

# Tissue engineering of bone: Clinical observations with adipose-derived stem cells, resorbable scaffolds, and growth factors

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Website:  
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DOI:  
10.4103/2231-0746.95308

Quick Response Code:



George K. B. Sándor

Professor of Tissue Engineering, Regea Institute for Regenerative Medicine, University of Tampere, Tampere, Finland

**Address for correspondence:**

Prof. George K.B. Sándor, Institute of Biomedical Technology, University of Tampere, Tampere, Finland.  
E-mail: george.sandor@uta.fi

## ABSTRACT

**Introduction:** Tissue engineering offers a simple, nonallergenic, and viable solution for the reconstruction of human tissues such as bone. With deeper understanding of the stem cell's pathobiology, the unique properties of these tissues can be effectively harnessed for the benefit of the patients. A primary source of mesenchymal stem cells (MSCs) for bone regeneration is from adipose tissue to provide adipose-derived stem cells (ASCs). The interdependency between adipogenesis and osteogenesis has been well established. The objective of this article is to present the preliminary clinical observation with reconstruction of craniofacial osseous defects larger than critical size with ASC. **Materials and Methods:** Patients with large craniofacial osseous defects only were included in this study. Autogenous fat from the anterior abdominal wall of the patients was harvested from 23 patients, taken to a central tissue banking laboratory and prepared. All patients were reconstructed with ASCs, resorbable scaffolds, and growth factor as required. Vascularized soft tissue beds were prepared for ectopic bone formation and later microvascular translocation as indicated. **Results:** 23 ASC seeded resorbable scaffolds have been combined with rhBMP-2 and successfully implanted into humans to reconstruct their jaws except for three failures. The failures included one infection and two cases of inadequate bone formation. **Discussion:** The technique of ASC-aided reconstruction of large defects still remains extremely sensitive as it takes longer duration and is costlier than the conventional standard immediate reconstruction. Preliminary results and clinical observations of these cases are extremely encouraging. In future, probably with evolving technological advances, ASC-aided reconstruction will be regularly used in clinical practise.

**Keywords:** Adipose-derived stem cells, growth factors, resorbable scaffold, tissue engineering

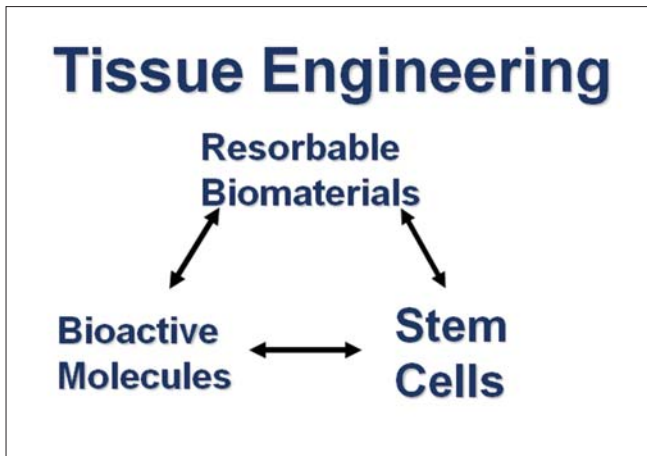
## INTRODUCTION

Tissue engineering efforts involve collaborations between groups of cell biologists, biochemists, biomaterial scientists, engineers, and clinicians.<sup>[1]</sup> In order to understand the complex role of the various components of tissue engineering, one can think of an equilateral triangle where stem cells, resorbable scaffolds, and bioactive molecules such as growth factors continuously interact with each other [Figure 1]. The science of tissue engineering is built upon the understanding of the nature of the interactions

between these three key components.<sup>[1]</sup>

### Sources of stem cells

The source of cells for tissue engineering depends on the requirements of the structure that is to be replaced. Human embryonic stem cells (hESCs) are pluripotent stem cells isolated from the inner mass of human blastocysts.<sup>[1]</sup> While hESCs have greatest potential due to their differentiation capacity, there are problems that must be solved before their clinical use in tissue engineering. The problems with hESCs include culturing hESC



**Figure 1:** An equilateral triangle illustrating three important components of tissue engineering and the continuous interaction between cells, resorbable scaffolds, and bioactive molecules or growth factors

without exposure to animal proteins (xeno-free media), avoidance of teratoma or hamartoma development, and immune rejection by the recipient host.<sup>[2]</sup> For the present time, it is adult stem cells that are used clinically. Those cells with the lowest morbidity in harvesting and those which still retain a degree of pluripotentiality would be the most advantageous in the tissue engineering of bone, for example.<sup>[1]</sup>

