulating factor
bFGF: basic fibroblast growth factor
TIMPS: tissue inhibitor of metalloproteinases
hAM-MSC: human amniotic membrane mesenchymal stem cells
BM-MSC: bone marrow mesenchymal stem cells
CAL: cell-assisted lipotransfer
GMP: good manufacturing practice

Introduction

Treating lesions in diabetic patients with peripheral arterial disease staged V-VI on the Rutherford scale is a challenge due to the complex physiopathology of the lesions and frequent background comorbidities. Among the complications of diabetes, ulcers represent the greatest social and economic burden, necessitating long outpatient follow-up and increased hospitalisations, as well as drastically lowering the QoL of affected patients (1). The lifetime risk of a diabetic patient developing an ulcer is 25%, and the lesion will lead to major or minor amputation in at least 14 % of patients affected (2). In ischaemic diabetic ulcers, treatment outcomes are compromised by a complex of factors, in particular co-morbidity, ischaemia and infection (3). Hence, a coordinated multidisciplinary approach is needed to achieve limb salvage (4).

The best conditions for effective lesion healing can be found immediately after inferior limb revascularisation. Nonetheless, the strategies commonly used for wound treatment seem not to affect the natural course of non-healing lesions, and often fail to lead to ulcer resolution within a reasonable time-frame. This means that there is a risk of not achieving timely primary patency. Nonetheless, thanks to a better understanding of the molecular events that lead to lesion persistence in diabetic subjects (5), several regenerative medicine products have found applications in chronic

wound therapy; engineered tissues, skin substitutes (6), growth factors such as PDGf, VEGF, GM-CSF, bFGF, and autologous products such as protein-rich plasma (PRP) have all produced encouraging results in this regard (7,8). The most promising field of study in regenerative medicine for diabetic ulcers, is, however, stem cell research (9). In particular, mesenchymal stem cells (MSCs) are widely available in adult tissues and have shown the capability to interfere with the pathological healing process in chronic wounds (10,11). Indeed, MSCs can affect all three phases of the normal healing process (inflammation, proliferation and remodelling) in an autocrine/paracrine fashion, and by differentiating into tissue-resident cells (12).
Fat grafts and amniotic membrane are homologous tissues commonly considered waste products. However, they are sources of not-expanded mesenchymal stem cells and various bioactive molecules that seem to promote chronic wound healing. As a consequence, each has been applied in different expressions of impaired healing in diabetic foot ulcers (DFUs) in both clinical and preclinical settings (13) over recent decades. We describe here our experience with 19 post-revascularization cases with particularly severe chronic arterial DFU (all classifiable as grade 2 and 3, stages C and D according to the Texas Wound Classification), which we treated using cryopreserved amniotic membrane (n=09) or enriched (n=2)/not enriched (n=6) fat grafting, both associated with common surgical wound-care procedures. Data concerning healing/not healing, healing time, amputation rate and ulcer relapse were recorded over 20-month follow up.

Aims

In diabetic vasculopathic patients, skin ulcers may compromise limb healing and salvage despite successful revascularisation. Hence, prompt lesion healing after revascularisation represents a critical end-point in the multidisciplinary care path that such patients receive. Although available medications maintain an ideal "macro-environment" for wound healing, they do not significantly modify its natural course. However, mesenchymal stem cells are known for their therapeutic potential in impaired healing processes, thanks to the production of several immunomodulatory, proangiogenic and chemotactic factors. We set out to document our experience with the use of two homologous, "waste" tissues rich in these progenitor cells, fat grafts and amniotic membrane, along with standard care, to enhance the healing of diabetic lower limb ulcers in arteriopathic patients.

