Autologous Buccal Pad of Fat Stromal Vascular Fraction Used To Regenerate Larger Defect Due to Maxillary Dentigerous Cyst

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Abstract

Cyst is a pathological cavity may or may not be lined by epithelium, contains fluid, semisolid material or gas and present in bone or soft tissue. Sometimes the cyst may involve important anatomical structure which requires careful enucleation and retaining anatomic integrity. Various modalities of treatments can be advocated like enucleation, marsupialization and resection. To bring the impacted canine by orthodontic movement good bone support is necessary in the osteolytic region. Hence we attempted bone regeneration with stem cells from autologous stromal vascular fractions SVF. We used SVF from buccal pad of fat with minimal manipulation to treat larger defect involving right maxilla and maxillary sinus due to dentigerous cyst. Good bone regeneration was achieved with 16 month of followup. Patient is now under orthodontic treatment to bring down the impacted canine.

Key word: *Dentigerous Cyst, Enucleation, Buccal pad of fat, SVF.*

Introduction

Cyst is a pathological cavity which may or may not be lined by epithelium, contains fluid, semisolid material or gas and present in bone or soft tissue[1]. Dentigerous cyst is lesion attached to the cervical region of an impacted tooth and cystic fluid forms between reduced enamel epithelium and enamel of the tooth crown. Usually dentigerous cysts are more common in mandible frequently in male and it is the second most common cyst of the jaws comprising 14-20% of jaw cyst. Mandibular 3rd molar followed by maxillary canine and occasionally supernumerary teeth or odontomas are involved in cyst formation [2, 3]. Dentigerous is usually associated with painless expansion, grow considerably in size of the jaw and cause facial asymmetry[4]. It occurs at any age group, but more common in the second and third decades[5]. Small cysts are usually easy to treat surgically. However, these are asymptomatic even when reaching considerable size and impacted tooth are displaced distance due to cystic pressure and treatment is more difficult one. Surgeries may results in removal of many teeth or tooth buds or affect the vitality of adjacent teeth.

SVF from Buccal pad of fat

According to Hausman, Dodson [6] the word adipocyte refers to the lipid filled cell, found in high concentration in various body locals. Basic adipose architecture contains some lipid filled adipocytes, connective tissue, blood vessels, pre adipocytes, monocytes and macrophages. A cell fraction that serves as the precursor to majority of adipocytes of the adult is called stromal vascular cell fraction (SVF). The pioneering work of Prunet-Marcassus et al. [7] suggested that adipose tissue contains a number of progenitor cells that gives rise to different phenotypes in

addition to a large portion of macrophages and hematopoietic progenitor cells.

The study was performed at the Department of Oral and Maxillofacial Surgery, KSR Dental College, Tiruchengode, Tamilnadu, India. This study was performed with written informed consent from his parents and after the approval of Institutional Ethical Committee - KSR Institute of Dental Science & Research (IEC-KSRIDSR) and in full accordance with the World Medical Association Declaration of Helsinki.

We took buccal pad of fat from right side of the patient's cheek (malar region) intra orally before surgery for dentigerous cyst under local anesthesia. The fat tissue was processed into SVF under Good Manufacturing practice (GMP) class clean rooms according to standard operating procedure at the Research Department of Biotechnology of KSR institution campus. After sending fat tissue for SVF isolation clinical procedure of cyst removal was carried out.

Isolation of SVF Fromhuman buccal pad fat [8]