One source of mesenchymal stem cells (MSCs) for bone regeneration is from adipose tissue to provide adipose-derived stem cells (ASCs).<sup>[2,3]</sup> This should not be surprising as there is interdependency between adipogenesis and osteogenesis.<sup>[2,3]</sup> When we look at a sample of bone marrow, we find osteogenic cells, hematopoietic cells, and fat cells. Certainly, the harvesting of adipose tissue is not morbid and may even be advantageous for some if liposuction was used as the harvesting method [Figure 2].<sup>[3-6]</sup>

MSCs are capable of multiple lineage differentiation including adipocytes, chondrocyte, and osteoblast pathways. MSC clones sequentially differentiate into adipocytes, dedifferentiate, and subsequently transdifferentiate into osteoblasts *in vitro*.<sup>[6]</sup> Using the tissue engineering model, it has been possible to harvest autogenous ASCs from patients having a liposuction procedure and used to seed a resorbable scaffold<sup>[7]</sup> that can be made using CAD/CAM technology to the precise dimensions of a missing segment of bone. The seeded cells could be stimulated by physical means such as vibration loading<sup>[8,9]</sup> or with growth factors such as bone morphogenetic proteins (BMPs) to guide the differentiation and growth of the cells.<sup>[10-13]</sup> Although major segments of human mandibular defects have been reconstructed with constructs containing growth factors such as rhBMP-7, reports regarding cellularized grafts are lacking.<sup>[14,15]</sup> While there has been much laboratory study of possible techniques,<sup>[16-18]</sup> most clinical reports describe occasional single case successes.<sup>[19-22]</sup> This study focuses on clinical experience of 23 patients with craniofacial osseous defects larger than critical size.

## MATERIALS AND METHODS

A total of 23 patients with craniofacial osseous defects larger



**Figure 2:** Liposuction is a convenient and nonmorbid way of harvesting adipose-derived stem cells for cell culturing and tissue engineering purposes

than critical size were studied in this case series. The study was conducted in accordance with the ethical principles of the Helsinki Declaration and approved by the Ethics Committee of the Pirkanmaa Hospital District, Tampere, Finland (R03058). Autogenous fat from the anterior abdominal wall of the patients was harvested from all 23 patients and taken to a central tissue banking laboratory [Figure 3] and prepared as described by Mesimäki *et al.*<sup>[20]</sup> All patients were reconstructed with ASCs, resorbable scaffolds, and growth factor as required.

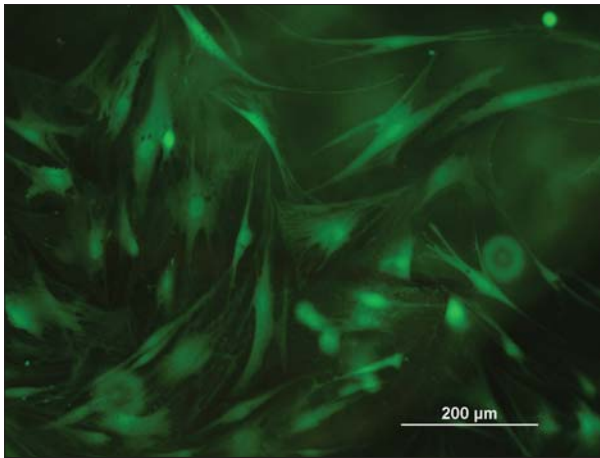
Vascularized soft tissue beds were prepared for ectopic bone formation and later microvascular translocation were performed as indicated. Cases where microvascular flaps were not indicated, recipient beds were prepared in well-vascularized areas of local muscle or between dura and pericranium. All patients were followed closely postoperatively and those who received jaw reconstructions were also treated with dental implants.

## RESULTS

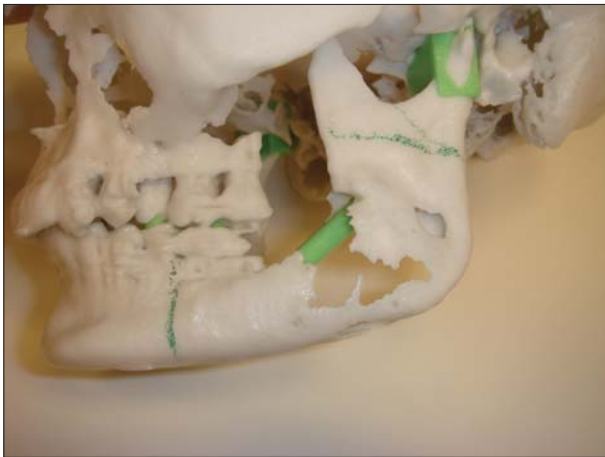
Once the cells populated the scaffold [Figure 3], the resulting bioimplant or construct was transplanted into the patient to restore the defect. This *ex vivo*-derived reconstruction had one major obstacle. The vitality of the bioimplant was entirely dependent on the vascularity of the recipient bed. To this end, vascularized free flaps such as rectus abdominis were used to help vascularize the constructs if they were in complex or hypoperfused wounds. To date, 23 ASC seeded resorbable scaffolds have been combined with rhBMP-2 and successfully implanted into humans to reconstruct their jaws [Figures 4 and 5]. The failures included one infection (4.3%) and two cases (8.6%) with inadequate bone formation.

