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HISTOLOGICAL STUDY TO COMPARE THE APPROPRIATE PARTICLE SIZE OF BETA TCP FOR SOCKET PRESERVATION

Oral Pathology

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ABSTRACT

Background: One the most used and potent synthetic bone graft substitute which is not only osteoconductive, but also osteoinductive which has been recently discovered and gain popularity is the β-tricalcium phosphate (β-TCP). Along with its cell-mediated resorption propertybeta TCP allow regeneration of full bone defect. Its clinical outcome is sometimes considered to be "unpredictable", possibly due to a poor understanding of β-TCP physico-chemical properties. **AIM:** Histological evaluation and effects of of beta TCP small and large particle at the site of defect in cases of grossly decayed teeth, bone defect and other required areas. **Material and Methods:** Total of 20 cases were included in the study (10 cases each of small and large particle). Trephine biopsy was performed and sent for histological evaluation. **Results:** Histological evaluation depicted the results on the basis of new none formation with residual bone graft material. Vascularity is studied in both the cases with areas of inflammation. **Conclusion:** Production of Mesenchymal stem cells progenitors plays a potent role in elucidating the effects of small Beta TCP particle and is responsible for the production of new bone.

KEYWORDS

Bone graft, particle size, tooth extraction, alveolar volume loss

INTRODUCTION:

Replacement of natural missing teeth with an implant has become a routine procedure in dentistry, with only one pre-requisite, which is, presence of enough bone at the surgical site to place an implant which can restore function and aesthetics. Autogenous bone is the gold standard due to its osteogenic, osteoinductive and osteoconductive properties, however its limited availability, very fast resorption and additional surgical site has decreased its rampant usage[1]. Also, other types of graft materials, such as xenograft and alloplasts have become popular due to their easy availability and reliable results. However, when it comes to predictable regeneration there are many factors one must consider, one being the particle size of the graft used, though not a widely studies topic, does play an important role in bone turnover.

The right kind of particle size has since long been in discussion. Mowlem way back in 1944 suggested that small particle size of the autogenous cancellous bone graft may enhance osteogenic effect[2]. Robinson also noted enhanced Osteogenesis, but also hypothesized that small particles in the bone Coagulum would resorb more readily[3].Usually, when a bone crusher is used to grind large chunks of autogenous bone collected intraorally, it produces small particle size4. In the literature,particle size of 100- 400 um has been found to be most appropriate[5,6]. It was suggested that these small particles may enhance osteogenesis compared to larger particles (1000 - 2000 um) due to enlarged surface area and ideal pore size between particles which allow for increased vascularization and osteogenesis to occur. Particles smaller than the aforementioned size get resorbed too fast for bone formation to occur. Particles that are too large may hinder vascularization and may be sequestered[7].

But recent research showed that large particle size (1000-2000µm) when used for lateral ridge augmentation resulted in greater ridge width gain at the level of the crest, however, vital bone formation was more extensive when small particles (250-1000µm) were used[8]. To study the particle size in this study authors chose a synthetic bone graft, β-TCP, it being synthetic in nature, demonstrates osteoconduction, osteoinduction, and cell-mediated resorption. It has also been used as a carrier for local administration of drugs (bone morphogenic proteins, antibiotics) at the defect site. Interestingly, with β-TCP there is production of more bone when the osteoclastic activity is more[9]. And the calcium released during resorption is used up for more boneformation[10]. Some of β-TCP has also shown osteoinductive activity[11-13], but for that to happen the graft should be first autoclaved. According to Kühlet al[14] there was no difference between autogenous bone alone or in combination with β-TCP while new bone formation was concerned. In this study, two different sizes were used, 0.25-1 small particle size and 1-2 large particle size.

MATERIALAND METHODS

All patients undergoing bone grafting followed by implant placement were counselled and only the cooperative patients were enrolled for the study. The following were the inclusion criterias:

1. Above 25 years of age.
2. Deficient bone, grossly destructed tooth, or any other indication for dental implant.
3. Patients with controlled medical conditions such as diabetes, etc.
4. Not allergic to any of the bio-materials used in the study.
5. Compliant towards the study.

Exclusion Criteria:

1. Patients with systemic diseases and/or presence of infections contraindicating periodontal surgery/implant placement.
2. Patients on medication known to interfere with tissue health and healing.
3. Pregnant or lactating females.
4. Patients with known habit of smoking and tobacco chewing.
5. Patients allergic to any product or to any of the medications used in the study.