Materials and Methods

Study population

Of the 200 type-II diabetic patients with ulcers who underwent inferior limb revascularisation at our hospital (Vascular Surgery Department, Santa Maria della Misericordia University Hospital, Udine, Italy) from January 2012 to August 2014, we offered the cases classifiable as grades 2 and 3 according to the Texas Wound Classification (TWD) amniotic membrane/fat grafting as a complementary treatment, alongside standard care procedures; 17 patients accepted (2 women, 15 men) (14). All lesions were classifiable as grade C or D on the TWD. Informed written consent was obtained, and the local Institutional Review Board approved our project. 11 patients had insulin-dependent diabetes, while 6 were undergoing oral therapy. Their average age was 64 years 9 months. The average lesion size was 20.3 cm2. 4 ulcers were on the leg, 10 on the foot, and 3 patients had ulcers on both foot and leg. The patients' main demographic and ulcer features are listed in Table 1.
Preoperative workup included complete clinical examination, photographic examination, and ulcer measurement. Right after revascularisation, patients underwent 2 weeks of negative pressure therapy to enhance local blood flow and wound cleaning (15). If necessary, antibiogram-based antibiotic therapy was prescribed until there was clinical evidence of infection eradication. Patients were then operated on following the protocol described below.

Surgical Technique

According to the standard operating protocol, all ulcers underwent surgical debridement under regional or general anaesthesia using an ultrasound system (SonicOne®)(16), until a viable plane was achieved. This was followed by excision of damaged wound edges with a scalpel and debridement of exposed bone. The choice to use either amniotic membrane or enriched fat over the wound was dictated mainly by ulcer shape and abdominal fat availability; it has been shown that abdominal fat, in particular that over the fascia of Scarpa, is a better source of progenitor cells (21). Adipose tissue was harvested and collected through a closed system exploiting the Water-jet Assisted Liposuction method (Body-Jet® system with LipoCollector® tool). For the 6 patients that presented minimal tendon or bone exposure (less than 10% of the wound area), the product obtained was injected at one cm deep into the ulcer edges and bed using a 1.9-mm blunt cannula connected to a 2.5 ml Luer-Lok syringe, taking care to distribute it in "micro-tunnel" deposits (17). The quantity of lipoaspirate injected varied from 10 to 30 ml. The technique was the same described in a previous paper, albeit without the use of PRP (18).

For 2 of the 11 patients who presented major tendon or bone exposure, washed fat was processed using the Celution®System-an EC-marked device (currently under review by the FDA and the European CAT) that enzymatically digests fat to isolate SVF and concentrate ADRC (19). The product (approximately 5 ml) was then resuspended in 20-30 ml of adipose tissue to create enriched fat. The enriched fat was grafted, at 1 cm depth, to the ulcer edges and bed. For the nine remaining patients, after the debridement, cryopreserved amniotic membrane, previously ordered from the nearest tissue bank (Fondazione Banca dei Tessuti di Treviso-ONLUS, Treviso, Italy), was positioned on the base of the ulcer and sutured in place like a skin graft.

After two weeks, all lesions showed valid granulation tissue, which we covered with a split-thickness skin graft harvested from the thigh or gluteus. In two cases, the skin graft was unnecessary because the ulcers had almost completely healed at two weeks.

Except for case 1, all surgical procedures were performed in day hospital. Patients were monitored in our outpatient services at least weekly until ulcer healing. After that, a check-up was scheduled at month 3, then every 6 months, for a total follow-up time of 20 months. The outcomes: healing/not healing, healing time, number of amputations, and ulcer relapse were recorded.

Results

The patients' average age was 64 years 9 months, and their average lesion size was 20.3 cm2. 4 ulcers were on the leg, 11 on the foot, and 2 patients had ulcers on both foot and leg. The patients' main demographic and ulcer features are listed in Table 1. Ulcer healing was achieved in 16 out of 19 patients, and this outcome was stable at the end of 20-month follow-up. Although subjective, we noticed that the newly formed tissue was particularly "healthy" and elastic. The added soft tissue thickness could have prevented traumatic or decubitus lesions in patients whose ulcer pathogenesis had a neurological component (lesions are rarely purely ischaemic).

In the remaining case, lesion worsening with vascular re-occlusion was recorded, and necessitated forefoot amputation. This case was particularly challenging-a 59-year-old woman with exposure of the first metatarsal-phalangeal and the first proximal inter-phalangeal joints of the right foot. After the initial revascularisation and two weeks of negative-pressure wound therapy (NPTW), she underwent surgical toilette and received an amniotic membrane. This led to temporary ulcer resolution, without recourse to skin grafting. After 1 year, however, vascular re-occlusion occurred, accompanied by the appearance of trophic lesions of the same foot. Despite attempts at recanalization, the lesion worsened, her general conditions deteriorated, and forefoot amputation was considered necessary.