## DISCUSSION

The materials used in hard and soft tissue regeneration include autogenous or allogeneous grafts, silicon, artificial bones besides a host of other materials. They have an inherent disadvantage of high cost, immunogenicity/allergenicity, and risk of transmitting infectious diseases besides a lot of other disadvantages. Literature



**Figure 3:** Live/dead staining of adipose stem cells. Green cells are viable; dead cells red



**Figure 4:** Large ameloblastoma defect of body and ramus of mandible outlined on rapid prototype and reconstructed using ASCs, BMP-2, and beta-TCP granular scaffold



**Figure 5:** Parasymphyseal 10 tooth sized ameloblastoma defect from rapid prototype and reconstructed using ASCs, BMP-2, and beta-TCP granular scaffold

has sporadic reports of successful cases that have been treated with ASCs.<sup>[23]</sup>

These clinical observations extend our limited knowledge regarding the potential use of ASCs for osseous tissue repair and regeneration. Although ASC has been used alone for reconstruction, this report probably is the largest series to report the synergistic effect of ASC, resorbable scaffolds (beta-TCP or bioactive glass), and growth factors (rhBMP2). The results indicate a 4.3% incidence of failure due to infection and approximately 9% due to less adequate bone formation than expected. It has been documented that ASCs have a capacity to undergo rapid osteogenic differentiation as early as 1 week *in vitro*.<sup>[24]</sup>

Defects of the skull<sup>[24]</sup> and jaws<sup>[25]</sup> have been either successfully reconstructed or accelerated healing with the use of hASCs.<sup>[26]</sup> hASC had earlier been used including combination with bone chips, the use of various osteoconductive scaffolds, and recombinant proteins. Mesimaki *et al.* used a novel microvascular flap with hASCs, a scaffold (beta-tricalcium phosphate) and rhBMP-2 to heal a large defect in the maxilla.<sup>[20]</sup> Similar combination of autogenous ASCs, BMP-2, and either beta-TCP or bioactive glass scaffolds was used in this series. The main advantage of ASCs is their ease of harvest and minimal morbidity. Harvesting bone marrow stem cells is far more painful and much more invasive. ASCs are hybrid grafts as they contain perivascular endothelial precursor cells and may also be angiogenic.

Another source of stem cells could be from suction trap aspirates of bone during mandibular third molar removal. This technique would allow stem cell harvesting during one of the most common oral surgical procedures performed today. Third molar removal also presents some new opportunities. The removed developing third molar follicle can yield follicular cells, cementoblast-like cells, and dental pulp stem cells which can also be cultured. The receptors of these cells are being characterized and this is an important first step in the understanding of these cells and their possible future utilization.

## CONCLUSION

The synergistic effects of ASC, TCP, and rhBMP2 have been proven useful in about 85% of the cases followed after reconstruction with these materials. The long-term success of this procedure needs to be verified using a large sample. Future directions need to be directed against more favorable cocktail of growth receptors, cytokines along with scaffold modification. Isolation and refining of the ASC also need to be simplified so as to make it a theater-side procedure.

## REFERENCES

1. Sándor GK, Suuronen R. Combining adipose-derived stem cells, resorbable scaffolds and growth factors: An overview of tissue engineering. *J Can Dent Assoc* 2008;74:167-70.
2. Grinnemo KH, Syven C, Hovatta O, Dellgren G, Corbascio M. Immunogenicity of human embryonic stem cells. *Cell Tissue Res* 2008;331:67-78.
3. Gimble JM, Zvonic S, Floyd ZE, Kassem M, Nuttall M. Playing with bone

