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# A novel autologous cell therapy approach for the acceleration of bone repair in human extraction socket healing

## Un'innovativa terapia cellulare autogena nell'accelerazione della rigenerazione ossea nell'alveolo postestrattivo umano

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## Summary

We evaluated the use of Bone Repair Cells (BRCs), an autologous construct of stem and progenitor cells, to the regeneration of bone in a human extraction socket model. 24 patients participated to this controlled randomized proof-of-concept clinical trial. Our results suggest that BRCs accelerate the early stages of osteogenesis and should be taken in consideration for larger scale clinical studies and for the treatment of larger craniofacial defects.

## Riassunto

Abbiamo sperimentato l'uso di Bone Repair Cells (BRC), una miscela autologa di cellule staminali e progenitrici, nella rigenerazione ossea umana in sede di estrazione. 24 pazienti hanno partecipato a questo studio clinico randomizzato controllato. I nostri risultati suggeriscono che le BRC accelerano gli stadi iniziali di oseoneogenesi e dovrebbero essere prese in considerazione nel trattamento di difetti craniofaciali maggiormente estesi.

## Introduction

Dental implants are commonly used in the treatment of most partial and complete edentulisms. In the attempt to achieve the highest survival rates and avoid short and long-term complications it is general consensus that implants ought to be inserted in adequate bone volumes. Unfortunately tooth loss results in horizontal and vertical loss of bone structure, which is faster in the first few months and progresses inexorably throughout life. When this happens, regenerative procedures allow clinicians to attain adequate implant placement in deficient alveolar ridges although, where indicated ridge preservation techniques should be adopted in the attempt to prevent or at least lessen the inevitable ridge resorption.

Several approaches have been evaluated for a faster and more robust bone formation in the extractive alveolus including the use barrier membranes, the use of autologous bone or bone substitutes of different derivation, and combinations of the aforementioned materials. Some approaches have been proven to reduce but not prevent changes in alveolar ridge volume. The use of mineralized grafts also generates concerns on the rate of resorption of these materials and its possible correlation with the long-term outcomes of the implants placed in the regenerated bone. While studies have shown the ability of bone regenerated using mineralized grafts to sustain loading and provide similar implant long-term results as pristine bone (Fiorellini *et al.*, 2003), it is reasonable to direct the effort of bone regenerative research to the creation of tissues that mimic nature as much as possible.

Tissue Engineering research is establishing innovative techniques for more predictable bone regeneration in the treatment of orofacial bony defects. In our study a Cell Therapy approach was evaluated in a human extraction socket healing model. This innovative approach utilizes a mixture of autologous bone marrow-derived cells expanded to concentrations not achievable in a simple bone marrow aspiration: Bone Repair Cells (BRCs). In this technique a bone marrow aspirate is harvested from the patient's posterior iliac crest 12 to 14 days prior to the surgical intervention. The cells are depleted from red blood cells and inoculated in a completely automated, closed cell cassette, which expands stem and progenitor cells trough a Single Pass Perfusion (SPP) System. BRCs are used for multiple applications in cardiovascular, neurological

and orthopedic surgery.

## Materials and Methods

This was a Phase I/II prospective controlled randomized proof-of-concept clinical trial investigating safety and regenerative potential of BRCs in the treatment of localized orofacial defects. The study protocol was approved by governing Institutional Review Boards and by the Food and Drug Administration (FDA) and was submitted to the NIH clinical trials database (NCT00755911). The clinical phase (September 2008 - December, 2010) included 24 adult patients of the University of Michigan School of Dentistry, who signed informed consent. Each patient required replacement of a premolar tooth indicated for extraction with dental implants. A computer generated randomization process assigned the subjects to test or control groups. The baseline for the study was set on the day of tooth extraction and socket grafting (Graph 1). *Closed and open bone measurements* were taken using individually fabricated occlusal surgical stents for enhanced reproducibility. Immediately prior to grafting the cell construct was adsorbed onto a commercially available gelatin sponge. The adsorbed sponge (or the carrier alone in the control group) was then transplanted into the extraction socket to the level of the interproximal bone. A bioabsorbable collagen barrier membrane was placed to contain the transplant and the flaps were coronally repositioned until reaching tension-free primary closure and sutured. Patients were instructed to rinse with 0.12% Chlorhexidine and not to brush in the area of the graft for 2 weeks. A postsurgical regimen of Amoxicillin 500 mg TID for 7 days and Ibuprofen 600 mg, QID for 3 days was prescribed to all patients. Postoperative visits were performed at 1, 2 and 4 weeks; the suture material was removed at 2 weeks. Re-entry procedures were performed 6 weeks (6 BRC patients and 6 controls) and 12 weeks (6 BRC patients and 6 controls) post-surgery. Closed and open bone clinical measurements were repeated on each subject and bone cores of approximately 2 x 7 mm in dimension will be removed with a trephine drill from the area corresponding to the center of the previous extraction socket. The *Misch bone density scale* was used as clinical assessment of the quality of the regenerated bone. The harvested bone cores were fixed in 10% neutral buffered formalin to allow for histomorphometric and µCT analyses. A dental implant was placed in the osteotomy prepared by bone core harvesting reaching implant insertion torque of 35 Ncm. Cases requiring *additional ridge augmentation procedures* at the time of implant placement were also documented. All implants were uncovered 5 months and restored 6 months after tooth extraction and were followed up for 1 year.

