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BONE TISSUE ENGINEERIN: USING STEM CELL AND TCP IN REGENERATION OF CRITICAL SIZE DEFECT IN THE MANDIBULE: A PILOT STUDY

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INTRODUCTION

Tissue engineering was initially introduced to describe the technology for producing tissue *in vitro(Samual,2008)*. Recently, the term regenerative medicine has been used to describe the development of technology and surgical procedures for the regeneration of tissue *in vivo(Samual,2008)*. The working goal of tissue engineering is the implementation of existing knowledge for the creation of a product (tissue) that can resemble autogenous tissue and able to act as a substitute for any lost tissue at any point in time. Futhermore, this technology often provides opportunities for the discovery of new scientific knowledge. In this regard, tissue engineering is likely to contribute important knowledge to the study of cell and molecular biology while adding to the fund of knowledge that can be drawn from the advancement of health care.

The goal of tissue engineering and regenerative medicine is to promote healing and ideally, true regeneration of tissue structure and function more predictably, more quickly, and less invasively than allowed by previous techniques (see table 1). Autogenous bone graft is the gold standard of reconstructive oral, craniofacial and general orthopedic surgeries, because it provides an osteoconductive matrix in addition to cells and growth-stimulating molecules. However, the quality of autogenous bone varies depending on the health status of the patient (e.g. those with osteopenia/ osteoporosis, diabetes or a history of smoking have poorer quality autogenous bone graft). Moreover, even in a healthy patient, autogenous bone graft has its disadvantages, such as limited supply, increased surgical operation time, postoperative pain and risk of surgical complication (e.g. infection). Historical perspective of tissue bioengineering originated with the introduction of three-dimensional biomaterial scaffolds to support the regeneration and to substitute autograft. Numerous matrices, including allogenic, xenogenic, and synthetic graft materials are available on the market for use in oral surgical procedures and orthopaedic surgery. The exact mechanism of the action of these matrices is the passive promotion of cells to migrate from the sides of the wound through the matrix, eventually leading to repair of the defect. There are other passive materials designed to act as physical guide or barrier for cells involved in the repair and regeneration process. The results of most of the passive methods are variable and depend on physical and chemical properties as well as the patient's individual healing response. The unpredictable outcome from the use of passive therapies inspired the research that led to the development of treatments designed to stimulate the cells responsible for regeneration. Furthermore, the robust challenge in tissue bioengineering is the implantation of mesenchymal stem cells (MSCs) in the scaffold in combination with the signalling molecules (Samuel,2008).

Table 1: Method of reconstruction of mandible

METHOD OF RECONSTRUCTION OF MANDIBULAR DEFECTS	COMMENT
Autogenous bone grafts	The gold standard (osteoinductive and conductive)
Allografts	Mechanical support and scaffold (osteoconductive)
Alloplasts	Temporary reconstructive method (complication).
Xenografts	Small bone defects (osteoconductive).
Destruction osteogenesis	Limitations; specific indications
BMPand other cytokines	Gives promising results but still under research.

Signaling molecules

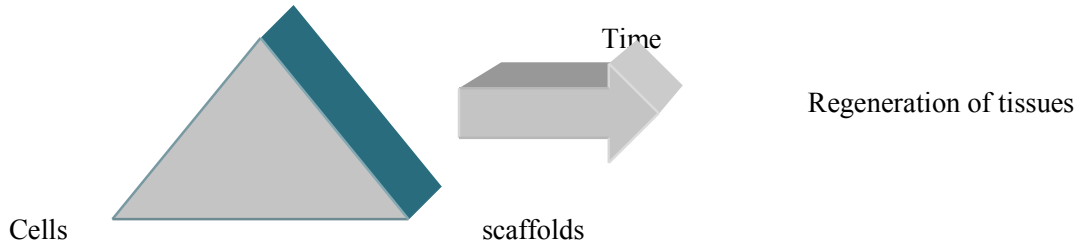


Fig 1: Elements for regeneration

This tissue engineering technique for bone regeneration combines three crucial elements to enhance regeneration: 1) signalling molecules (e.g. PDGF, BMP); 2) cells (e.g. MSC, osteoblasts, fibroblasts); 3) conductive scaffolds (e.g. CaPO4, Collagen) as in Fig 1.

