

Adipose-Derived Mesenchymal Stem Cells Transplantation for Socket Preservation: A Clinical Report

Tabanella Giorgio^{1*}, Ferlosio Amedeo², Orlandi Augusto³ and Gennai Alessandro⁴

¹O.R.E.C.-Oral Reconstruction and Education Center, Via Rovereto, Rome, Italy

²Biomedicine and Prevention Department, Tor Vergata University of Rome, Rome, Italy

³Biomedicine and Prevention Department Tor Vergata University of Rome, Rome, Italy

⁴Plastic, Reconstructive, and Aesthetic Surgeon, Specialized in General Surgery, Contract Professor at the University of Camerino, Italy

***Corresponding Author:** Tabanella Giorgio, O.R.E.C.-Oral Reconstruction and Education Center, Via Rovereto, Rome, Italy.

Received: February 18, 2019; **Published:** April 22, 2019

Abstract

Introduction: Tooth loss may be associated to a significant tridimensional alveolar bone collapse that can impede a proper implant prosthetic driven insertion. Socket preservation is an effective technique that has been proposed to minimize bone resorption however there is still a lack of a well-defined protocol since different biomaterials can be utilized. Recent developments in the field of tissue engineering have brought to clinician's attention the use of stem cells for tissue regeneration. A new technique is then described on three consecutive patients seeking alveolar bone maintenance after tooth extraction.

Case Presentation: Three consecutive patients needing extraction and endosseous implant placement were enrolled in the study. Simultaneous tooth extraction and socket preservation with autotransplanted adipose-derived mesenchymal stem cells was performed on each patient.

Conclusion: Adipose-derived mesenchymal stem cells transplantation may be suitable to reduce bone remodeling following tooth extraction.

Keywords: Socket Preservation; Stem Cells; Mesenchymal Stem Cells; Bone Augmentation; Dental Implant; Transplantation

Introduction

Tooth extraction may be associated to a significant loss of alveolar bone [1-3]. It is well known that the amount of bone resorption after tooth extraction may be related to several factors such as tissue biotype [4], presence of an acute infection and simultaneous extractions of adjacent teeth [5].

Several papers have been investigating different biomaterials to be used for socket preservation thus limiting the amount of alveolar bone loss [6-8]. However, there is still no scientific evidence to provide clear guidelines in regards to the type of biomaterial and surgical procedure that should be used to prevent bone collapse after extraction [7]. Araujo, *et al.* [3] demonstrated that alveolar bone resorption already occurs during the first week and is mainly localized on the buccal plate. This cascade of events is of importance when an endosseous dental implants has to be inserted in an esthetic area since there is an urgent need to preserve the residual bone volume [9]. It is reported up to 50% of bucco-lingual bone loss during the first year after extraction [10] and an average bone loss of 3.6 mm horizontally and 2.15 mm vertically [11].

Innovative developments in tissue engineering have brought new applications in the field of tissue regeneration. The therapeutic potential of human multipotent mesenchymal stem cells (MSCs), which are harvested from adipose tissue, has generated increasing interest. In fact, mesenchymal stem cells can differentiate into a variety of tissue types: bone, cartilage and nerve tissue. The main advantage of adipose-derived stem cells compared to other sources is the large number of cells that can be easily and quickly selected from adipose tissue [12]. Furthermore, growth factors secreted by MSCs might also promote neovascularization [11].

In this manuscript we described a new approach for socket preservation: the use of adipose-derived mesenchymal stem cells transplantation in three consecutive patients.

Case Report and Methodology

The study was approved by the Ethical Committee (on March 31st 2017, protocol #764). Three consecutive patients were selected for this pilot study. Patients were informed about the purpose of the study and then the first three consecutive patients who signed the informed consent were enrolled. No exclusion criteria were considered. All the extracted teeth were maxillary molars (Figure 1 and 2). The extractions were due to advanced periodontal disease in two patients and deep caries in one patient. Participants received an antibiotic therapy in association to tooth extraction (1 tab of augmentin twice a day for 6 days). The target area where a tooth has to be extracted was infiltrated with local anesthesia (articaine 4% 1:100.000). Prior to tooth removal adipose tissue was harvested from the suprapubic region in two of the three patients and from the pretrochanteric area in the third patient.