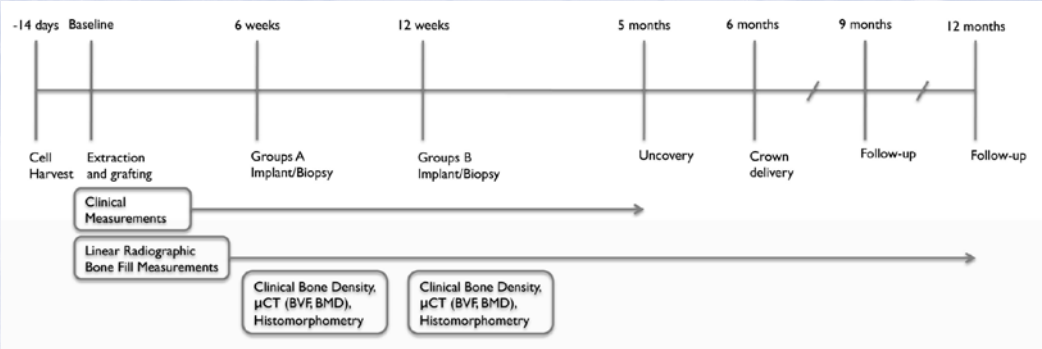
At baseline and re-entry appointments, *standardized periapical radiographs* were obtained using a long-cone technique, an individually fabricated occlusal stent for reproducibility of angulation and positioning of the digital sensor and an aluminum step wedge for correction of the gamma. Measurements from baseline images were compared to corresponding follow-up images to determine bone gain in all patients.

Prior to demineralization and sectioning, fixed harvested bone cores were scanned for a *Micro-Computed Tomographic analysis* allowing for the measurement of Bone Volume Fraction (BVF; %) and Bone Mineral Density (BMD; mg/cc) of each sample.

Sectioned samples where then stained with Hematoxylin and Eosin and Masson's Trichrome stains for *histomorphometric analysis*. Bone Area over Tissue Area (BA/TA, %) was calculated for each sample on the most representative slide (center of the bone core).

Data was analyzed with ANOVA test and Tukey post-hoc test and by Student-*t* test.

Graph 1: Study Timeline



## Results

In order to reduce variability in extraction socket size/morphology we included patients requiring extraction of solely premolar teeth. In addition the morphology of the socket defects where measured to ensure proper randomization of the samples between groups. Soft tissue measurements encompassed keratinized gingiva width and thickness, while hard tissue measurements included extraction socket width both in the bucco-lingual and mesio-distal dimensions, buccal and lingual plates thicknesses, socket height and presence of dehiscences or fenestrations. Statistical analysis on Baseline Clinical measurements shows that the randomization process was successful in providing defects with similar characteristics.

The evaluation of alveolar ridge volume changes was performed both on soft and hard tissue parameters. The location of the soft tissues in relation to the stent was measured on the buccal and lingual sites while hard tissue measurements were taken at the mesial, distal, buccal and lingual sites of the extraction socket. The analysis showed that the highest ridge volume change occurs at 12 wks but the test groups seemed to experience lower degree of resorption. This trend achieved significance when comparing loss of lingual vertical dimension between the control group at 12 wks and the test group at the same time-point (Table 1).