Application of bone engineering in craniofacial regions for different purposes

1- Enhancement of osseointegration of dental implants: There was a positive impact of different BMP on the rate and quality of osseointegration and peri-implant bone regeneration.

2- Repair of craniofacial defects: Various studies have been conducted to explore the role of BMP for reconstruction of calvarial defects. Different preparations of BMPs and carriers have been utilised to reconstruct mandibular defects; they showed variations in result in relation to percentage of bone formation and its mechanical properties (Marukawa et al,2002). In addition, Sinus floor augmentation and experimentally induced cleft palate defect showed excellent bone regeneration with application of BMPs(Van den Bergh et al,2000).

3-Contradictory results have been reported for the use of rhBMP-7 to repair periapical and periodontal defects (Shabahang et al,1999).

4- The results of attempts to obtain more mature bone in a short time by merging the osteoinduction and distraction osteogenesis supported the potential benefits of this strategy in achieving a better quality of bone (Abu-Serriah,2000).

The purpose of the study

The purpose of the study is to test the effect of the combination of mesenchymal stem cells (MSCs) and BMP7 produced by recombinant DNA technology (rhBMP7) incorporated into scaffold bone tri-calcium phosphate TCP to regenerate a critical size defect in the mandibular bone of rabbits

The objective of the study

The objective of the study is to assess the quality and quantity of bone regeneration in two study groups. The first group (A) the reconstruction of critical size mandibular bone defect will be carried out with a combination of MSCs and rh BMP7 incorporated in TCP bone scaffold, whereas the second group will be utilized a mixture of MSCs incorporated in TCP.

The study will be carried out with 8 rabbits, randomly allocated into group 1 or group 2 after the creation of a critical size defect under general anesthesia. During the experimental life-time of the animals the assessment will be made by plain radiographic and cone beam CT monitoring of regenerated bone after the administration of bone marker. The animals will be sacrificed after 3 months and the explanted mandibles will be subjected for plain films, cone beam CT measurements, mechanical testing, and histomorphometrical examination.

Why study the mandibular bone?

The mandible is an important jaw bone in the maxillofacial skeleton. This bone is frequently resected in cancer surgery and is difficult to construct because of masticatory muscle attachment, relatively poor blood supply and the high potential for contamination with oral flora.

Why critical size defect?

Critical size defect (CSD) has been defined as the minimal osseous defect in a particular bone in a species of animal that would not heal spontaneously during the lifetime of the animal; it can heal by fibrous union and not by bony union (Schmitz , 1986). Preparation of a bone defect that is of a size incapable of bone regeneration by natural processes is called a true CSD (Abu-Serriah, 2000). Based on the published literature, it was suggested that the CSD of the body of the mandible of large mammal might be at least 25% of its total length.

Why TCP as bone scaffolding?

The most important parameters involved in scaffolding, i.e. 1) porosity; 2) pore size; 3) biomaterial stiffness; 4) biomaterial resorption kinetics; 5) scaffold pre-seeding; and 6) provision of temporary load-sharing to withstand the forces of mastication, were investigated

TCP is compatible to tissues and superior to other synthetic materials (Xin et al, 2005). Histological examinations confirmed that the implant is resorbed and concomitantly replaced by normal bone (Scott Metsger et al, 1982). Its porosity provided an osteoconductive scaffold, which favoured the internal growth of cells and vessels (Gan et al, 2008). In many studies, TCP has shown promising results for both small and large defects (Ekholm et al, 2006).

Why MSCs?

Bone marrow-derived mesenchymal stem cells (MSCs) are multipotential cells that are capable of differentiating into, at a minimum, osteoblasts, chondrocytes, adipocytes, tenocytes, and myoblasts. The proliferative capacity of MSCs allows a small volume of extracted bone marrow to be expanded i to large quantity when isolated and cultured. Thus, MSCs are held to be readily available and an abundant source of cells for tissue-engineering applications.

