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Microfractured Lipoaspirate May Help Oral Bone and Soft Tissue Regeneration: a Case Report

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Abstract

Background: **Among most of the mesenchymal stem cells sources, adipose tissue represents an ideal source because of the easy access and the simple isolation procedures. We developed an innovative technique (Lipogems®) to obtain microfragmented fat tissue transfer. This adipose tissue houses intact stromal vascular niche and mesenchymal stem cells with high regenerative capacity.**

Objectives: **Aim of this case report is to show a novel Lipogems® application in a difficult case of oral surgery.**

Materials and Methods: **We treated a difficult patient with localized oral bone atrophy with Lipogems® micro fat grafting technique in combination with swine cortico-cancellous bone mix Gen-Os. The patient was followed-up for 12 months.**

Results: **As in other surgical applications, we observed an excellent tissue healing and absence of inflammation and infection, and a significant subjective pain reduction within the grafted areas. The postoperative radiographic evaluation and the histological slides showed homogeneous redefinition of bone defects, and intensive osteointegration.**

Conclusion: **This case report suggests that Lipogems® may improve the healing, osteointegration and stability of the implants in newly formed bone. More studies are required to confirm the efficacy of Lipogems® in oral surgery on larger scale.**

Keywords: Lipogems®, Lipoaspirate, Adipose tissue, Oral surgery.

Introduction

The adipose tissue represents a very attractive source of mesenchymal stem cells (MSCs) and supports the healing and regeneration of many tissues[1-5]. Adipose tissue harvesting is greatly facilitated by the technique of liposuction, which is relatively non-invasive and results in minimal discomfort of the patient and it is easily performed under local anesthesia[6,7].

Adipose derived mesenchymal stem cells are routinely obtained enzymatically from fat lipoaspirates as SVF[8] and/or may undergo prolonged *ex vivo* expansion, with significant senescence and decline in multipotency, which leads to clinical results below the expectations[9]. Besides, these techniques have complex regulatory issues[10].

Objective of this case report is to show how micro-fractured fat transplantation obtained with the new device Lipogems® is safe and effective in the treatment of an oral bone defect.

Material and methods

Harvesting and processing of the fat tissue

Lipogems System has been developed to improve the classical fat graft lipofilling technique[5] with the aim of providing transplantable clusters of lipoaspirate with reduced size for the improvement of their post-transplant engraftment[2,11]. The system consists of a disposable kit for the aspiration, processing and reinjection of adipose tissue in both human and veterinary medicine[12-17]. Before harvesting the fat, the selected site is injected by local anesthesia and adrenaline at high dilution[12]. Its core is a disposable and closed device filled with saline solution that pro-

gressively reduces the size of the clusters of adipose tissue by means of mild mechanical forces and eliminates oil and blood residues responsible for the inflammation processes. The technique is gentle and intra-operatively provides micro-fractured fat in a short time (15-20 minutes), without expansion and/or enzymatic treatment[12,17]. The vasculo-stromal niches of Lipogems® survive in the site of the injection and improve tissue health. To induce new bone formation, we mixed Lipogems® with the swine cortico-cancellous bone mix Gen-Os (http://www.ostralos.com/webapps/category/102540/336204/99644) which has been demonstrated to have a high osteoconductive activity. We selected Gen-Os because of its highest similarity with the human bone in terms of actual density, mineral content, infrared spectroscopy, FTIR and XRD diffractograms. Gen-Os expands up to 50% in volume after hydration with sterile saline: hydrated collagen contained in each granule also increases biomaterial adhesiveness.