The average healing time for the other 16 patients was 25.3 days after the last operation (either skin grafting or homologous tissue placement in the case of the two patients who did not require a skin graft to enhance re-epithelisation).

At the end of follow up period, 16 patients showed complete ulcer healing. Pervious revascularisation was achieved after a single intervention in 8 cases, while 8 patients were treated for vascular re-occlusion, which did not, however, subsequently relapse. There were no major complications to record. Two representative cases are presented below.

Case 1
Figure 1 shows an extensive lesion of the dorsal aspect of the left foot in a 67-year-old patient with severe PAD presenting pluricomplicated insulin-dependent diabetes with several comorbidities (hypertension, dyslipidaemia), who had previously undergone amputation of the 4th and 5th toes. Femoral-popliteal bypass plus tibial vessel PTA restored blood flow to the wound. After two weeks of VAC therapy, the ulcer was debrided and an amniotic membrane was positioned; 15 days later, the granulating lesion was covered by a split-thickness skin graft. The last picture shows the appearance of the healed area, with stable soft tissue coverage of noble structures and good skin trophism at 1 year.
Case 2
Figure 2 shows an ulcer on a transmetatarsal amputation stump of the left first ray in a patient who had previously undergone three revascularisation procedures. After 2 weeks of NPWT, we debrided the wound and grafted 10 ml of adipose tissue. 1 month after the skin graft (E), the wound showed complete re-epithelialisation.

Discussion
As a pathogenic factor of diabetic lower limb ulcers, PAD is associated with prolonged healing time, increased risk of amputation (25% at 6 months) and impaired healing ability (22). Although patent revascularisation has proven to increase the likelihood of healing in such patients (23), diabetics are still characterised by a lower patency rate when compared to people without diabetes. Furthermore, poor blood flow is not the only factor that has to be take into account when trying to promote the healing of such challenging lesions and save the limb (24). Indeed, there are over 100 known physiological factors that contribute to wound healing deficiencies in individuals with diabetes; these include decreased growth factor production, impaired angiogenic and immune responses, keratinocyte and fibroblast migration and proliferation, collagen accumulation, alteration in the quantity of granulation tissue and number of epidermal nerves, and impaired balance between the accumulation of extracellular matrix (ECM) and metalloproteinase (MMP) activity (25).
Hence, the advanced wound dressings available today are designed to create and maintain an ideal "macro-environment" to aid wound healing. That being said, they do not significantly alter its natural course. For this reason, different regenerative medicine products, proven to support the treatment of chronic lesions by influencing the micro-environment described above, may be considered. In particular, the amniotic membrane (AM) has a long history of application for regenerative purposes (26). It consists of viable cells, collagen, and an extracellular matrix organised into multiple layers, and contains helpful biomolecules such as cytokines, growth factor and some function-specific molecular elements (defensins, MMPs and tissue inhibitor of metalloproteinases (TIMPS)). Within the amniotic membrane, mesenchymal stem cells (hAM-MSCs) act together with the aforementioned factors to enhance wound healing; MSCs have demonstrated a paracrine function mediated through the production of various immunomodulator, angiogenic, antiapoptotic and chemotactic factors. Furthermore, it has been shown that, under proper stimulation, these cells can differentiate into different mesenchymal lineage cells such as keratinocytes, pericytes and endothelial cells.
Adult stem cells do not pose the ethical problems that preclude the use of embryonic tissue. Indeed, they are present in virtually all post-natal organs, generally treated as waste products. Moreover, both cryopreserved and dehydrated AM has been successfully used for the treatment of chronic wounds, demonstrating antibacterial properties, determining a reduction of inflammation and scar-tissue formation, and providing a substrate for cellular proliferation and migration.

In diabetic patients, there is scarce bone marrow mesenchymal stem cell (BM-MSC) migration to wound sites (27). This physiological response to ischaemia highlights the importance of progenitor cells in wound healing. In fact, some prospective studies indicate the superiority of amniotic membrane with respect to skin substitutes or standard care, from both clinical and resource utilisation standpoints, in the treatment of diabetic ulcers of the lower extremities.