Figure 1: Pre-operative clinical view. The first maxillary right molar shows a deep caries associated to periodontal disease and mucogingival recession.

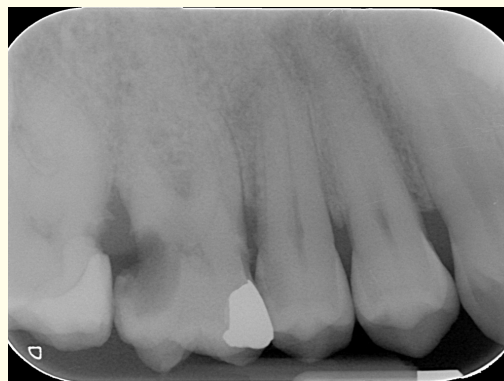


Figure 2: The peri-apical radiograph is showing a deep caries and a distal periodontal crater.

Fat preparation

Solution of lidocaine (400 mg), sodium bicarbonate (5 mEq) and epinephrine (1 mg), was prepared in a Cold Ringer's lactate solution (500 mL) and was infiltrated into the autologous collection site(s) (Figure 3).



Figure 3: The retrochanteric area is infiltrated with lidocaine.

Harvesting procedure

Adequate skin antisepsis was executed by the application of povidone iodine before the surgical procedure. The Superficial Enhanced Fluid Fat Injection (SEFFI) [13] as a tissue harvesting technique was performed by manual aspiration of the autologous fat with a 10-mL syringe mounted on a 20 cm long cannula with multi-perforated ports of either 0.5 mm or 0.8 mm in diameter (Figure 4). Manual micro tissue aspiration was performed 15 minutes after the infiltration of the prepared solution in the donor site.



Figure 4: Adipose tissue is gently harvested by aspiration.

Tissue preparation

The aspirated material was then gently cleaned with cold Ringer's solution and left to decanter in a vertical position for about 2 minutes inside the syringes. The liquid part, collected at the bottom of the syringes, was then eliminated and the whole procedure was repeated again to ensure the rinsing of the adipose tissue and the elimination of the anesthetic solution and blood, designed to facilitate tissue precipitation (Figure 5).

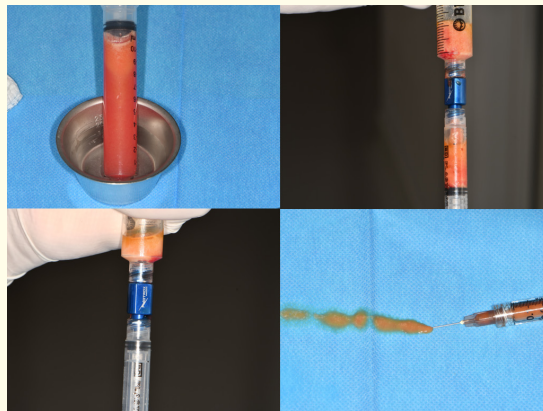


Figure 5: The aspirated material is cleaned with cold Ringer’s solution.

Extraction

A sharp dissection of the supragingival fibers was achieved by using a 15C blade and an ultrasonic device. After gentle luxation one tooth for each patient was extracted and the processed stem cells were positioned into the socket (Figure 6) and covered with spongy collagen (Figure 6) which was sutured on the attached gingiva. After allowing the complete wound closure, four months after socket preservation (Figure 7) a bone biopsy was performed with a 2 mm internal diameter trephine bur (Figure 8-10). The implant site was then prepared with a combination of osteotomes, twist and tapered drills to allow the insertion of an endosseous dental implant of 4.3 mm of diameter (Nobel Replace Groovy CC RP- Nobel Biocare AB).