Histological analysis

Three months after the surgical procedure, the carrot for the histological analysis were extracted from the cortical/medullary – vestibular/lingual direction, more or less in the center of the area regenerated with a cutter Trefine ∅ 3 mm. Samples had cylindrical shape, about 7 mm long axis and 2.5 mm diameter. The specimens were fixed at room temperature for 2 days in 10% phosphate buffered formaldehyde pH 6.9, and dehydrated overnight in ethanol prior to a three-step impregnation in a methylmethacrylate (MMA) monomer (Merk) for at least 3 days. For embedding, specimen blocks were stored in MMA 80%, Plastoid N 20% (Rohm Pharma, Germany) for 2 h in uncapped vials under vacuum and embedded in capped 10 mL glass vials at 37°C overnight. After the polymerization, the glass vials were removed and moistened sections (50 μm) were cut using a Leica SP 1600 Saw Microtome with a rotating diamond saw blade for high-quality sample preparation of hard materials for microscopic analysis. After the cutting, slides were smoothed on a Mecapol 220 U (Presi, Grenoble, France) using the LD polishing methods with diamond abrasives and mounted on polyethylene slides. The cutting was performed on the long axis of the carrots; the polished sections were stained using fucsin/light-green and Toluidin blue (Sigma) for histological evaluation.

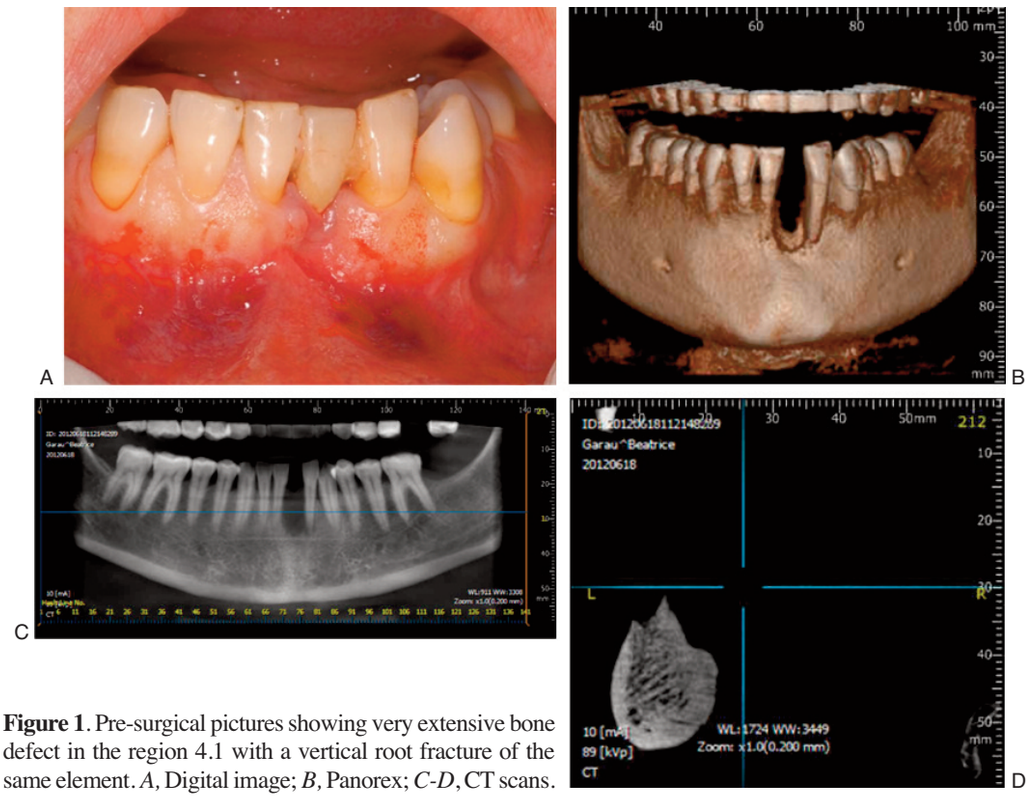


Figure 1. Pre-surgical pictures showing very extensive bone defect in the region 4.1 with a vertical root fracture of the same element. *A*, Digital image; *B*, Panorex; *C-D*, CT scans.

Ethics

The procedures mentioned in this case reports are in accordance with the ethical standards and with the Helsinki Declaration of 1975, as revised in 2000. The patient has been informed about risks, benefits and alternative options to the proposed treatment. She expressed and signed the informed consent form.