After complete medical and dental evaluation, patients were randomly divided into large and small particles groups:

Group I: large particles,10 cases each

Group II: small particles,`10 cases each

Procedure:

The surgical procedure was done following all aseptic norms of the surgery, any grossly destructed teeth requiring implant was extracted and socket preservation was done usingpowerbone (bone graft)powerbone TURKEY. The socket was covered with a collagen plug. and sutures were placed to keep the collagen plug in place. (Patient was prescribed with mouthwash,Bluem ,Holland)

Sutures were removed after 10 days and the healing was uneventful. By 4 months, at the time of implant placement, bonecore was taken with a trephine bur of size 3.2mm for the histological examination and the implant fixture was placed at the same surgical visit

Histological Evaluation:

Histopathology of (large) size 1 -2 mm bone graft particle

METHODOLOGY:

3.2 mm trephine core biopsies were obtained and fixed immediately in

neutral buffered formalinsolutionfor 24-48 hours. The specimens were processed after decalcifyinginmilddecalcifying agent (10% EDTA,pH 7.4). The tissues were processed using standard tissue processing laboratory protocol ofdehydration, clearing and infiltration with paraffin wax. Embedding and tissue block preparation wasdone with paraffin wax. 4 micron thick sections were stained with Hematoxylin and Eosin stains. Theslides thus obtained were viewed inresearch microscope (Olympus BX53) and digital images werecaptured in low and highmagnification (Olympus EPL3).

Methodology for small size graft particle:0.25-1m

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RESULTS:

Since Bone is not completely inorganic in nature is 65% inorganic and 35% organic various stages of mineralization can be appreciated under Microscopic analysis. Decalcification procedure is used to study the relationship of soft and hard tissue together. Therefore submitted decalcified section of large beta TCP particle was stained with standard Hematoxylin and Eosin stain and showed well formed areas of mineralized bone with cellular components composed of osteoblasts, osteocytes, osteoid and vascular connective tissue. Abundantareas of mature bone formation with varying degrees of mineralization within a fibrocellularconnectivetissue stroma with minimal residues of remnant graft material were evident. The mature bone showedlamellations, lined by bone lining cells and entrappedosteocytes with in the osteocytic lacunae. Thesections also showed focal areas showing residual graft particle at the graft- new bone interface. Verymild diffuse inflammatory infiltrate was seen suggesting satisfactory uptake of the grafted biomaterialin the absence of remarkable host inflammatory reaction. Areas showing new bone formation withentrapped osteocytes within the osteocytic lacunae at higher magnification were also seen. Vascularityand areas of new blood vessel formation is not appreciated in the histology of the section.

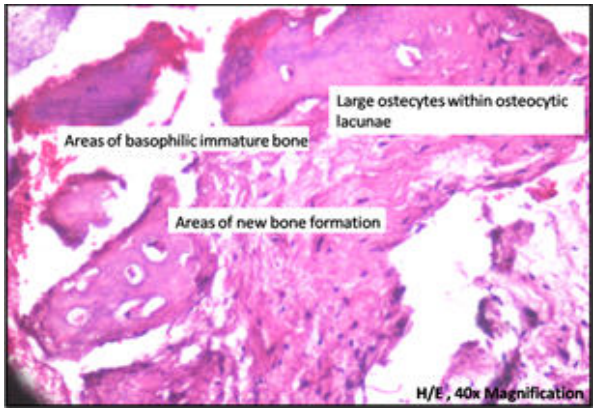

Figure1. Hematoxylin and Eosin stained section of Large Beta TCP particle with areas of new bone formation with areas of no new blood vessel formation. (40x magnification)

For Small Bone Graft Particles

The Hematoxylin and Eosin stained section showed well formed areas of Bony Trabeculae with varyingstages of Mineralization. Section showed abundant areas of basophilic immature bony trabeculaeswithlarge number of entrapped osteocytes line by osteoblatic rimming and vascular connective tissue. Focalareas showed the presence of new bone formation and areas of remenant graft material at the graft-new bone interface. The mature bone showed lamellations, lined by bone lining cells and entrappedosteocytes with in the osteocytic lacunae. Dense infiltration of diffuse inflammatory infiltrate was seensuggesting satisfactory uptake of the grafted biomaterial. Vascularity and areas of new blood vesselformation is appreciated in the histology of the section

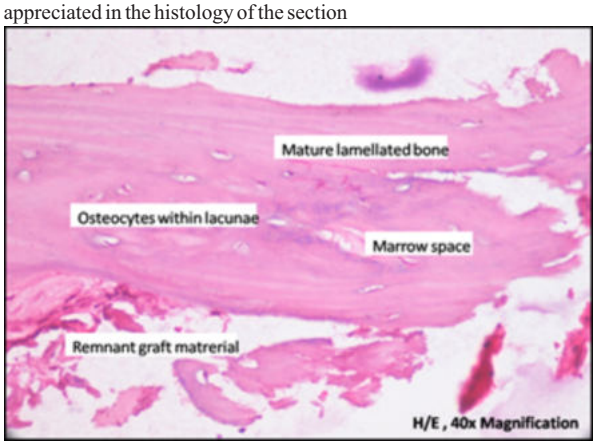

Figure 2: Hematoxylin and eosin stained section of small particle Beta TCP showing areas of new bone formation and areas of vascularity and inflammatory infiltrate. (40xmagnification)

DISCUSSION:

In one of the early discussions on bone augmentation, Rivault[15] stated that, as a rule, the graft particles get resorbed and then replaced by a new mineralized tissue along the walls of living host tissue, showing that 'host cellular elements' were more important than the graft material used, which only provides mineral salts, hence lies the importance of particle size[3]. This statement cannot be considered correct when it comes to autogenous bone as it is osteoinductive, osteogenic and osteoconductive, but due to aforementioned disadvantages its use has decreased. But when a different graft material is being used for augmentation then the intrinsic property of the graft type must be considered in selecting large or small particle size.