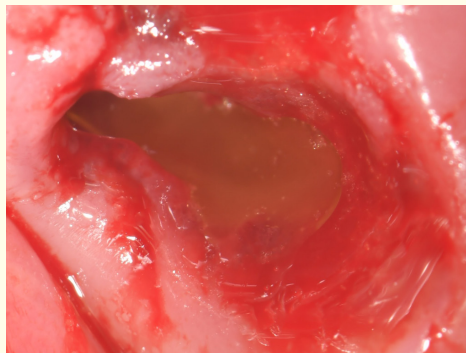


Figure 6: The stem cells are transplanted into the socket and Spongy collagen and criss cross sutures are used to stabilize the grafted material

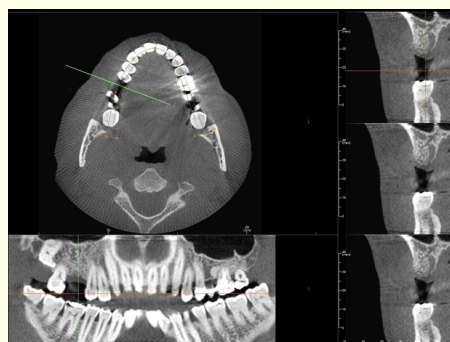


Figure 7: Four months after transplantation the CBCT sagittal cuts are revealing the regenerated bone with a trabecular aspect.

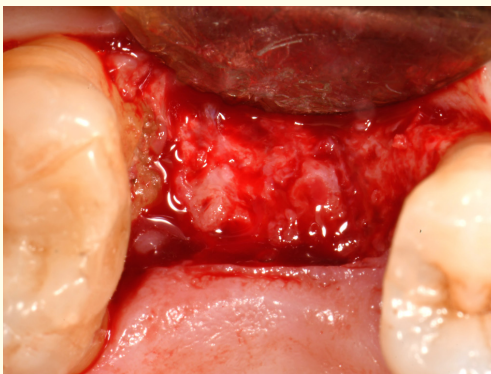


Figure 8: The intrasurgical picture is showing a newly formed spongy bone and an overall well-maintained alveolus.

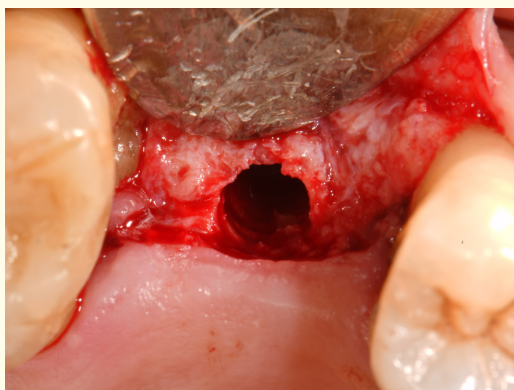


Figure 9: A trephine bur is used to harvest a bone biopsy for histological analysis.

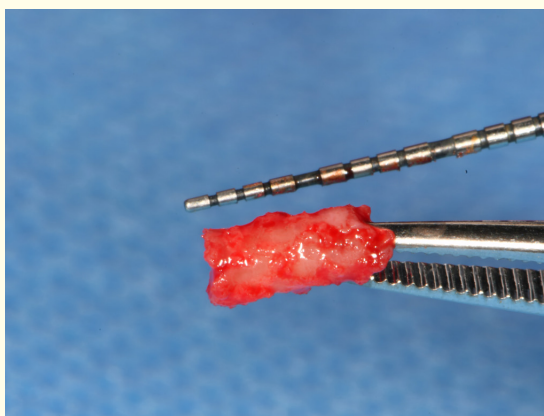


Figure 10: A bone biopsy of 7 mm is harvested.

Histotechnical preparation

The tissue samples were fixed in 10% neutral buffered formalin for almost 24h and then decalcified with a diaphath solution (microdec, EDTA-based) for two hours (two passage of 1 hour each). Finally, they were embedded in paraffin wax and 4 µm serial sections were cut at different levels and stained with haematoxylin and eosin for each block. The slides were examined with a Nikon Eclipse E1200 light microscope and pictures taken with a Nikon camera system.