The experimental protocol was approved by the Institutional Review Board KSR institute of Dental science and research ,Tiruchengode. The adipose tissue was processed within 2 hours of sample collection to retain the viability of the cells. The fat was processed for isolation of Stromal Vascular Fraction as previously described by Gandi et al. [8] with few modifications. Briefly, after rinsing in normal saline collected fat (6gms) was placed in a sterile petri dish, and was minced into small (4-5 mm) pieces with surgical scissors and scalpel. After removal of connective tissue the minced sample was transferred in centrifuge tube containing 10 ml of Type I Collagenase - Animal free origin (17100-Gibco® Life Technologies™, NY, USA). The adipose tissue-collagenase suspension was incubated at 37°C 30 to 60 mins with intermittent vigorous shaking for every 10 minutes. After incubation the suspension was neutralized by equal volume of complete medium, consisting of Mesen PRO medium supplemented with 20% of autologous serum. Then the content was centrifuged at 4000g for 10 min to pellet the MSC-rich dense stromal vascular fraction. After centrifugation the adipocyte and fat appeared as a yellow oily layer at the top of the tube. This supernatant containing the oily layer and collagenase solution was decanted with the help of a transfer pipette. The pellet was suspended in 2 ml of sterile 160mM NH4Cl and incubated at room temperature (RT) for 10 min to lyse the red blood cells. The samples were transferred to new centrifuge tube and centrifuged at 300g for 10 min to pellet the RBC free SVF. The SVF pellet was then resuspended in HBSS and filtered through a 40 ?m strainer (352340, Corning). The cell number & viability of SVF was calculated by tryphan blue dye exclusion assay as per the protocols of Darlington [9]. Following that about 15 × 106SVF cells/ml, was suspended in 4 ml of patients PRP prepared and taken for administrating after the clinical procedure of cyst removal.

Case Report:

14 year old boy reported to the department of oral & maxillofacial surgery with complaints of swelling in the right side of the face lateral to nose and swelling in the anterior part of upper jaw. On examination extra oral swelling 3 x 2 cm size present in lateral to right side of nose and obliteration of right nasolabial fold causing facial asymmetry (Fig 1). Intra oral examination revealed non-fluctuant swelling with obliteration of right labial sulcus from midline to 1st premolar region. Missing 13 and retained 53 (deciduous canine). Grade II mobility in 11, 12 and both these teeth are tilted towards the mid line. Duration of swelling was 10 months. Patient treated elsewhere for this complaint for the past 3 months and root canal opened in 11 and 12 there. OPG (Fig 2) I.O periapical X-Ray revealed larger cystic lesion involving right maxillary region with impacted 13, lying over 14, 15 region, also impacted supernumerary tooth lying right to lateral nasal aperture and roots of 11, 12 tilted towards the distal side. Aspiration showed straw colored fluid. Both clinical and radiographic findings suggested the provisional diagnosis of dentigerous cyst.

We discussed treatment plan with patient regarding preserving 11 (Right central incisor), 12 (Right lateral incisor) and to bring the impacted canine to normal anatomical position by orthodontic treatment. To bring the teeth from the impacted position which is very horizontally

placed, needs good bony support. Since the patient has dentigerous cyst there was bony erotion. So we wanted to regenerate bone. We decided to do cell based therapy with SVF from buccal pad of fat.

Treatment:

First step, we tried to reduce the size of cystic lesion by doing marsupialisation. Under local anesthesia (LA), we removed 53 (maxillary deciduous canine) and entered into cystic cavity, removed small portion of cystic lining for biopsy and to maintain the patency of opening. Small portion of ryles tube with multiple perforations were placed inside the cystic cavity through extracted socket of 53 (maxillary deciduous canine) and one end of the tube sutured with oral mucosa to stabilize the drain. Drain maintained for 1½ months, often irrigated with betadine solution through drain. During this time, root canal of 11, 12 filled. Biopsy result was diagnosed asdentigerous cyst.

Under LA (posterior superior alveolar nerve block) through intraoral approach, small incision was placed the right maxillary buccal sulcus region above last molar area, enter the buccal space, exposed part of buccal pad of fat (BPF). BPF mobilized taken out by slowly pulling out by artery forceps. Around 10 cc (6g) of fat taken out (Fig 3&4) and placed in the normal saline and immediately sent to the Good Manufacturing practice (GMP) class clean rooms according to standard operating procedure at the Bio - technology lab at KSR institution campus. Intraoral wound closed with 3-0 black silk. Time taken for processing to get SVF was around 2½ hours.

After an hour of harvesting BPF, under LA (bilateral infra orbital nerve block, nasopalatine nerve block and right side greater palatine nerve block) two relieving incision made, one on between 22 and 23, second on between 15 and 16 followed by gingival sulcular incision made from 22 to 15. Mucoperiosteal flap raised to expose the cystic lesion. Expanded, perforated buccal cortical bone 11, 12 and 13 region to expose the cystic lesion. Cyst enucleated by piece meal along with impacted supernumerary tooth. All the wall of maxillary sinus and around 11, 12 thoroughly checked for pathological lining and confirmed the devoid of lining (Fig 5). During this procedure, 15 ml of blood taken from patient sent for processing to prepare platelet rich plasma (PRP) and platelet rich fibrin (PRF).

