International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Sinus Lift: Future Matters???

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Abstract: In modern implant dentistry sinus lift procedures getting worldwide acceptance from dental professionals. Grafting of the maxillary sinus has become a highly predictable surgical technique for site development and implant reconstruction. This review explains the different future approaches for sinus augmentations.

Keywords: Sinus augmentation, stromal stem cell preparation from iliac bone marrow aspirate, tissue engineering approach, use of tissue-engineered bone cells & gene therapy

1. Introduction

Sinus augmentation procedures quite gaining a lot of interest in recent years for the management of the atrophic maxilla. Pneumatization of maxillary sinus causes insufficient vertical bone volume on posterior maxilla. So the restoration of edentulous posterior maxilla with dental implants is challenging due to a deficient posterior alveolar ridge, unfavorable bone quality and increased pneumatization of the maxillary sinus. Mainly two approaches i.e the crestal approach and the lateral window approach have been used. Traditional approaches includes the sinus lift with use of PRF, PRF+bone graft, without bone graft & certain growth factors. This review explain the future directions and what other approaches may help for clinician for augmenting the sinus floor with various different methods like, stromal stem cell preparation from iliac bone marrow aspirate, tissue engineering approach, use of tissue-engineered bone cells & gene therapy.

2. Stromal stem cell preparation from iliac bone marrow aspirate for sinus grafting

Interest in the osteogenic potential of bone marrow aspirate dates back at least to 1869, when Goujon reported the first use of autologous bone marrow to form bone, and has continued ever since.[1]Chutro demonstrated the use of marrow containing bone graft for long bone fracture repair.[2]McGaw and Harbin later established the role of bone marrow in osteogenic regeneration.[3]Friedenstein and colleagues isolated and cultured what they called "bone marrow fibroblasts" in vitro and later established their osteogenic potential.[4-6] Castro-Maloaspina et al purified these cell populations,[7] and Caplan[8] and Haynesworth et al[9] first identified them as progenitors by their ability to differentiate into osteoblasts, chondroblasts, myoblasts, and diverse other phenotypes. Owen discovered that when transplanted under the capsule of a kidney, these cells produced bone, cartilage, fat, and other tissues.[10-11]Procktop first proposed the term *stromal stem cell* to describe its role as a multipotent precursor cell for nonhematopoietic tissues.[12]

Bone-forming stem cells may be characterized as either *adherent* or *nonadherent.* Because they adhere to plastic surfaces and can be easily separated for expansion, adherent stem cells are generally the type obtained for culture. Nonadherent osteoblast lineage cells, the so-called lining cells that appear to be quiescent, recently were reported to be present in peripheral blood in as much as 2% of mononuclear cells. The proportion of circulatory osteoblastforming cells was found to be greater in growing adolescents than in adults and greater also in individuals with conditions associated with accelerated osseous wound healing, such as a bone fracture repair or bone graft reconstruction.[13] Osteoblasts are thought to be present in the circulation not only as a source of bone-forming cells, but *primarily* to function through the circulation under certain specific conditions.

However, it is clear that, as Yoshikawa et al and others have shown, isolated and cultured mesenchymal stem cells implanted into critical-sized bone defects demonstrate profound osteogenesis.[14-19]Early reports, especially in the area of maxillofacial *surgery,* have been promising. But what of the use of simple autologous bone marrow-immediately injected following aspiration into an osseous defect? Might there be a synergistic effect from the multiple cellular constituents of fresh marrow?