Measurements

Intrasurgical measurements were registered with a caliper (Caliper Castroviejo long 0-40mm straight, Hu-Friedy Chicago USA). The coronal margin of the buccal plate was considered as a point of reference for the measurements of the soft tissue. After allowing the socket to heal for 4 months, the measurements were taken again with the same caliper. A periodontal probe (North Carolina 15 UNC color coded probe, Hu-Friedy Chicago USA) was used to register the amount of buccal keratinized tissue (Table 1).

Patient	Bucco-Lingual (MM)	Bucco-Lingual at 4 Months (MM)	Mesio-Distal (MM)	Mucosa Thickness (Average in MM)	Mucosa Thickness at 4 Months (MM)	Keratinized Mucosa (Average in MM)	Keratinized Mucosa at 4 Months (MM)
1	7.0	6.5	9.0	4.5	4.0	3.7	4.0
2	12.0	11.5	6.0	2.5	3.5	2.5	3.5
3	16.0	16.5	10.0	2.5	4.0	5.7	7.0

Table 1: Average data reporting measurements at baseline and after 4 months of healing.

Results

Patient reported no swelling neither pain at the donor as well as recipient site. None of the patients needed any medication for pain control. The macroscopic evaluation of the site at the time of second surgery revealed the presence of a unique bony architecture with spongy bone and larger trabecular spaces. However, the healed bone was dense and the achieved primary stability ranged from 75 to 79 ISQ values. The healed bone was more rigid and less susceptible to be condensed with osteotomes compared to the usual bone density which is found in the maxillary molar region.

Microscopic evaluation demonstrated small biopsies of trabecular bone constituted by mature lamellar bone lined by flat osteoblasts and sparse ossifying “blue lines” (Figure 11-13). The final peri-apical radiograph is showing the balance between the definitive screw-retained zirconia restoration and the healed bone after stem cells transplantation (Figure 14).

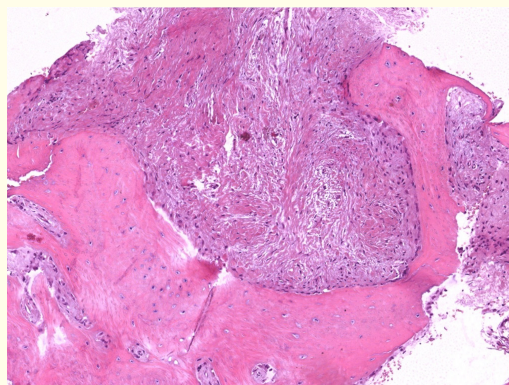


Figure 11: Microscopic evaluation demonstrated small biopsies of trabecular bone at 400X.

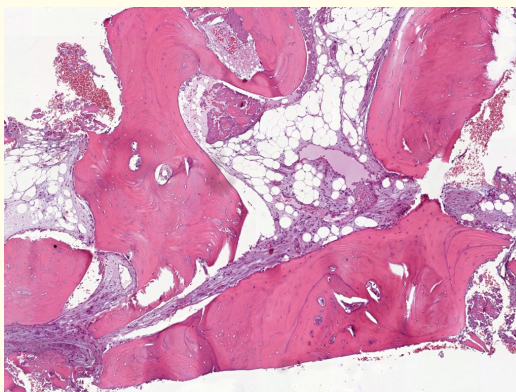


Figure 12: Mature lamellar bone is lined by flat osteoblasts at 400X.

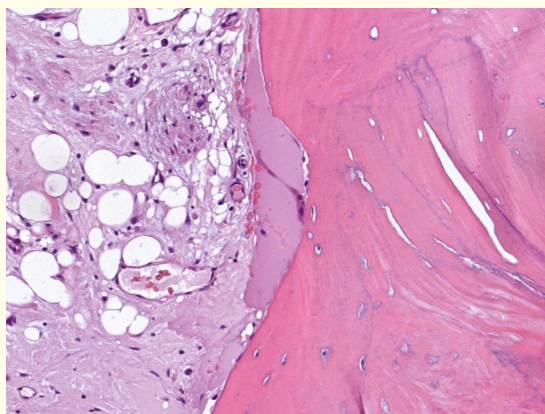


Figure 13: Sparse ossifying "blue lines" are evident at 4 months after stem cells transplantation at 400X.