Exposed crown of 13 and orthodontic button placed with ligature wire on the 13 for the orthodontic movement. Mean time processed BPF-SVF, PRP and PRF were ready for placement. After achieving the hemostasis, , sybografts (SYBOGRAFT-T-PLUG is a sterile synthetic beta Tricalcium phosphate) plug 25mm x 12mm size 3 in number placed on inner side of the strut of the buccal cortical plate over the 12 with resorbable suture (Fig 6). SVF and PRP mixed placed over the sybograft which absorbed these materials. And PRF placed over the sybografts and placed inside the cystic cavity. Wound closed with 3-0 vicryl. No blood or fluid came out through the nostril proper post-operative instructions given to avoid maxillary sinus infection and oroantral communication formation. Post-operative days were uneventful. Post-operative intra oral perapical X ray taken 7 day (Fig 7) Periodic checkup and radiographs-OPG and maxillary occlusal view (Fig 8&9) are taken to confirm bone formation. Grade II mobility present in 11 and 12 was not noticed after 3 weeks' time. After 1½ month of operation, orthodontic treatment started. With the 16 month follow up, bone formation is good, teeth 11 and 12 are stabilized without mobility, space created for 13, extra oral swelling came down to normal and no maxillary sinus infection. Patient is now under orthodontic treatment.

Discussion

Management of the cyst by various modalities like enucleation with or without removal of impacted tooth, marsupialization and resection. Cyst size and site, patient age, teeth involved and involvement of vital structures will play a major role in deciding above treatment plan. For extensive cyst involving many teeth in young patient, treatment plan may be decompression (marsupalization) followed by Enucleation. In this situation surgical access should be maintained for longer duration and recalled the patient very frequently.

Anjana et al. [10] in their article noted that dentigerous cyst very rarely occurs in maxillary

anterior region. They suggested marsupalization is first treatment option for younger age groups and advised radiographic review for every 6 months for the first 2 years is mandatory. Since the remnant of cystic lining can undergo ameloblastic changes.

Riordan et al. [11] has reported that adipose tissue is a rich and very convenient source of cells for regenerative medicine therapeutic approaches. In human adipose tissue are taken from abdominal area and also from buccal pad of fat in face region. It is easy and safe also to access these adipose sources than bone marrow sources and also have more MSC than bone marrow. The SVF of adipose tissue is known to contain mesenchymal stem cell, T regulatory cells, endothelial precursor cells, preadipocytes, as well as anti-inflammatory macrophages. Equine and canine studies demonstrating anti-inflammatory and regenerative effects. SVF contains large number of MSC like cells that could be induced to differentiate into adipogenic, chondrognic, myogenic and osteogenic lineages. Also monocytes/ macrophages present in SVF provide immune modulatory effects.

Sándor et al. [12] report describes the replacement of a 10 cm anteriormandibular ameloblastoma resection defect, reproducing the original anatomy of the chin, using a tissue engineered construct consisting of B - tricalcium phosphate (B - TCP) granules, recombinant human bone morphogenic protein 2 (BMP -2) & good manufacturing practical - level autologous Adipose Stem Cell (ASCs). Unlike prior reports, 1 - step in situ bone form ation was used without the need for anectopic bone formation step. There constructed defect was rehabilitated with a dental implant supported over denture. A subcutaneous a diposet issue sample wash arvested from the anterior abdominal wall of the patient before resection ASCs was isolated and expanded ex-vivo over the next 3 weeks. The expanded cells were seeded onto a scaffold consisting of BTCP and BMP -2 and the cell viability was evaluated. The construct was implanted into the parasymphy seal defect.