Like AM, adipose tissue is a generous source of MSCs, specifically adipose-derived stem cells (ADSCs), located within the stromal vascular fraction. Adipose tissue is a widely available, easy to obtain, and ADSCs (like MSCs from other sources) possess the ability to promote angiogenesis, stimulate dermal fibroblast proliferation, and differentiate into multiple lineages upon proper stimuli (28-30), making them ideal cells for the treatment of non-healing wounds (31).

Traditionally used as a filler, since the early 2000s enriched and unenriched fat grafting has been employed in plastic surgery to assist the healing of radiation damage, degenerative conditions (Perry-Romberg syndrome), nerve injury and neuropathic pain, and for cosmetic purposes (32-36). Enriched fat grafting, known as cell-assisted lipotransfer (CAL), consists of adding enzymatically digested SVF, obtained from a part of the lipoaspirate, to the other part of harvested fat. This converts it into an ADSC-rich product with higher regenerative potential. Indeed, in a previous study we demonstrated the superiority of CAL vs. standard lipotransfer in breast reconstruction; CAL improved graft survival and enhanced progenitor cell concentration, which is why we prefer it to simple fat grafting for more severe cases (37-40).

Indeed, direct grafting of lipoaspirated fat circumvents several regulatory issues (e.g. GMP rules) and avoids cell manipulation, which could modify the properties of ADSCs. Moreover, the Celution® system is already undergoing investigation by both the FDA and CAT. Furthermore, ADSCs have been shown to exert a proangiogenic effect in animal models, and there are several preclinical and clinical studies involving the use of expanded and-not expanded ADSCs to stimulate neoangiogenesis in critical limb ischaemia either completed or ongoing (41-42). This proangiogenic effect could be enhanced by the hypoxic conditions typical of arteriopathic diabetes (43), which is encouraging, as deep ulcers in diabetic patients affected by severe PAD are typically hard to treat. In such patients, re-establishing blood flow to the lesion and eradication of any infection are two of the three cornerstones to achieving limb salvage.

Hence, a multidisciplinary approach is fundamental to avoid amputation, the risk of which will also depend on the grade and stage of the wound. In our patient series, both amniotic membrane and fat grafting, alongside proper bed preparation and standard plastic surgery techniques, were confirmed to be viable tools for enhancing wound healing in patients suffering from TWC-grade 2-3 chronic diabetic ulcer. As carriers of progenitor cells, cytokines and angiogenic and immunomodulatory factors, and preserving their tissue-specific structure, these homologous, non-immunogenic, "waste" tissues are also easy to administer, requiring minimal surgical skill, and are adaptable to wound and patient features. Except for one patient, our cases showed stable healing at 20-month follow-up, even when vascular re-occlusion had occurred.

The use of homologous tissues and derivatives for regenerative purposes (especially in chronic wounds) is becoming more and more common, and plastic surgeons are slowly gaining confidence in them, thanks to encouraging results obtained from the early clinical reports. However, their widespread use is still limited by a lack of studies with higher statistical significance. Indeed, it is difficult to enrol homogeneous populations affected by lesions that are so diversified and complex for prospective, randomised studies.

That being said, outcomes such as our own, albeit in a small patient series, help make the case for using such tissues to promote wound healing in arteriopathic diabetic patients with deep lower limb ulcers.

The different techniques proposed, share similar mechanisms of action but it would be interesting to differentiate how they affect healing in a larger series, as well as determining the differences between the different healed tissue, and their uses in non-revascularized patients. Indeed, it is hoped

that such techniques will eventually obviate the need for amputation and therefore preserve patient QoL, as well as reducing the burden on healthcare systems, in ever-greater numbers of patients.

Conclusions

As part of a multidisciplinary approach to deep ulcers in patients with concomitant diabetes and peripheral artery disease, amniotic membrane and fat grafting after revascularisation appears to promote timely, effective and stable healing, and should therefore be considered before resorting to amputation. Indeed, the use of "waste" human tissues rich in mesenchymal stem cells seems to promote the healing of extensive lesions in a time-frame more likely to fall within limits of primary and secondary patency in these extreme revascularisation cases.