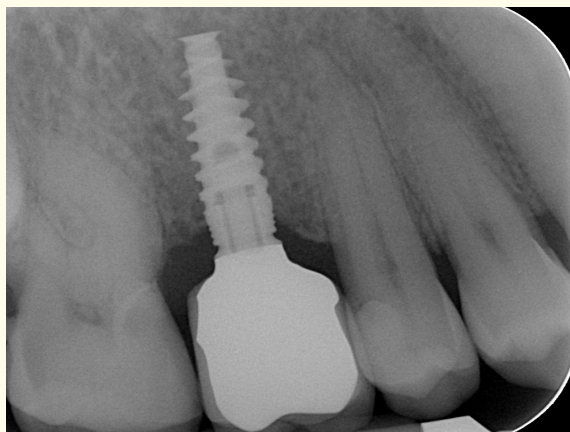


Figure 14: Final peri-apical radiograph.

Discussion

Tridimensional implant prosthetic positioning is crucial for a correct emergence profile as well as and biomimetic and long-lasting results [14]. Furthermore, the maintenance of socket volumes after tooth extraction is influencing the correct implant positioning [15]. However, the healing of extraction sockets involves bone remodeling that inevitably leads to atrophic changes of the alveolar ridge. Then research has been conducted to define a protocol to preserve the alveolar bone following extraction [8]. However up to date there is no evidence that describes a biomaterial as superior to others for the maintenance of socket volumes [16]. Besides there is a clear evidence in the literature that shows the presence of remnants of grafting materials after an average of 3 to 9 months from socket preservation [17-24]. The data reported in this article however describes 100% vital bone after using adipose-derived mesenchymal stem cells as a novel approach for socket preservation. The histological analysis reported a vital bone tissue since the presence of osteocytes nuclei. However, the absence of clear osteoid synthesis prevent us to evidence new bone formation. The more evident changes have been appreciated in bone marrow tissue mainly replaced by scarring at diverse times of developing from necrosis of fat tissue to early granulation tissue synthesis evident as blue substance deposition in extracellular milieu. Moreover, a cellular collagenized stroma is apparent in other pictures with many elongated fibroblasts embedded in pink collagen fibers. Nevertheless, a real inflammatory response is no present. Only rare multinucleated giant cells have been observed particularly near fat necrosis. Finally, the clinical data (Table 1) demonstrated the overall dimensional stability of the sockets treated with mesenchymal stem cells at 4 months. The minor bucco-lingual loss was evident in smoker patients (two of the three patients). There was also a trend of increased soft tissue thickness as well as higher band of keratinized mucosa but this may be related to the soft tissue manipulation executed at second stage surgery rather than to the stem cells transplantation.

Conclusion

Although adipose-derived mesenchymal stem cells could be utilized as an alternative to biomaterials further research is need to draw any conclusion.