3. Bone Marrow Aspiration Technique

The technique of using bone marrow aspirate to treat various maxillofacial defects was developed by Boyne,[20] and although it has never gained widespread acceptance, the technique has been used ever since in oral and maxillofacial reconstruction, alveolar cleft repair, and periodontics. With the patient in a lateral decubitus or prone position, the posterior hip is prepped and draped in a sterile manner. The posterosuperior iliac spine is palpated, and a percutaneous needle entry is made 2 cm below the spine and 3 cm lateral to the midline all the way to the bone using an 11 -gauge needle. After punching through the cortex, blood is drawn from the marrow space, usually in 2- to 3-mL aliquots. The needle is then redirected without being completely removed, and the process is repeated around the posterior iliac wing. An alternative approach is to insert the needle superior-

inferiorly adjacent to the posterosuperior iliac spine, where the cortex is thinner and the marrow space can easily be found with a multiport needle. A 15-gauge needle can be used to aspirate marrow from the anterior iliac crest, which is perhaps the easiest area to access. After 2 mL of blood is withdrawn, the needle is redirected dorsally, caudally, and medially within the marrow space. (Continuous aspiration in one site should be avoided because it will yield mostly venous blood.) Most stem cells are derived in the first 2 mL. About 10 mL of aspirate is recommended for most sinus graft situations. The aspirate is then placed into the centrifuge for cell separation.[21-22] Post aspiration discomfort is usually limited to 1 to 3 days.

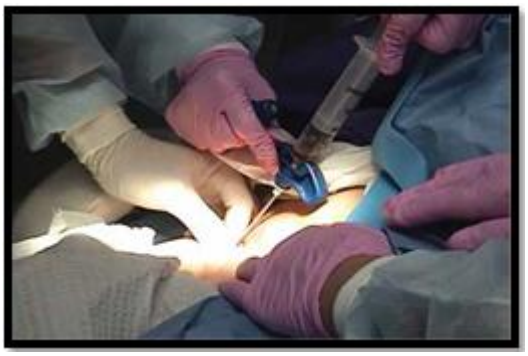

Figure 1: Obtaining marrow aspirate from anterior iliac crest

Graft Preparation

Following cell separation, biphase porous β-tricalcium phosphate (β-TCP) is mixed with the cell concentrate. β-TCP has been shown to be a favorable cell attachment vehicle for bone formation.[23-25] The cell preparation is placed beneath the sinus membrane, and the wound is closed with resorbable sutures. Dental implants can be placed immediately or 3 to 4 months later, according to standard osseointegration protocols. This unstudied technique may prove to be as successful as using autograft alone, and it appears to be equally effective in the elderly and younger patient

Therefore, Stromal stem cells derived from iliac bone marrow aspirate and concentrated by centrifugation can serve as a promising adjunct to initiate osteogenesis at the sinus floor. Using β-TCP as a space-maintaining carrier, bone harvesting is avoided while autogenous inductivity is preserved.

Tissue Engineering for Maxillary Sinus Augmentation:-

Bone augmentation in preparation for implant placement is usually carried out with autograft, allograft, or alloplast. [26-29] Regardless of the location of the donor site, when autograft is used, the potential for morbidity must be considered. Another consideration is the limited availability of intraoral bone that is suitable for grafting. Alloplastic materials are unsuitable in situations where vascularity is compromised, as is often the case in sinus grafting. Because it causes little or no donor site morbidity, tissue engineering for bony augmentation of the maxillary sinus floor offers a significant advantage over conventional grafting.

Ideally, this procedure is performed under local anesthesia using autologous bone with osteogenic capacity. Tissue engineering that involves the use of living tissue in vivo represents a new concept in cell culture technology. Compared with conventional cell cultures, the development of engineered tissues depends on the three-dimensional arrangement of cells and the formation or synthesis of an appropriate extracellular matrix, as in a combined alveolar and sinus defect. Current tissue-engineering methods use resorbable biomaterials, tissue encapsulation, and perfusion cultures and give major consideration to scaffolding of biomaterials to define a three-dimensional shape or to guide matrix formation. Naturally derived and synthetic polymers, composites, ceramics, and bone morphogenetic proteins, as well as cellular systems, are now under study. For the sinus graft, a carrier material for maintenance under the sinus membrane is essential.