References

1. Sekhar MS, Thomas RR, Unnikrishnan MK, Vijayanarayana K, Rodrigues GS. Impact of diabetic foot ulcer on health-related quality of life: A cross-sectional study. Semin Vasc Surg. 2015;28(3-4):165-171.
2. American Diabetes Association. Economic costs of diabetes in the U.S. In 2007. Diabetes care. 2008;31(3): 596-615.
3. Gershater MA, Löndahl M, Nyberg P Larsson J, Thörne J, Eneroth M et al. Complexity of factors related to outcome of neuropathic and neuroischaemic/ischaemic diabetic foot ulcers: a cohort study. Diabetologia. 2009;52(3):398-407.
4. Alexandrescu V, Hubermont G, Coessens V, Philips Y, Guillaumie B, Ngongang C et al. Why a multidisciplinary team may represent a key factor for lowering the inferior limb loss rate in diabetic. Acta Chir Belg. 2009;109(6):694-700.
5. Falanga V. Wound healing and its impairment in diabetic foot. Lancet. 2005;366(9498):1736-1743.
6. Snyder DL, Sullivan N, Schoelles KM. Skin substitutes for treating chronic wounds. Rockville (MD): Agency for Healthcare Research and Quality (US); 2012 Dec.
7. Barrientos S, Brem H, Stojadinovic O, Tomic-Canic M. Clinical application of growth factors and cytokines in wound healing. Wound Repair Regen. 2014;22(5):569-578.
8. Kontopodis N, Tavlas E, Papadopoulos G, Pantidis D, Kafetzakis A, Chalkiadakis G et al. Effectiveness of platelet-rich plasma to enhance healing of diabetic foot ulcers in patients with concomitant peripheral arterial disease and critical limb ischemia. Int J Low Extrem Wounds. 2016;15(1):45-51.
9. Blumberg SN, Berger A, Hwang L, Pastar I, Warren SM, Chen W. The role of stem cells in the treatment of diabetic foot ulcers. Diabetes Res Clin Pract. 2012;96(1):1 - 9.
10. Da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci. 2006;119(Pt 11):2204-2213.
11. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells. 2007;25(10):2648-59.
12. Caplan AI. Why are MSCs therapeutic? New data: new insight. J Pathol. 2009;217(2):318-324.
13. Han SK, Kim HR, Kim WK. The treatment of diabetic foot ulcers with ulcultured, processed lipoaspirated cells: a pilot study. Wound Repair Regen. 2010;18(4):342-348.
14. Armstrong DG, Lavery LA and Harkless LB. Validation of a Diabetic Wound Classification System: The contribution of depth, infection, and ischemia to risk of amputation. Diabetes Care 1998; 21(5): 855-859.