Case report

Forty-five (45) years old female with a very localized bone defect, 3 walls, in the region 4.1, which resulted in a vertical root fracture of the same element. The clinical history showed diabetes type I on insulin therapy. All the biochemical parameters were stable. Considering the systemic disease of the patient (Figure 1) and evaluating all the possible therapeutic options to repair the localized bone defect, Lipogems® mixed with Gen-Os appeared to be the most suitable, because of its non-invasiveness and the possibility that Lipogems® might lower the risk of complications[15].

First surgical procedure

We harvested and processed the subcutaneous fat with Lipogems® as previously described[14]. We then mixed the micro-fragmented fat with the scaffold Gen-Os, a swine cortico-cancellous bone mix with high osteoconductive activity (http://www.ostralos.com/webapps/category/102540/336204/99644).

We performed a full-thickness 1 cm vertical incision between the first premolar and the lower right canine (4.4-4.3), with a 2/4 Molt, and gently proceeded in the direction of the defect in order to realize a horizontal tunnel. At the point 4.1, we accurately detached the periosteum on all the walls of the defect keeping it as intact as possible and stretching it in order to have enough free space for the insertion of the mix Lipogems/Gen-Os.

To harvest, compact and place in site the mix, we used a special syringe for biomaterials. We closed the tunnel with sutures in GoreTex CV5 (Figure 2).

We post-surgically treated the patient for 5 days with 1200 mg Bacampicilline (2 capsules per day), 400 mg Dexibuprofene (2 capsules per day), and 20 mg Pantoprazole (1 capsule per day).

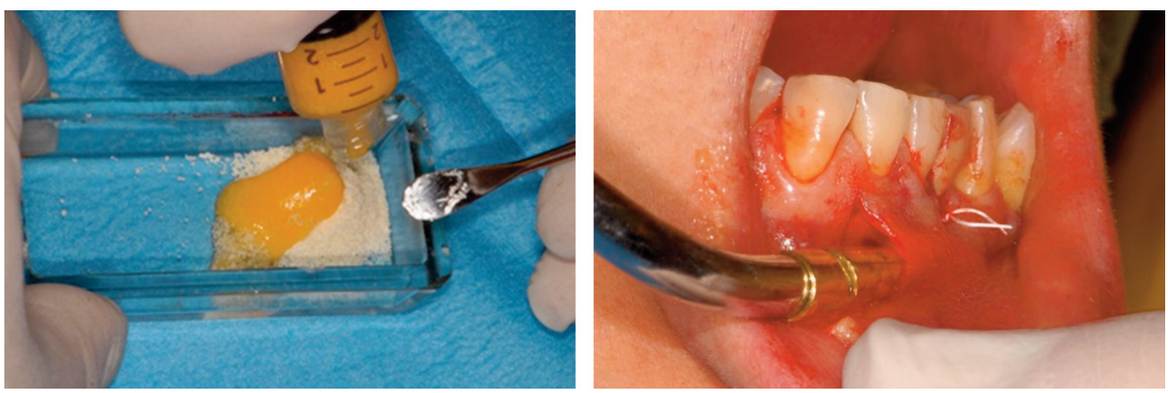
Seven days after the treatment, we observed good healing of the soft tissue and absence of edema. After the removal of the sutures at day 15, we observed a perfect tropism of the surrounding tissue. The clinical picture was excellent with a consistent improvement and acceleration of the healing process of the soft tissues and bone density, measured with the high rating score scale Hunsfield, of 850 at the second month and 980 the third month after surgery (Figure 3)[18].

Second surgical procedure

After 3 months, we implanted the prosthesis into the regenerated defect. Because of the paucity of the inter-radicular space, we selected a direct implant "One-piece" with a diameter of 2.6 mm. We placed the implant in 35 Ncm of Torque and, immediately after, positioned a passive prosthesis keeping it in a light static and dynamic occlusion.

The CT scan performed three months after the implant of the prosthesis showed a complete bone regrowth (Figure 4).

In summary, we evaluated the efficacy of our approach according to clinical, histological and radiological parameters. In details, 7 days after the treatment, we observed a very satisfactory healing of the soft tissue and absence of edema. The patient did not suffer any disturbance in the grafted area and reported a modest pain in the lumbosacral area



A B

Figure 2. Lipogems® is mixed with Gen-Os *(A)* and inserted in the periosteal tunnel *(B)*.