Using rhBMP7

RhBMP7 is an acidic extra-cellular protein that is firmly bound and possesses osteoinductive properties, although it is clearly separable from bone collagen. BMP can be isolated from several sources, including bovines, humans, rats, monkeys, and osteogenic osteosarcoma. With regard to BMPs produced by recombinant DNA technology (rhBMPs), the vast majority of research has been carried out using rhBMP2(Abu-Serriah,2000). RhBMP7 was mainly applied for the reconstruction of long bone. The authors have had experience in using rhBMP7 in craniofacial applications since 2000, and they have achieved promising results. Naudi et al (2002) conducted a study to assess radiographically, mechanically and histomorphometrically the quality and quantity of bone regeneration in a critical-size osteoperiosteal mandibular discontinuity defect following the application of bone morphogenetic protein 7 (rhOP-1) on a tricalcium phosphate (TCP) scaffolding. The investigation was conducted on ten adult white New Zealand rabbits (3.0-4.0kg). In all cases a unilateral straight body ostectomy was performed anterior to the first premolar following insertion of a bicortical screw to reinforce the lower border. The segment of bone was removed with the covering periosteum and incisor tooth (Figures 1-3). In seven cases the critical-size defect was filled with rhOP-1 on a prefabricated scaffolding of TCP (Group 1) (Figure 4). In the remaining cases the defect was filled with the TCP alone (Group 2). Radiographic assessments with plain radiographs were carried out at 0, 4, 8, and 12 weeks follow-up. Three months post-operatively the animals were sacrificed, the mandibles removed and the surgical sites were assessed with cone beam CT radiography, mechanical testing and histomorphometric analysis .Clinically, the regenerated bone (Figure 5) appeared to be continuous with the adjacent bone, was remodelled to a size commensurate with the defect size and there were no incidences of infection.

Radiographic findings

There was complete fusion of the graft with the proximal bone segment with what again appeared to have a radio density similar to that of bone. The width of the remodelled graft was similar in size to the contralateral non-operated side but appeared deficient interiorly, probably due to the lack of the presence of an incisor tooth within the

graft. In group 2 there was no bridging of the gap between the graft and the proximal bone and the graft was not remodelled to a width similar to that of the contra lateral side. Neither was there any development of a visible outer cortex radio graphically.

Histomorphometric analysis using image analysis software showed statistically significantly higher amounts of bone regeneration ($p<0.014$) within the surgical sites of group 1, with minimal bone regeneration in the cases in group 2.

Conclusions

The restoration of critical-size mandibular defects in rabbit mandibles using a combination of rhOP-1(rh BMP7) with TCP scaffolding was successful. This may suggest future clinical applications**.**

In summary, the complexity of skeletal tissues has been a hindrance to the development of an effective regeneration system. Nevertheless, significant steps are being taken regarding the use of progenitor stem cells, adequate scaffold materials and growth factors or bioactive agents. Our goal is to construct material with a single system of such properties-structural support, cell support and controlled release of signals. Using multipotential stem cell combined with TCP and rhBMP7 to regenerate bone in critical size defect in the mandibule a new technique was developed, which produce a biomaterial in which bone marrow provides a rich source of cells from MSC to cells that have already committed down the osteogenic lineage and were destined to become osteoblasts. The robust effect with presence of BMP namely rhBMP7 would improve the quality of the bone regenerate. Therefore, with the three core properties, the new biomaterial would serve as a bone substitute in bone regeneration.

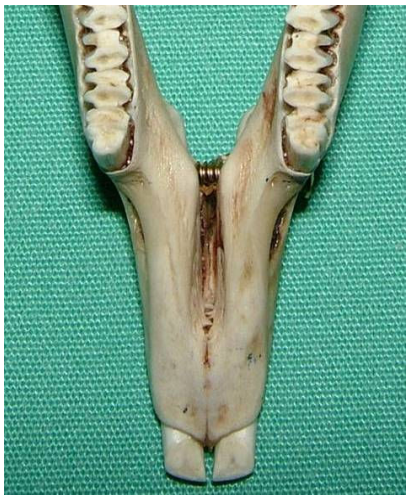
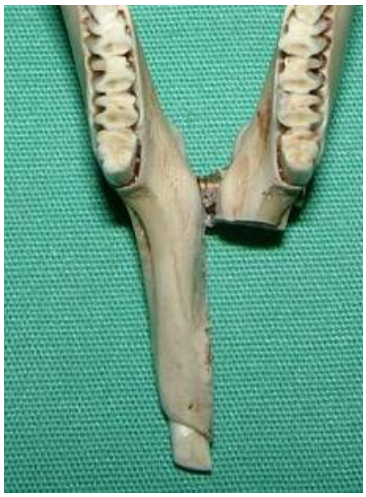