Allografts, are readily available but, they do not produce the inorganic calcium or scaffolding necessary for bone regeneration but provide excellent source of collagen type I[16]. so, even if large particles are used for bone regeneration, it will produce predictable results. A study found that even the farthest particles of freeze-dried bone allograft from the host-graft interface wereembedded in new bone[17].In 2010, Chackartchi et al[18] found no clinical or histological differences between the small and large particle size of DBBM. Similar results were observed by Pereyra et al[19], high rate pf bone formation was observed regardless of the particle size Bio-Oss® S (0.25-1 mm) and Bio-Oss L (1-2 mm). Fucini et al[20]too noted that results did not differ significantly when particles 250 to 500 µm or 850 to 1000 µm were used to treat periodontal defects.Xenograft, on the other hand lacks organic component, inorganically provide a natural architectural matrix as well as a high source of calcium[21]and also, helps in maintaining the physical dimensions of the augmented bone during the remodeling phases[22]. One must also consider resorption rate of xenograft, in a study by Degidi et al[23] even after 8 years of placement of bovine bone, 6.2% of the xenograft could be found at the site and in another study 17.3% of the graft even after 11 years of its placement[24]. Hence, in author's opinion, a small particle size should be considered when using a xenograft, this however, has not been confirmed in any of the clinical trials or histological studies, only observations/small case series/animal studies happen to highlight[25]. In a study comparing two different particle sizes of demineralized bovine bone mineral (DBBM) for sinus floor augmentation, authors stated that both the sizes act similarly when it comes to new bone formation[26]. When it comes to alloplasts, large particlesare suggested for lateral ridge augmentation[27]. Hall et al[28] compared two different size range bioactive glass, 300 to 355 micronsand other one with large size range 90 to 710 microns with demineralized freeze-dried bone allograft and stated that size of the graft particle is not significant. His results showed higher percent bone-to- implant contact and percent bone height fill in with the use of DFDBA.

For osteoconduction to occur, presence of differentiated mesenchymal cells becomes paramount[29,30]. This can be made possible by maintaining adequate blood supply. In the histology of large bone particles, we cannot appreciate vascularity, as much as it can be appreciated in the histology of small graft particles. Small particle size means more surface area, facilitating growth factors to form new blood vessels and accelerating differentiation of mesenchymal cells into

osteoblasts[31]. Another thing to be understood is the that fact that in all the studies aforementioned authors have used different graft type for regeneration of different bone at different sites, this shows that different bone particles exhibit different bone healing abilities which is also site dependant. However, there is no literature with large case series, studying one type of bone graft in different sites to state which particle size is suitable for which site for regeneration[32-35].

Another pointer to ponder upon in achieving vascularization is the space between graft particles, which should be more than 100 µm[36,37]. Research has shown that there is greater bone formation when there is spaces of 300 to 500 µm between particles as compared to 50 to 100 µm of space38,39. This depends upon the porous structure of the graft particle and was found to be a significant factor in osteoconduction and synostosis[40,41].Studies have demonstrated that materials with high porosity have a positive impact on bone formation[42].In literature, two different pore sizes, <5 µm in diameter and >100 µm in diameterhave been studied[43]. Micropores tend to increase the surface area of the graft while macroporestend to enhance material resorption and boost osteoinduction[44]. Presence of open and interconnected macropores are vital for blood vessel formation, cell migration, diffusion of nutrients, and circulation of pro-osteogenic factors[45]. β-TCP scaffolds with interconnected pore structure (ihTCP) presents higher new bone formation volume[46]. β-TCP being a porous graft material, has the ability to boost the infiltration of MSCs, thus enhancing new bone formation[47].In an experimental study while comparing porosity between β-TCP blocks with 58.1 ± 1.7% porosity and dense β-TCP blocks group with 10.9 ± 2.3 porosity, former showed 200-fold higher new bone formation just in 4 weeks[48]. This shows that particle size and a porous structure are crucial for new bone formation, however the porous structure is dependent upon the particle size.

Literature also suggests use of large particle size, Urist et al[49] reported that particles size of 1000 to 2000 µm were more effective in bone formation, whereas, smaller size of 250 to 420 µm interrupted chondrogenesis and ossification. Another author Cornu et al[50] stated that large particles of fresh-frozen bone have higher stiffness than smaller morsels during impaction. Our study is in consistency with the literature which states that small particle size enhances bone regeneration.

CONCLUSION:

Macrophages are vital regulators of skeletal remodeling and osseous repair. Based on histological study it is safe to state that small particles size aids in production of more number of residents MSC's which further leads to more vascularisation with osteogenic differentiation, hence more bone formation. Therefore it has been putforth that bone osteogenesis initiated by MSCs with artificial bone may create a perfect combination, and make artificial bone a better scaffold with potential of osteoconduction and osteoinduction.

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Conflict Of Interest: none

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