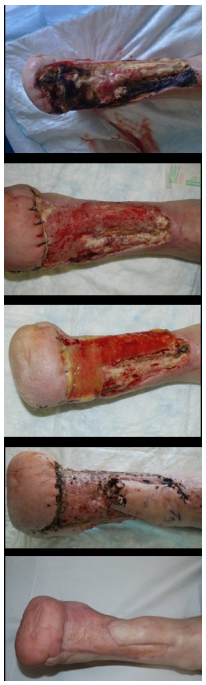
15. Téot L, Guillot-Masanovic M, Miquel P Truchetet F, Meaume S, Dompmartin A et al. Clinical impact of negative-pressure wound therapy: A 1,126-patient observational prospective study. Wound Repair Regen. 2014;22(3):341-50.
16. Maan ZN, Januszyk M , Rennert RC et al. Non contact, low-frequency ultrasound therapy enhances neovascularization and wound healing in diabetic mice. Plast Reconstr Surg. 2014;134(3):402e-411e.
17. Coleman SR. Structural fat grafting. Aesthet Surg J. 1998;18(5):387-389.
18. Salemi S, Rinaldi C, Manna F, Guarneri GF, Parodi PC. Reconstruction of lower leg skin ulcer with autologous adipose tissue and platelet-rich plasma. J Plast Reconst Aesthet Surg. 2008;61(12):1565-1567.
19. Fraser JK, Hicok KC, Shanahan R, Zhu M, Miller S, Arm DM. The Celution® System: Automated Processing of Adipose-Derived Regenerative Cells in a Functionally Closed System. Adv Wound Care. 2014;3(1):38-45.
20. Hasdmir M, Agir H, Eren GG, Aksu MG, Alagoz MS, Duruksu G et al.. Adipose-derived stem cells improve survival of random pattern cutaneous flaps in radiation damaged skin. J Craniofac Surg. 2015;26(5):1450-1455.
21. Schipper BM, Marra KG, Zhang W, Donnenberg AD, Rubin JP. Regional anatomic and age effects on cell function of human adipose-derived stem cells. Ann Plast Surg. 2008;60(5):538-544.
22. Elgzyri T, Larsson J, Nyberg P, Thörne J, Eriksson KF, Apelqvist J. Early revascularization after admittance to a diabetic foot center affects the healing probability of ischemic foot ulcer in patients with diabetes. Euro J Vasc Endovasc Surg. 2014;48(4):440-446.
23. Mills JL Sr. Update and validation of the Society for Vascular Surgery wound, ischemia, and foot infection threatened limb classification system. Semin Vasc Surg. 2014;27(1):16-22.
24. Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. J Clin Invest. 2007;117(5):1219-1222.
25. Riccio M, Pangrazi PP, Parodi PC Vaienti L, Marchesini A, Neuendorf AD et al. The amnion muscle combined graft (AMCG) conduits: a new alternative in the repair of wide substance loss of peripheral nerves. Microsurgery. 2014;34(8):616-622.
26. Tepper OM, Carr J , Allen RJ Jr Chang CC, Lin CD, Tanaka R et al. Decreased circulating progenitor cell number and failed mechanisms of stromal cell-derived factor 1alpha mediated bone marrow mobilization impair diabetic tissue repair. Diabetes. 2010;59(8):1974-1983.
27. Lee RH, Kim B, Choi I Kim H, Choi HS, Suh K et al. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. Cell Physiol Biochem. 2004;14(4-6):311-324.
28. Zuk PA, Zhu M, Ashjian P De Ugarte DA, Huang JI, Mizuno H et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2015(12);13. 4279-4295
29. Fraser JK, Wulur I, Alfonso Z, Hedrick MH. Fat tissue: an underappreciated source of stem cells for biotechnology. Trends Biotechnol. 2006(4);24:150-154.
30. Hassan WU, Greiser U, Wan W. Role of adipose-derived stem cells in wound healing. Wound Repair Regen. 2014(3); 22:313-325.
31. Matsumoto D, Sato K, Gonda K et al. Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. Tissue Eng. 2006;12(12):3375-3382.
32. Koh KS, Oh TS, Kim H Chung IW, Lee KW, Lee HB et al. Clinical application of human adipose tissue-derived mesenchymal stem cells in progressive hemifacial atrophy (Parry-Romberg disease) with microfat grafting techniques using 3-dimensional computed tomography and 3-dimensional camera. Ann Plast Surg. 2012;69(3):331-337.
33. Vaienti L, Gazzola R, Villani F, Parodi PC. Perineural fat grafting in the treatment of painful neuromas. Tech Hand Up Extrem Surg. 2012;16(1):52-55.
34. Moseley TA1, Zhu M, Hedrick MH. Adipose-derived stem and progenitor cells as fillers in plastic and reconstructive surgery. Plast Reconstr Surg, 2006;118(3 Suppl):121S-128S.