Figure 1.



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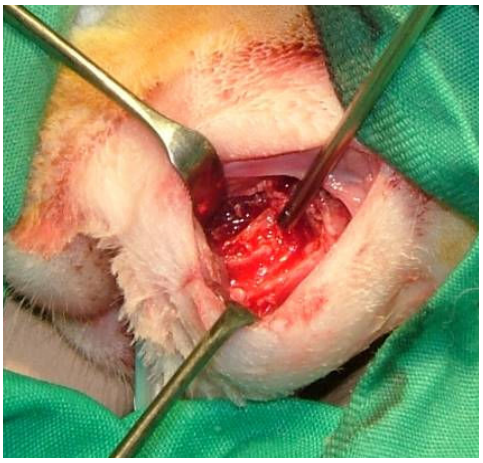

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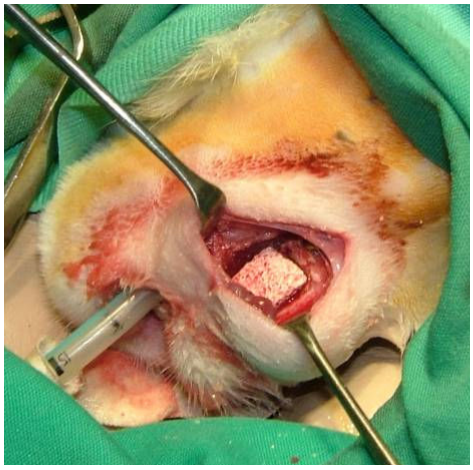

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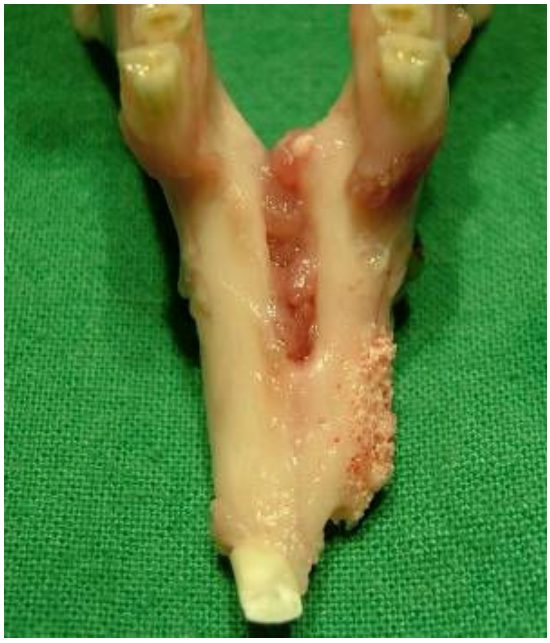

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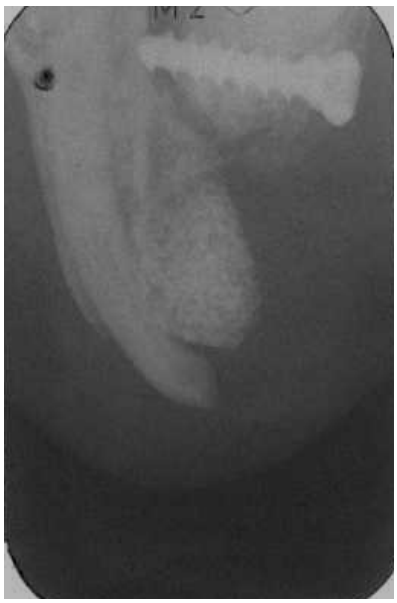