Bibliography

1. Amler MH, *et al.* "Histological and histochemical investigation of human alveolar socket healing in undisturbed extraction wounds". *Journal of the American Dental Association* 61 (1960): 32-42.
2. Trombelli L, *et al.* "Modeling and remodeling of human extraction sockets". *Journal of Clinical Periodontology* 35.7 (2008): 630-639.
3. Araujo MG and J Lindhe. "Dimensional ridge alterations following tooth extraction. An experimental study in the dog". *Journal of Clinical Periodontology* 32.2 (2005): 212-218.
4. Chappuis V, *et al.* "Ridge alterations post-extraction in the esthetic zone: A 3-D analysis with CBCT". *Journal of Dental Research* 92.12 (2013): 195S-201S.
5. Al-Askar M, *et al.* "Effect of Single and Contiguous Teeth Extractions on Alveolar Bone Remodeling: A Study in Dogs". *Clinical Implant Dentistry and Related Research* 15.4 (2011): 569-575.
6. Horváth A, *et al.* "Alveolar ridge preservation. A systematic review". *Clinical Oral Investigations* 17.2 (2013): 341-363.
7. Vignoletti F, *et al.* "Surgical protocols for ridge preservation after tooth extraction. A systematic review". *Clinical Oral Implants Research* 23.5 (2012): 22-38.
8. Hämmerle CH, *et al.* "Evidence-based knowledge on the biology and treatment of extraction sockets". *Clinical Oral Implants Research* 23.5 (2012): 80-82.
9. Jung RE, *et al.* "Alveolar ridge preservation in the esthetic zone". *Periodontology 2000* 77.1 (2018): 165-175.
10. Schropp L, *et al.* "Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study". *International Journal of Periodontics and Restorative Dentistry* 23.4 (2003): 313-323.

11. Ten Heggeler JMAG., *et al.* "Effect of socket preservation therapies following tooth extraction in non-molar regions in humans: a systematic review". *Clinical Oral Implants Research* 22.8 (2011): 779-788.
12. Tobita M and Mizuno H. "Adipose-derived stem cells and periodontal tissue engineering". *International Journal of Oral and Maxillofacial Implants* 28.6 (2013): 487-493.
13. Gennai A., *et al.* "Skin Rejuvenation and Volume Enhancement with the Micro Superficial Enhanced Fluid Fat Injection (M-SEFFI) for Skin Aging of the Periocular and Perioral Regions". *Aesthetic Surgery Journal* 37.1 (2017): 14-23.
14. Bidra AS. "Surgical and prosthodontic consequences of inadequate treatment planning for fixed implant-supported prosthesis in the edentulous mandible". *Journal of Oral and Maxillofacial Surgery* 68.10 (2010): 2528-2536.
15. Block MS. "Dental Extractions and Preservation of Space for Implant Placement in Molar Sites". *Oral and Maxillofacial Surgery Clinics of North America* 27.3 (2015): 353-362.
16. Chan HL., *et al.* "Alterations in bone quality after socket preservation with grafting materials: a systematic review". *International Journal of Oral and Maxillofacial Implants* 28.3 (2013): 710-720.
17. Pelegri AA., *et al.* "Clinical and histomorphometric evaluation of extraction sockets treated with an autologous bone marrow graft". *Clinical Oral Implants Research* 21.5 (2010): 535-542.
18. Froum S., *et al.* "Histological comparison of healing extraction sockets implanted with bioactive glass or demineralized freeze-dried bone allograft: a pilot study". *Journal of Periodontology* 73.1 (2002): 94-102.
19. Aimetti M., *et al.* "Clinical and histologic healing of human extraction sockets filled with calcium sulfate". *International Journal of Oral and Maxillofacial Implants* 24.5 (2009): 902-909.
20. Crespi R., *et al.* "Dental implants placed in extraction sites grafted with different bone substitutes: radiographic evaluation at 24 months". *Journal of Periodontology* 80.10 (2009): 1616-1621.
21. Crespi R., *et al.* "Comparison of magnesium-enriched hydroxyapatite and porcine bone in human extraction socket healing: a histologic and histomorphometric evaluation". *International Journal of Oral and Maxillofacial Implants* 26.5 (2011): 1057-1062.
22. Carmagnola D., *et al.* "Healing of human extraction sockets filled with Bio-Oss". *Clinical Oral Implants Research* 14.2 (2003): 137-143.
23. Heberer S., *et al.* "Healing of ungrafted and grafted extraction sockets after 12 weeks: a prospective clinical study". *International Journal of Oral and Maxillofacial Implants* 26.2 (2011): 385-392.
24. Barone A., *et al.* "Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorphometric study". *Journal of Periodontology* 79.8 (2008): 1370-1377.

Volume 18 Issue 5 May 2019

©All rights reserved by Tabanella Giorgio., *et al.*