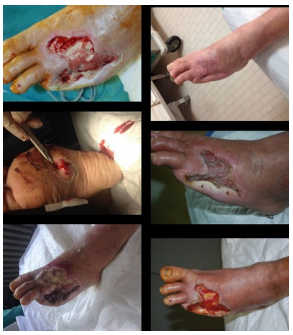
35. Coleman SR. Hand rejuvenation with structural fat grafting. Plast Reconstr Surg. 2002;110(7):1731-1744.
36. Piccinno MS, Veronesi E, Loschi P Pignatti M, Murgia A, Grisendi G et al. Adipose stromal/stem cells assist fat transplantation reducing necrosis and increasing graft performance. Apoptosis. 2013;18(10):1274-1289.
37. Domenis R, Lazzaro L, Calabrese S Mangoni D, Gallelli A, Bourkoula E et al. Adipose tissue derived stem cells: in vitro and in vivo analysis of standard and three commercially available cell-assisted lipotransfer techniques. Stem Cell Res Ther. 2015;5(6):2.
38. Lee HC, An SG, Lee HW Park JS, Cha KS, Hong TJ et al. Safety and Effect of Adipose Tissue-Derived Stem Cell Implantation in Patients With Critical Limb Ischemia. Circ J. 2012;76(7):1750-1760.
· 39. Hong SJ, Jia SX, Ping Xie Xu W, Leung KP, Mustoe TA et al. Topically Delivered Adipose Derived Stem Cells Show an Activated-Fibroblast Phenotype and Enhance Granulation Tissue Formation in Skin Wounds. PLoS One. 2013;8(1):e55640.
40. Moon MH, Kim SY, Kim YJ Moon MH, Kim SY, Kim YJ et al. Human adipose tissue-derived stem cells improve postnatal revascularization in a mouse model of hindlimb ischemia. Cell Physiol Biochem. 2006;17(5-6):279-290.
41. Cho HH, Kim YJ, Kim JT V Song JS, Shin KK, Bae YC et al. The role of chemokines in proangiogenic action induced by human adipose tissue-derived mesenchymal stem cells in the murine model of hindlimb ischaemia. Cell Physiol Biochem. 2009;24(5-6):511-518.
42. Chung HM, Won CH, Sung JH. Responses of adipose-derived stem cells during hypoxia: enhanced skin-regenerative potential. Expert Opin Biol Ther. 2009;9(12):1499-1508.
43. Liu Y, Xu Y, Fang F. Therapeutic Efficacy of Stem Cell-based Therapy in Peripheral Arterial Disease: A Meta-Analysis. PLoS One. 2015;10(4):e0125032

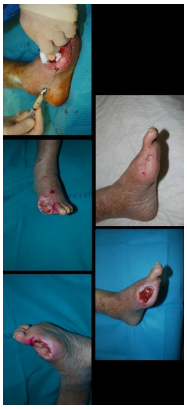
Figure 1: An extensive, non-healing full-thickness ulcer of the left forefoot in a 67-year-old insulin-dependent diabetic arteriopathic patient (A-B). After two weeks of NPTW (C) the lesion was granulating but the margins of the wound were not progressing satisfactory. (D) The wound one day after surgical debridement and amniotic membrane positioning. (E) Two weeks after covering with a split-thickness skin graft. (F) The aspect of the foot after 1 year.

Figure 2: (A-B) Ulcer occurring over a left first ray amputation stump in a 69-y.o. man with a background of multiple comorbidities. After 2 weeks of NPWT and surgical toilette, we grafted 10 ml of adipose tissue harvested from the abdominal region under local anaesthesia (C). (D) The appearance of the wound 1 week after the procedure. (E) Complete re-epithelisation one month after skin grafting.

Figure 3: (A-B) Ulcer occurred over a left first ray amputation stump in a 69 yo man with a multiple comorbidities background. After 2 weeks of NPWT and surgical toilette, we grafted 10 ml of adipose tissue harvested from the abdominal region in local anaesthesia (C). (D) The aspect of the wound 1 week after the procedure. (E) Complete reepithelization one month after skin grafting.







Average age Sex	64,9 yo
Females Males	n=2 n=17
DM II OT ID	 n=7 n=12
COPD	n= 7
IC	n= 5
CRF	n= 3
Dyslipidaemia	n= 18
HT	n= 19
Average ulcer area	20,3 cm2
Ulcer Classification (TWC) Grade: II III Stage: C D	 n= 4 n=15 n=9 n=10

Ulcer localization	n=4
Foot	n=12
Leg	n=3
Foot and leg	

Table 1: Main patient and ulcer features. OT: oral therapy; ID: insulin-dependent; COPD: chronic obstructive pulmonary disease; IC: ischaemic cardiopathy; CKF: chronic kidney failure; HT: hypertension