Periosteum is now known to have cell populations that contain chondroprogenitor and osteoprogenitor cells that can be isolated in tissue culture and used to form cartilage and bone. The use of cultured periosteal cells for tissue engineering to repair bone and cartilage was first described by Rich et al[30] in 1994 and by Breitbart et al[31] in 1998.

Deshmukh et al 2015[32] evaluated the Bilateral maxillary sinus floor augmentation with tissue-engineered autologous osteoblasts and demineralized freeze-dried bone and stated that These cells can be harvested from a person, multiplied outside his body using bioengineering principles and technologies and later introduced into a tissue defect& concluded that tissue engineering makes it possible to fill larger volumes of the sinus cavity and provides predictable bone formation as compared to alloplasts and allografts. Tissue engineering also reduces donor site morbidity and makes the procedure more acceptable to the patient.

There remains also the critical question of how to supply cells embedded within large cell-polymer constructs while at the same time maintaining sufficient oxygen and nutrients to sustain survival and proliferation, allowing time for the integration of the developing tissue within the surrounding tissue. This may be the main cause for failure, ie, insufficient vascular support of the graft. One possible solution to this problem may be the application of vascular endothelial growth factor (VEGF) to achieve a higher initial angiogenic response and long-term stabilization of capillary-like structures.[33-34] Therefore, Ongoing efforts toward the development of tissueengineered materials are aimed at reducing or eliminating donor site morbidity and gaining materials with mechanical properties equal to or better than those currently in use. Intensive experimentation is taking place to create tissue-engineered hard tissue components such as bone, cartilage, or both.

Use of Tissue-Engineered Bone Cells for Sinus Augmentation

Implant-borne tooth restorations have become a standard of care in modern dentistry. For dental implant placement, the presence of sufficient bone volume is a crucial prerequisite. Predictable bone regeneration of large alveolard efects, such as those resulting from ablative periodontal disease or traumatic injury, pose a significant clinical challenge, particularly when accompanied by significant vertical bone loss. Of the various techniques for reconstructing a deficient

alveolar ridge, autogenous bone grafting is predictable, well-documented, and unequivocally accepted asthe standard of care. Nonetheless, autografting is sometimes associated with substantial morbidity in the form of infection, malformation, pain, and loss of function.[35] Alternatives to autogenous bone harvesting, such as allograft, xenograft, and alloplast bone substitutes, are associatedwith other risks and/or potential problems.[36-37] For example, allografts and xenografts carry some risk for disease transmission and have diminished bone-forming capacity. Synthetic materials have a higher incidence of infection, rejection, and extrusion than alloplasts and allografts, and their long-term interaction with host physiology remains uncertain. Because of these limitations and drawbacks, efforts are underway to find an autogenous alternative to conventional bone grafting that is minimally or non-invasive.[38] Anew technology has been developed via tissue engineering to establish a type of "injectable bone" resulting from morphogenesis of osteoinductive cells derived from cellcultures plated onto biocompatible scaffolds and augmented with growth factors.[39] In recent animal studies,[40-41]bone formation was consistently demonstrated in grafted efects using mesenchymal stem cells (MSCs), the growth factors in platelet-rich plasma (PRP), and/or a carrier of beta-tricalcium phosphate (β-TCP). Based on the results of these experimental studies, a human study was conducted using tissue-engineered injectable bone for sinus augmentation simultaneous to the placement of dental implants.