Clinical evaluation of bone density was assessed using the *Misch Scale*. This is a clinical measure attained during the preparation of the implant osteotomy which classifies bone densities in 4 classes from D1 to D4 (Misch *et al*., 1987). Using this assessment higher bone densities were found in the treatment groups compared to controls (Table 2).

Table 1: Clinical measurements

| | Gingival Recession (Buccal) | Gingival Recession (Lingual) | Alveolar Ridge Rersorption (Mesial) | Alveolar Ridge Rersorption (Distal) | Alveolar Ridge Rersorption (Buccal) | Alveolar Ridge Rersorption (Lingual) |
|---|---|---|---|---|---|---|
| Control 6 wks | 0,20 ± 0,60 | 0,70 ± 0,51 | 0,83 ± 0,78 | 0,67 ± 0,65 | 0,40 ± 0,37 | 0,67 ± 0,42 |
| BRC 6 wks | -0,50 ± 0,50 | 0,83 ± 0,44 | 0,50 ± 0,26 | -0,50 ± 0,37 | 0,50 ± 0,76 | 0,67 ± 0,31 |
| Control 12 wks | 2,25 ± 0,25 | 2,67 ± 0,33 | 1,67 ± 0,81 | 0,75 ± 0,28 | 1,92 ± 0,49 | 3,42 ± 1,01* |
| BRC 12 wks | 2,00 ± 0,56 | 1,58 ± 0,44 | 0,92 ± 0,37 | 1,00 ± 0,62 | -1,08 ± 1,97 | 0,50 ± 0,77* |

* = $p < 0,05$

Table 2: Misch Bone Density Scale

| | D1 | D2 | D3 | D4 |
|---|---|---|---|---|
| Control 6 wks | 0 | 0 | 5 | 1 |
| BRC 6 wks | 0 | 4 | 1 | 1 |
| Control 12 wks | 0 | 1 | 5 | 0 |
| BRC 12 wks | 0 | 4 | 2 | 0 |

Small size dehiscences or fenestrations of the dental implant at the time of placement were treated by the use of osseous grafting only; larger-sized defects required the use of both osseous grafts and barrier membranes. Both control groups experienced higher need for additional procedures. 5 out of 6 control cases that were reentered at 6 weeks required augmentation and two of these required the use of both osseous graft and collagen membrane. Half of the control cases reentered at 12 weeks required augmentation, two of which required both osseous graft and collagen membrane. In each test group two out of six patients were grafted at the time of implant placement and only one out of six cases required the use of membranes in combination of bone substitutes (Table 3).

Table 3: Need for Additional Bone Grafting

| | Bone graft alone | Membrane + Bone Grafting | Total |
|---|---|---|---|
| Control 6 wks | 3 | 2 | 5 |
| BRC 6 wks | 1 | 1 | 2 |
| Control 12 wks | 1 | 2 | 3 |
| BRC 12 wks | 1 | 1 | 2 |

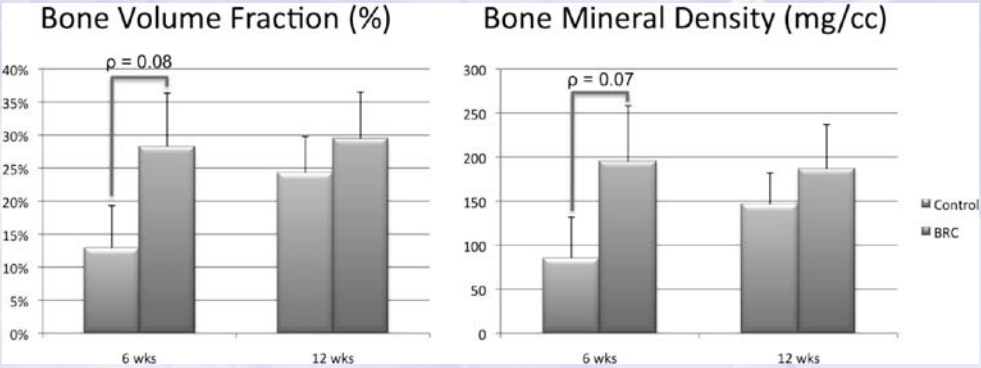
*Linear Radiographic bone fill measures* were taken as a percentage of the regenerated bone over the size of the initial defect. This measure showed statistical significance when comparing treatment and control groups at 6 weeks suggesting a possible role of the grafting material in the acceleration of the early stages of bone regeneration (Table 4).
This result was confirmed by the *Micro-Computed Tomography analysis* of the bone cores where 6 weeks test samples showed a 2 fold increase of Bone Volume Fraction (BVF; %) and an almost 3-fold increase in Bone Mineral Density (BMD; mg/cc) when compared to control samples at the same timepoint (Graph 2). Although this difference did not reach statistical significance it should still be considered meaningful given the small N that generally characterizes pilot studies.
*Histomorphometric analysis* demonstrated no significant difference between groups although at 6 weeks higher values were found in the BRC group than in the control group (Table 4). Interestingly histological evaluation of BRC-treated site at 6 weeks showed high degree of cellularity, maturation and vascularity of the regenerated bone at this early timepoint.
Another analysis performed was the correlation between Alkaline Phosphatase activity of the cells prior to implantation as assessed in our previous published paper (Kaigler *et al.*, 2010) and clinical outcomes assessed with the uCT evaluation. A direct correlation was found between these measures. This is interesting for future studies as a mean to evaluate the regenerative potential of BRCs prior to implantation and use this correlation as a predictor of clinical success.