- and fat. *J Cell Biochem* 2006;98:251-66.
4. Pittenger MF, MacKay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, *et al.* Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143-7.
  5. Wolf NS, Penn PE, Rao D, McKee MD. Intracolonial plasticity for bone, smooth muscle, and adipocytes lineages in bone marrow stroma fibroblastoid cells. *Exp Cell Res* 2003;290:346-57.
  6. Song L, Tuan RS. Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. *FASEB J* 2004;18:980-2.
  7. Suuronen R, Asikainen A. Biodegradable materials as tools to fasten bone in face and jaw surgery. *Duodecim* 2004;120:2002-7.
  8. Jan A, Sándor GK, Iera D, Mhawi A, Peel SA, Clokie CM. Hyperbaric oxygen results in an increase in rabbit calvarial critical sized defects. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:144-9.
  9. Tirkkonen L, Halonen H, Hyttinen J, Kuokkanen H, Sievänen H, Koivisto AM, *et al.* The effect of vibration loading on adipose stem cell number, viability and differentiation toward bone-forming cells. *J R Soc Interface* 2011;8:1736-47.
  10. Hu ZM, Peel SA, Ho SK, Sándor GK, Clokie CM. Role of bovine bone morphogenetic proteins in bone matrix protein and osteoblast-related gene expression during rat bone marrow stromal cell differentiation. *J Craniofac Surg* 2005;16:1006-14.
  11. Fok TC, Jan A, Peel SA, Clokie CM, Sándor GK. Hyperbaric oxygen results in an increase in vascular endothelial growth factor (VEGF) protein expression in rabbit calvarial critical sized defects. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008;105:417-22.
  12. Lindholm TC, Peel SA, Clokie CM, Sándor GK. Cortical bone grafts used to culture bone cells to be used for increasing efficacy of bone morphogenetic proteins in tissue engineered bone substitutes. *J Oral Maxillofac Surg* 2003;61 Suppl 1:S74.
  13. Barr T, McNamara AJ, Sándor GK, Clokie CM, Peel SA. Comparison of the osteoinductivity of bioimplants containing recombinant human bone morphogenetic proteins 2 (Infuse) and 7 (OP-1). *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;109:531-40.
  14. Moghadam HG, Urist MR, Sándor GK, Clokie CM. Successful mandibular reconstruction using a BMP bioimplant. *J Craniofac Surg* 2001;12:119-27.
  15. Clokie CM, Sándor GK. Reconstruction of 10 major mandibular defects using bioimplants containing BMP-7. *J Can Dent Assoc* 2008;74:67-72.
  16. Terheyden H, Knak C, Jepsen S, Palmie S, Rueger DR. Mandibular reconstruction with a prefabricated vascularized bone graft using recombinant human osteogenic protein-1: An experimental study in miniature pigs. Part I: Prefabrication. *Int J Oral Maxillofac Surg* 2001;30:373-9.
  17. Terheyden H, Warnke P, Dunsche A, Jepsen S, Brenner W, Palmie S, *et al.* Mandibular reconstruction with prefabricated vascularized bone grafts using recombinant human osteogenic protein-1: An experimental study in miniature pigs. Part II: Transplantation. *Int J Oral Maxillofac Surg* 2001;30:469-78.
  18. Terheyden H, Menzel C, Wang H, Springer IN, Rueger DR, Acil Y. Prefabrication of vascularized bone grafts using recombinant human osteogenic protein-1--part 3: Dosage of rhOP-1, the use of external and internal scaffolds. *Int J Oral Maxillofac Surg* 2004;33:164-72.
  19. Torroni A. Engineered bone grafts and bone flaps for maxillofacial defects: State of the art. *J Oral Maxillofac Surg* 2009;67:1121-7.
  20. Mesimäki K, Lindroos B, Törnwall J, Mauno J, Lindqvist C, Kontio R, *et al.* Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. *Int J Oral Maxillofac Surg* 2009;38:201-9.
  21. Warnke PH, Springer IN, Wiltfang J, Acil Y, Eufinger H, Wehmöller M, *et al.* Growth and transplantation of a custom vascularised bone graft in a man. *Lancet* 2004;364:766-70.
  22. Warnke PH, Wiltfang J, Springer I, Acil Y, Bolte H, Kosmahl M, *et al.* Man as living bioreactor: Fate of an exogenously prepared customized tissue-engineered mandible. *Biomaterials* 2006;27:3163-7.
  23. Mara KG, Rubin JP. The potential of adipose-derived stem cells in craniofacial repair and regeneration. *Birth Defects Res C Embryo Today* 2012;96:95-7.
  24. Lendeckel S, Jödicke A, Christophis P, Heidinger K, Wolff J, Fraser JK, *et al.* Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: Case report. *J Craniofac Surg* 2004;32:370-3.
  25. Kulakov AA, Goldshtein DV, Grigoryan AS, Rzhaniyeva AA, Alekseeva IS, Arutyunyan IV, *et al.* Clinical study of the efficiency of combined cell transplant on the basis of multipotent mesenchymal stromal adipose tissue cells in patients with pronounced deficit of the maxillary and mandibular bone tissue. *Bull Exp Biol Med* 2008;146:522-5.
  26. Mao JJ, Giannobile WV, Helms JA, Hollister SJ, Krebsbach PH, Longaker MT, *et al.* Craniofacial tissue engineering by stem cells. *J Dent Res* 2006;85:966-79.

**Cite this article as:** Sándor GK. Tissue engineering of bone: Clinical observations with adipose-derived stem cells, resorbable scaffolds, and growth factors. *Ann Maxillofac Surg* 2012;2:8-11.

**Source of Support:** Nil, **Conflict of Interest:** None declared.

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