Generally these cases were treated by extraction of 11, 12 because of more bone loss around the 11, 12 and grade II mobility, followed by cyst enucleation with removal of impacted 13 and supernumerary tooth and primary closure with or without bone grafts, which lead to loss of teeth and bone. Extraction of many teeth with loss of bone results in difficult of placing the fixed prosthesis. So we planned to preserve the 11, 12, and 13 and regenerate the bone with autologous buccal pad of fat SVF. The main advantage of our treatment plan for this situation, we moved the pathological lining completely along with the impacted supernumerary tooth on the same visit, avoiding the possibility of tumor formation, without multiple visits. Along with loss of teeth and repeated radiographic evaluation are also prevented .When comparing with use of BMP (Bone Morphogenic Protein) which is used for bone regeneration in these kind of defects, SVF is very economical and safe for the patients. Natural anatomy, esthetics and function of preserving the natural teeth were possible by this technique.

Conclusion

Various modalities of treatments can be advocated like enucleation, marsupialization and resection for the management of cystic lesions. We used autologous buccal pad of fat-stromal vascular fractions (SVF) with minimal manipulation to treat larger defect involving right maxilla due to dentigerous cyst involving maxillary sinus to avoid extraction of permanent teeth and to bring impacted canine in anatomical position. This required good bone support. In this case Good bone regeneration achieved with in 16 month follow up. Patient is now under orthodontic treatment. Autologous buccal pad of fat-stromal vascular fractions (SVF) is produce promising results in quality bone regeneration in jaw bones. This type of bone regeneration should be tried with multi-center study to make it for regular practice.

Figures

Fig 1. Pre-Operative

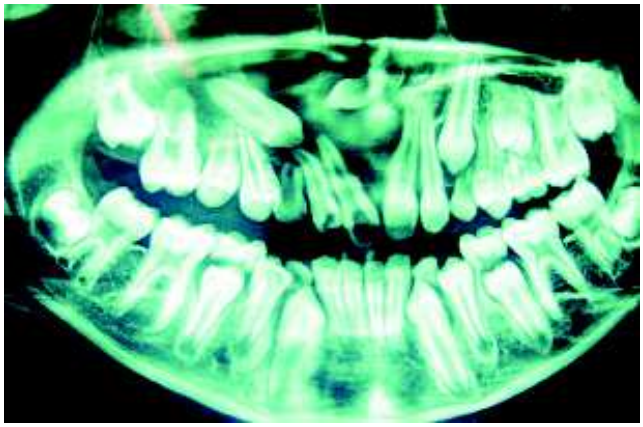

Fig 2. Pre-Operative OPG

Fig 3. Harvesting buccal pad of fat

Fig4.Harvested fat for SVF processing

Fig 5. Intra operative cyst enucleation

Fig 6. Placement of graft material along with SVF & PRP

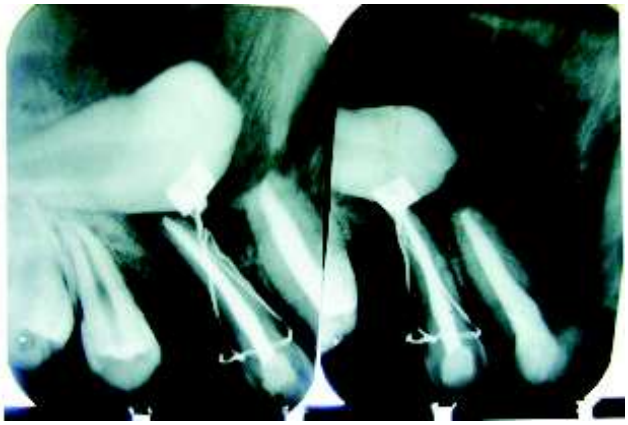

Fig 7. Immediate post-operativeIOPA radiograph

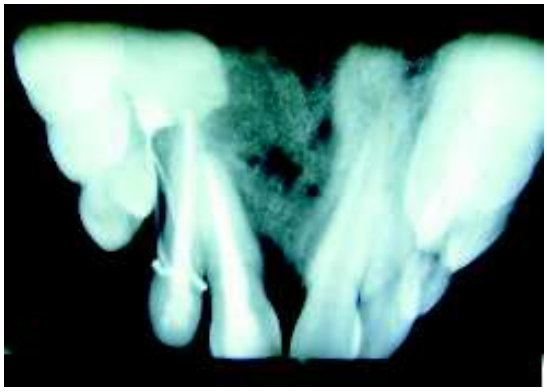

Fig 8.Occlusal view.

Fig 9. Postoperative OPG (4 Months)

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