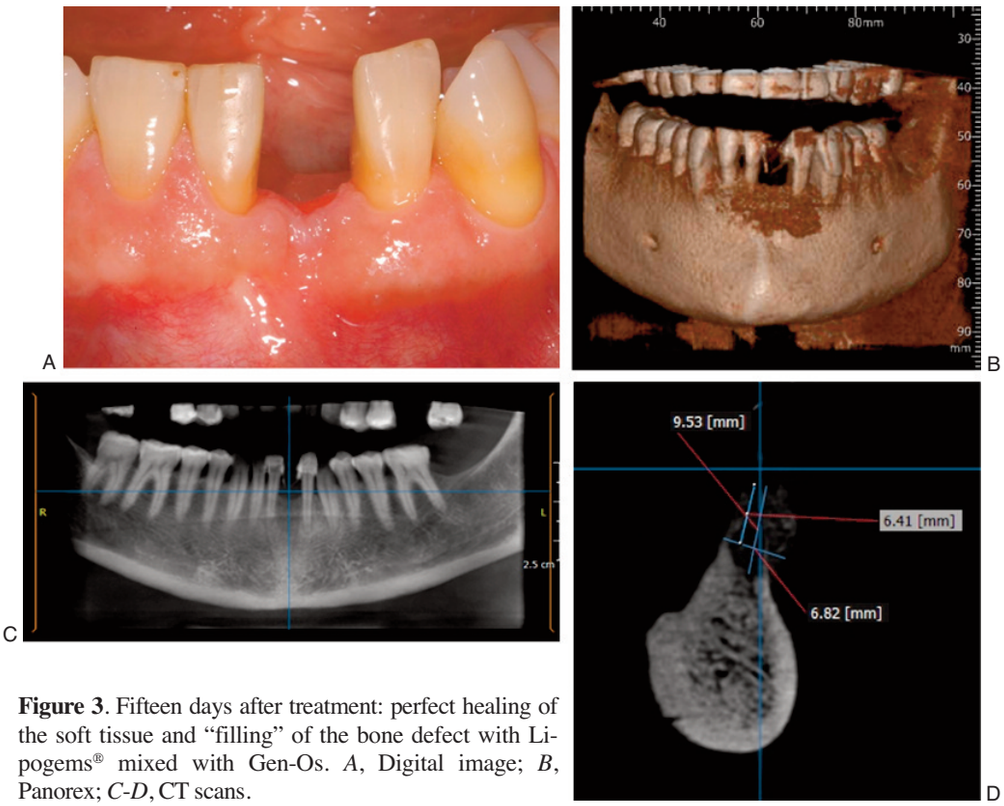


Figure 3. Fifteen days after treatment: perfect healing of the soft tissue and "filling" of the bone defect with Lipogems® mixed with Gen-Os. *A*, Digital image; *B*, Panorex; *C-D*, CT scans.

where we harvested the fat. After the removal of the sutures at day 15, we observed a perfect tropism of the surrounding tissue. The clinical picture was excellent with a consistent improvement and acceleration of the healing process of the soft tissues and bone density, as observed in similar cases previously treated without Lipogems®.

The follow-up at one, two, three, six, and twelve months also revealed an early and intensive osteosynthesis, which is well documented also by the histological analysis done on samples at three months after the second surgical procedure (Figures 5 and 6). Indeed, Fucsin/Light Green staining revealed the formation of mineralized bone stained in green and indicated in Figures as lb (lamellar bone) when mature, and wb (woven bone) when immature; soft/newly formed tissue is revealed in red and indicated in Figures as ft (fibrous tissue) when characterized by fibroblasts in collagen fibres and mt (mineralizing tissue) when it is not yet mineralized but the organization of cells and extracellular matrix looks like immature bone and the staining from red became fuchsia. We observed excellent tissue neo-formation with no indications of inflammatory responses after 3 months from the second surgical procedure. After the treatment with Lipogems®, the areas previously de-