4. Cell based therapy for sinus augmentation

A. Cell Preparation Technique

One month prior to surgery, MSCs were isolated from the patient's iliac crest via marrow aspirate (10 mL), according to a reported method.[42] Briefly, the basal medium, low-glucose Dulbecco's modified Eagle's medium (DMEM), and growth supplements were obtained from BioWhittaker Molecular Applications. Three supplements were used for inducing osteogenesis: Dexamethasone (Dex), sodium β-glycerophosphate (β-GP), and L-ascorbic acid 2-phosphate (AsAP). These were obtained from Sigma Chemical. The cells were incubated at 37°C in a humidified atmosphere containing 95% air and 5% carbon dioxide. The MSCs were replated at densities of 3.1 x 10^3 cells/cm2 in 0.2 mL/cm^2 of control medium. The presence of bone-differentiated cells was confirmed via detection of alkaline phosphatase activity using p-nitrophenylphosphatase as a substrateThe cultured cells were trypsinized and readied for implantation.

B. Platelet-rich plasma preparation

Preoperative hematologic assessment included a complete blood count (CBC) with platelet levels. PRP was extracted 1 day prior to surgery. The PRP was isolated in a 200-mL collection bag containing anticoagulant citrate under sterilized conditions at the blood transfusion service department. To develop the PRP, the blood was first centrifuged for 10 minutes at 1,100 rpm. Subsequently, the buffy coat (containing the platelets and leukocytes) was taken up. A second centrifugation (2,500 rpm for 5 minutes) was performed to combine the platelets into a single pellet, and the plasma supernatant, which was platelet-poor plasma (PPP), was removed. The resulting pellet of platelets, the buffy coat/plasma fraction (PRP), was re-suspended in the residual 20 mL of plasma to create a platelet gel. The PRP was stored at 20°C in a conventional shaker until needed.

The performance of a sinus augmentation onlayosteoplasty using MSCs, PRP, and/or β-TCP (tissue-engineered "bone") with simultaneous implant placement suggest that tissue-engineered cells can yield adequate bone volume for simultaneous implant placement. The results of the investigation in recent years indicate that tissue engineered bone used for maxillary sinus augmentation with simultaneous implant placement in patients provides stable and predictable results and bodes well for the future development of tissue engineering in the coming decade.

Gene Therapy of Growth Factors for Tissue Engineering

Tooth loss, often a consequence of trauma or disease, can lead to the destruction of nearly half of the original tooth supporting (or alveolar) bone.[43] A variety of techniques have been developed to restore alveolar bone prior to or at the time of dental implant placement, including osseous grafting and guided bone regeneration.[44] However, lack of predictability and an inability to achieve volumetric bone changes beyond the "envelope" of the alveolus limit these reconstructive approaches. Advances in the field of tissue engineering and biomimetics offer significant potential to regenerate craniofacial structures using biologic mediators and matrices that mimic the tissue's original formative processes.[45-46] Tissue engineering of alveolar bone using gene therapeutic approaches may offer potential for optimizing the delivery of growth-promoting molecules at implant osteotomy sites.[47-48] the application of gene delivery methods for regeneration and repair of soft tissue and bone in periodontal, peri-implant, and sinus floor augmentation procedures is the future for upcoming researchers. Which include the role of bone morphogenetic proteins (BMPs) and platelet-derived growth factors (PDGFs) in dentistry and for reconstructive craniofacial procedures.

BMPs belong to the powerful superfamily of transforming growth factor beta (TGF-β) that regulate cartilage and bone formation during embryonic development and regeneration in postnatal life.[49] Recent studies have demonstrated the expression of BMPs during distraction osteogenesis, tooth development,[50-51] and periodontal repair.[52-54]BMP-7, also known as osteogenic protein-1 (OP-1), is a multifunctional member of the BMP family that handles multiple roles in bone formation and regeneration. BMP-7 stimulates bone regeneration around teeth,[55]endosseousdental implants,[56] and in maxillary sinus floor augmentation procedures.[57] Though encouraging, results of recent studies demonstrate only partial or inadequate regeneration, thus highlighting the need for improved methods of growth factor delivery.