Table 4

| | Linear Radiographic Bone Fill | BA/TA (%) |
|---|---|---|
| Control 6 wks | 55,31 ± 3,20%* | 19,6 ± 4,2% |
| BRC 6 wks | 78,86 ± 1,03%* | 28,8 ± 9,1% |
| Control 12 wks | 74,64 ± 3,34% | 35,1 ± 3,2% |
| BRC 12 wks | 80,06 ± 2,04% | 35,2 ± 8,9% |

* = p < 0,05

Graph 2.



## Discussion/Conclusion

When attemting the regeneration of a new tissue cells, growth factors, scaffold and the formation of new vessels play a fundamental role on the healing process in a simultaneous and temporal timeframe (Taba *et al*., 2005). Cell transplantation offers beneficial advantages in the attempt of regenerating biologic tissues making the repopulation of defects from host cells a less significant player. In the engineering of new bone structures several studies have evaluated the use of both somatic and stem cells. The goal of the implanted *somatic cells* is not only that of depositing the tissue they are committed to, but also that of orchestrating this whole process through the release of cytokines and growth factors. The use of *stem cells* in bone regeneration has an adjunctive benefit over the use of somatic cells. Once transplanted in the grafted site stem cells maintain their ability to differentiating into different phenotypes orchestrating the regeneration of bone by providing osteogenic cells as well as "supportive osteogenic cells". Supportive osteogenic cells are cells that do not directly create bone but that facilitate bone depositions creating structures that are needed to allow this process. The use of these particular populations of stem cells provides a source for osteogenic cells as well as cells that will create the vascular network and fibroblasts required in the development of the new tissue.

A study by Marei et al. intended to regenerate bone by implanting an engineered porous scaffold seeded with bone marrow mesenchymal stem cells (BMSCs) in a rabbit extraction socket, the result of their study show interesting promise in the area of dentoalveolar surgery (Marei *et al*., 2005). De Kok and coworkers evaluated the use of Mesenchimal Stem Cells (MSCs) in canine extraction sockets infused in hydroxyapatite/tricalcium phosphate (HA/TCP) cylinders and concluded that an alveolar socket model may be an appropriate model for initial clinical investigation of MSC-mediated bone repair (De Kok *et al*., 2005). In humans Mesenchymal stem cells (MSCs) isolated from a patient's iliac crest marrow aspirates in combination with Platelet-rich plasma (PRP) were successful in the regeneration of periodontal tissue (Yamada *et al*., 2006). A recent paper utilized fresh bone marrow aspirates as grafting material for extraction sockets. 15 test sites and 15 control sites were treated and titanium screws were used as reference point for clinical measurements. 6 months after grafting test sites demonstrated lower degree of alveolar ridge resorption and no need for additional augmentation while the control group required additional augmentation in 5 out of 15 cases. Histomorphometrical evaluation revealed no significant difference between test and control sites (Pelegrine *et al*., 2010).

This was the first study evaluating Bone Repair Cells in the treatment of human extraction sockets. This Cell Therapy regenerative approach accelerates the early stages of osteogenesis is safe for use in localized orofacial bony defects. This pilot study establishes preliminary evidence for consideration of larger scale clinical studies and for the use of BRC therapy in the treatment of larger craniofacial defects.

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