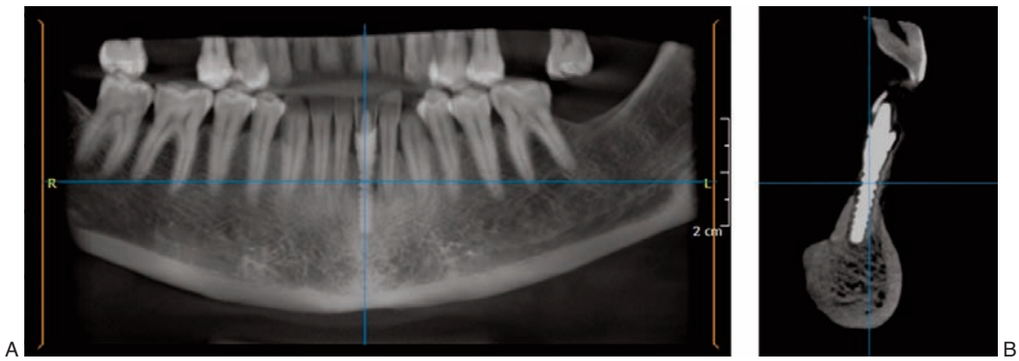


Figure 4. CT scan showing complete bone regrowth after 3 months.

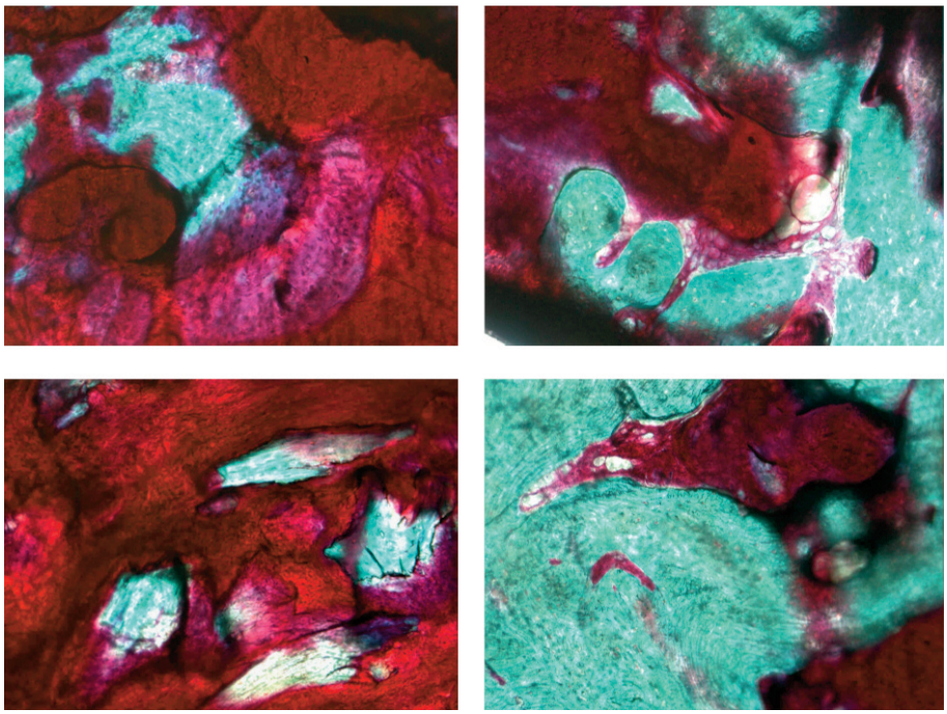


Figure 5. Histological sections of the tissue formed after three months from the second surgery. lb) lamellar bone evidenced by ordered disposition of osteocytes embedded in an orderly mineralized matrix; wb) woven bone, primary bone, with random distribution of osteocytes in a disorganized mineralized matrix; ft) dense fibrous tissue; mt) connective tissue partially mineralized; at) adipose tissue. Original magnification 100x; Fucsin/Light Green staining.

void of bone appeared almost fully jointed with connective tissue rich in fibrous matrix that occasionally loses its organization in parallel fibres turning into the typical structure of the newly formed bone. In addition, the newly formed tissue replaced the lipoaspirate and the particulate injected. Only in some areas, few residues of well-organized and vascularized adipose tissue are present as shown in Figures 5B and 5D and at higher magnification in Figure 6B.