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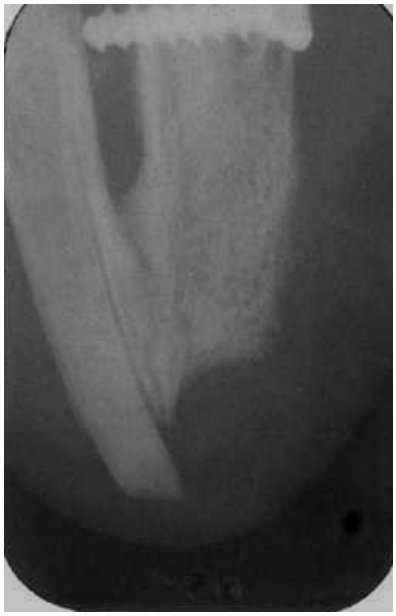

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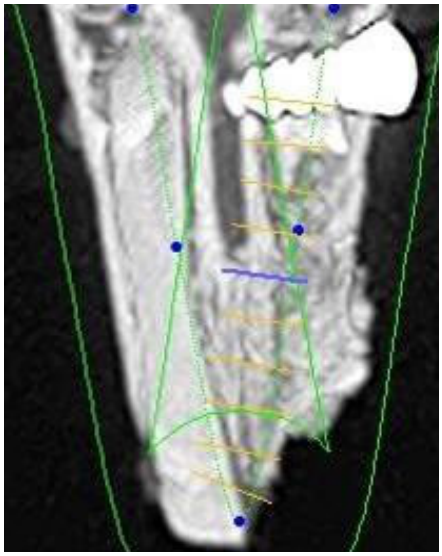

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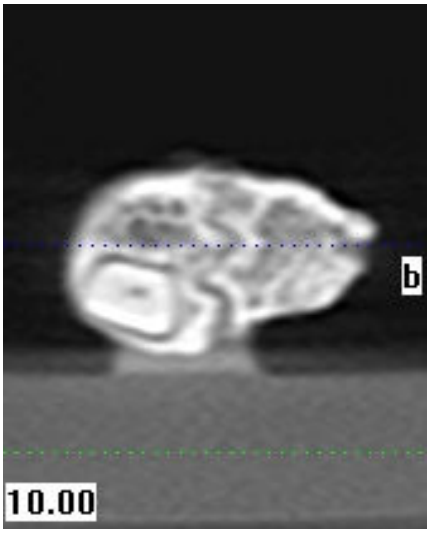

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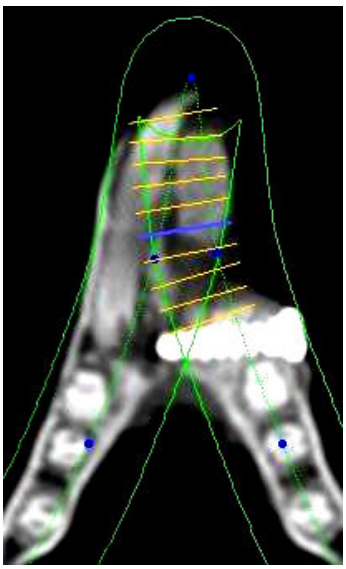

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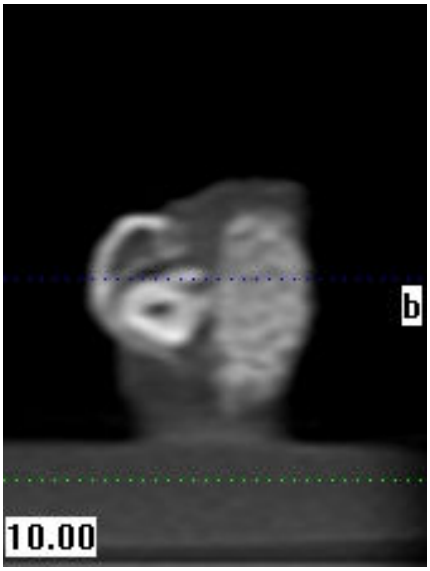

Figure 11.

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