Gene Transfer for Clinical Treatment Protocols

Since the half-life of growth factors in vivo is transient (on the order of minutes to hours), complete bone regeneration is not a certain outcome of conventional surgical therapy. Typically, high concentrations of growth factors arerequired to promote tissue regeneration.[58] Therefore, supplemental local growth factor production via gene transfer may be superior to bolus delivery methods.[59]In very basic terms,

gene therapy refers to the insertionof genes, directly or indirectly, into targeted cells alongwith a matrix to promote aspecific biologic effect in an individual. Typically, the aim is to supplement a defective mutant allele with a functional one, but it can also be used to induce a more favorable host response. Targeting cells for gene therapy requires the use of vectors, or direct delivery methods, to induce transfection.[60] By incorporating DNA in a matrix, gene therapy prolongs the bioavailability of such molecules.[61-62]Many vector systems are available to deliver DNA sequences of genes of interest, including plasmids, adenovirus, adeno-associated virus, and retrovirus. The choice of vector is based on the duration of delivery and the adverse effects associated with each vector.[63]Incorporating DNA into scaffolding matrices must allow for transfection of a sufficient number of cells to produce inductive doses of the desired protein. For example, incorporation of PDGF-encoded plasmid DNA into polyflactic co-glycolic acid) (PLGA) scaffolds showed encouraging results in sustained release of plasmid DNA, which led to transfection of cells and sustained production of the protein of interest for 28 days or longer. In addition, in vivo delivery of the PDGF-encoded plasmid DNA promoted matrix deposition and blood vessel formation in the developing tissue at 4 weeks.[64] In the future, combined DNA and cell therapy may be possible by transducing cells with the DNA of interest to obtain optimal numbers and then seeding the cells in the scaffolds. The cell-gene scaffolds could then be implanted in the wound to provide sustained release for regeneration of tissues and organs.

Gene Therapy for Alveolar Bone-Engineering Applications

The use of topical BMP to promote osteogenesis has been studied in a variety of bony sites over the past 5 years[65-66] In the craniofacial complex, an ex vivo approach was used for repair of periodontal alveolar bone; Jin et al[67] used BMP-7 gene transfer to stimulate new alveolar bone, tooth root cementum, and periodontal ligaments. Ad-BMP-7 or its antagonist, Ad-Noggin, transduced syngeneic dermal fibroblasts that were seeded on gelatin carriers and transplanted into large alveolar bone defects. Repair of the periodontal defects was observed as a process of rapid chondrogenesis followed by osteogenesis, cementogenesis, and predictable bridging of the bone defect. Conversely, Noggin (competitive BMP antagonist) gene transfer blocked alveolar bone and cementum repair, both in osseous defects and in tissueengineeredcementum.[68]More recently, gene delivery of BMP-7 via an adenoviral vector combined with a collagen matrix was used to repair large alveolar bone defects associated with implants at extraction sockets.[69]

PDGF has demonstrated strong potential in regenerationof gingiva,[70] alveolar bone,[71] and cementum[72] in a variety of wound-healing models. Alveolar bone defects treated with adenovirus encoding PDGF-B yielded strong evidence of bone and cementum regeneration beyond that of control vectors, including a nearly four-fold increase in bone bridging and a six-fold increase in tooth-lining cemental repair. In addition, sustained and localized expression of the luciferase reporter gene at the periodontal lesions was confirmed for a period of up to 21 days after gene transfer.

5. Conclusion

Many advances have been made over the past decade in the reconstruction of complex oral and craniofacial bone defects. In particular, developments in polymeric and ceramic scaffolding systems for cell, protein, and gene delivery have undergone significant growth. A cell-gene scaffolding system has been developed to re-engineer periodontal tissue with successful outcomes.[73]The targeting of signaling molecules or growth factors by gene therapy to the craniofacial complex has led to significant new information regarding bioactive molecules that promote tissue repair in both medicine and dentistry.

Further advancements in the field of gene therapy will continue to rely heavily on multidisciplinary approaches that combine the expertise of engineering, dentistry, medicine, and infectious disease specialists in repairing complex soft and hard tissue wounds

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