Discussion

During the last two decades, treatment with implants become one of the first options for the prosthetic rehabilitation of partially edentulous jaws. There is a critical need to find new therapeutic approaches for the treatment of the atrophic alveolar bone, especially when the bone defects prevent immediate dental restoration. Up to now, several surgical options have been adopted for the reconstruction of a full-thickness

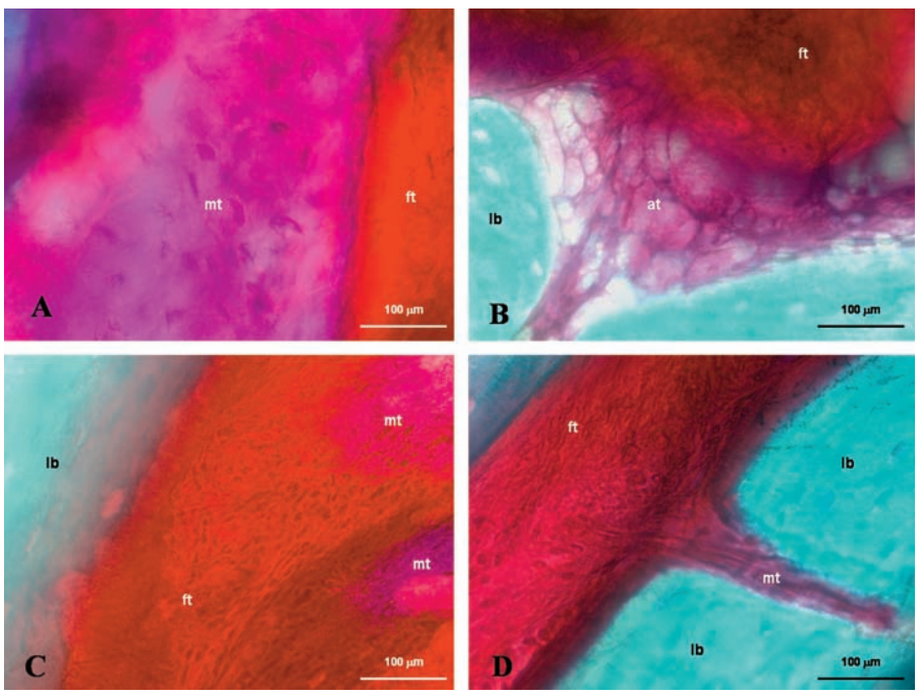


Figure 6. Histological images at 400X magnification of the areas included in white squares of the images reported in Figure 5.

alveolar defect, such as autologous bone grafts, allografts, xenografts, alloplastic materials, barrier membranes for guided bone regeneration (GBR), growth factors, platelet rich plasma (PRP) and distraction osteogenesis[19]. All of them aim to obtain enough bone for an optimal implant stabilization and a good osteointegration. The gold standard is the autologous bone graft[20], because of its osteogenic potential, biomechanical properties, and the biocompatibility. Nevertheless, the limit of the autologous bone graft is the technical difficulty and the morbidity of the harvested bone site. The use of allograft (human cadavers bone)[21] or xenograft (material derived from animals)[24] as well as synthetic bone substitutes (hydroxyapatite – HP) and bioactive glasses (alone or in combination with autogenous bone grafts) have been also proposed. Because of their osteoconductive properties, all of these approaches can provide the framework for bone formation, but not bone formation itself, as they are not osteoinductive. On the contrary, platelet rich plasma (PRP) and bone morphogenetic proteins (BMPs) have the ability to promote and stimulate bone development[23] and this can be attributed to the pro-angiogenic, -proliferative, and -differentiating effects on osteoblasts[24]. PRP is obtained by sequestering and concentrating platelets from whole blood[25] and it is very important in wound healing because it promotes coagulation and supplies growth factors[26]. Published data on PRP show controversial results, some indicating an improvement of the amount and density of the bone formation[24], some others showing no significant effects, possibly because of the low PRP concentration[27,28]. Therefore, the unpredictable amount of bone promotion and the variability in the levels of growth factors, make this approach still not completely standardized[27-29].

Recently, MSCs seeded onto porous ceramics scaffold with osteoconductive properties[30-32] have been proposed as an alternative method. Published data indicate sub-optimal results due to low rate of HP resorption and the poor plasticity, thus worsening the implantation procedure and even increasing the risk of infection[33]. Other studies using a combination of fibrin glue, MSCs and PRP reported good results. Indeed, neovascularization of bone with accelerated healing and earlier new bone formation were assessed[34,35]. Despite the good results, this method has the disadvantage of being time consuming, as it requires bone marrow aspiration, cell isolation and expansion[33]. An ideal human MSC source should be abundant, harvested with minimally invasive procedures, and should provide a cell population retaining good viability and differentiation potential despite donor age; adipose tissue incorporates all these features[9]. The therapeutic and healing effect of fat grafting is now widely recognized and attributed to the stromal vascular fraction, which contains the stem cell compartment, responsible for tissue remodeling[13].

The chance of clinical translation of the multilineage potential of adipose tissue derived MSCs is delayed by the poor/negligible cell survival within cryopreserved lipoaspirates, the difficulty of *ex vivo* expansion, and the complexity of current Good Manufacturing Practice (cGMP) requirements for expanded cells. Hence, availability of a minimally manipulated, autologous, hMSC/pericyte-enriched fat product would have remarkable biomedical and clinical relevance. Recently, Prof. Tremolada developed and patented an innovative system, named Lipogems®, providing a non-expanded, ready-to-use fat product. The system uses mild mechanical forces in a completely closed system, avoiding the use of enzymes, additives, and other manipulations. Differently from unprocessed lipoaspirate, the non-expanded Lipogems® product encompasses a remarkably preserved vascular stroma with slit-like capillaries wedged between adipocytes and stromal stalks containing vascular channels with evident lumina[36]. The entire procedure is fast, safe, and not subjected to the regulatory restrictions imposed by the Good Manufacturing Practice Guidelines. In this case report, we aimed to show how micro-fractured fat transplantation obtained with Lipogems® applied in combination with cortico-cancellous inorganic bone of pig (xenografts) could improve the healing, osteointegration and stability of the implant. The results are very promising: no infection within the site of implant, halved healing time, no periodontal inflammation and retraction, and good osteointegration and stability of the implant.

We are aware that this case report has a number of limitations, and we certainly know that more studies are required to confirm the efficacy of Lipogems® in oral surgery on larger scale. We anticipate that other patients with combined bone defects (horizontal/vertical) have been treated with Lipogems® mini-invasive technique and the results, although partial, are very promising. At this stage, we cannot demonstrate the efficacy of our approach with a comparative histological analysis at defined endpoints in terms of differences in the rate of neoosteogenesis and maturation of newly formed bone tissue compared with control cases treated with traditional approaches[37-40]. Based on our experience,

we can assert that the treatment with Lipogems® constantly improved the healing rate of the soft tissues within the surgical incision. In addition, we observed an excellent tissue tropism even starting from day 2, with little or no post-surgical edema, and abundant induced neo-vascularization. The histological analysis after 3 months revealed a level of maturation of the bone tissue, which is usually detectable after 6 months or more.

To confirm these data, we are setting a randomized double-blind clinical trial possibly performed bilaterally, with a test site and contralateral site control. Anyhow, as soon as the cases actually under treatment with Lipogems® will be concluded, we will extend this preliminary report with additional results.

Conclusions

Many technical approaches to restore bone defects in oral surgery have been suggested and discussed, and different results in terms of osteointegration, rate of infection, healing rate, and stability of the implant are reported. This case report suggests that Lipogems® micro-fractured fat may be a safe and effective option to improve bone regeneration. Of course, more studies are required to confirm this technique in oral surgery on larger scale and in different clinical situations.

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