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Periodontal Regeneration by Autologous Bone Marrow Mononuclear Cells Embedded in A Novel Thermo Reversible Gelation Polymer - Report with 36 Months Follow-Up

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Abstract

Regeneration of bony defects caused by periodontal disease continues to be a challenge for clinicians. Application of stem cells from different tissue sources and scaffolds for regeneration have been reported in animal models but clinical studies with long term follow-ups are limited. Herein we report the three years follow-up of the application of autologous bone marrow mononuclear cells (BMMNCs) embedded in a thermo-reversible gelation polymer (TGP) for periodontal regeneration. A 23-year female patient with advanced periodontitis (class IV gingival recession, probing pocket depth (PPD) of 5 mm and 6 mm in relation to mandibular lateral and central incisors respectively, and clinical attachment level (CAL) of 13 mm) correlated with radiographic evidence of severe horizontal bone loss extending up to the apex of mandibular incisors was selected for the treatment. After debridement, the defect was implanted with BMMNCs impregnated in TGP. Then the clinical parameters and radiographic evaluation were made at periodic intervals of 6, 12, 24 and 36 months. At six months, significant improvement with the clinical parameters (PPD had reduced to 2 mm, clinical attachment level had improved by 6 mm) was observed. At 36 months, the radiograph revealed bone regeneration with improvement in vertical and horizontal bone height. Transplantation of BMMNCs in a novel TGP is safe and results in a relatively significant and stable clinical outcome in horizontal alveolar bony defects.

Keywords: Periodontitis, Alveolar Bone Loss, Regeneration, Stem Cells, Tissue Scaffolds

Introduction

Osseous grafting and guided tissue regeneration (GTR) are established techniques for periodontal regeneration; however several recommendations for improvement have been suggested [1-3]. Recently, use of bone marrow stem cells [4], periodontal ligament cells [5], alveolar periosteal cells [6], dental

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pulp stem cells [7], periapical follicular stem cells [8] and scaffolds such as Fibrin Glue [4], Tricalcium phosphate (β-TCP), Hydroxyl apatite [5], and Collagen [6] have been experimented. Clinically autologous [9] and allogenic mesenchymal stem cells [10] with scaffolds have been reported but with limited long-term efficacy. Herein we report a three years follow-up of application of Bone Marrow Mono-Nuclear Cells (BMMNCs) embedded in a Thermo-reversible Gelation polymer (TGP) in advanced periodontitis.

Case Description

A 23-year-old female patient reported with severe mobility of mandibular incisors during January 2009. Medical and dental histories were taken. Intraoral examination disclosed poor oral hygiene [OHI(s): Score 3.6], and periodontal examination revealed Probing Pocket Depth (PPD) of 6 mm and 5 mm in relation to mandibular central and lateral incisors respectively, with class IV gingival recession (GR), and grade III mobility of mandibular central incisors. Clinical Attachment Level (CAL) was 13 mm with radiographic evidence of severe horizontal bone loss extending up to the apex of mandibular incisors (figure 1).

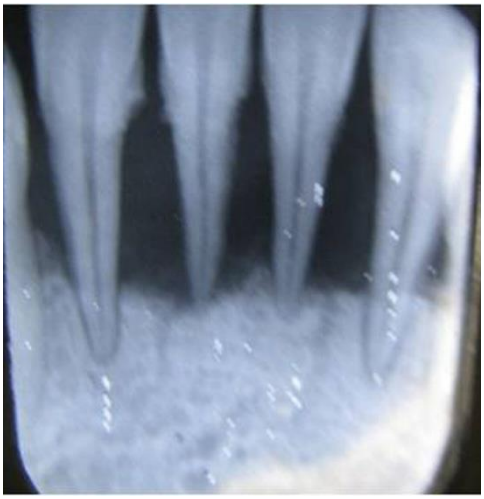

Figure 1. Preoperative radiograph showing horizontal bone loss extending up to the apex of the mandibular incisors.

Patient was informed about the surgery, graft material, and uncertainties of using a new regenerative method. Written consent was obtained in accordance with the Helsinki Declaration. The Institutional Ethical Committee approved the protocol prior to study.

Thermo-reversible Gelation Polymer (TGP)

TGP (Mebiol® Nichi-In Biosciences, Chennai, Tamil Nadu, India) is a copolymer composed of thermoresponsive polymer blocks [poly(N-isopropylacrylamide-co-n-butyl methacrylate) and hydrophilic polymer blocks (polyethylene glycol [PEG]) [11]. It was provided in a lyophilized sterilized form in a flask. Later, 4 ml of TGP was dissolved in 4 ml normal saline and refrigerated at 4°C until further application.

Bone Marrow Aspiration and BMMNCs Isolation

Thirty ml of bone marrow was aspirated from the iliac crest under local anaesthesia. It was transported in anticoagulant acid citrate dextrose bags under cold chain preservation to the cell processing facility. The sample was processed under cGMP SOP's class 10000 clean room and class 100 Biosafety cabinets. It was subjected to Ficoll gradient centrifugation procedure and BMMNCs were collected by removing the Buffy coat. The viability of cells was checked using Trypan Blue Dye exclusion method using Neubaur's Hemocytometer. A portion of the processed cells was subjected to Flowcytometry analysis for quantifying the CD34+/CD45- cells by appropriate Fluorescein Isothiocyanate (FITC) antibodies (Becton Dickinson, Jan Jose, USA). The Endotoxin test was also carried out using Limulus Amebocyte Lysate (LAL) kit method for confirming the sterility [12].

Surgical Procedure and Cell transplantation

The initial periodontal treatment (scaling, root planing and oral hygiene instructions) was completed and extra coronal temporary splinting of mandibular anteriors was carried out. Endodontic therapy was

performed on #24 and 25. Four weeks elapsed between the non-surgical and surgical phases. Under adequate local anesthesia full thickness mucoperiosteal flap was raised in relation to mandibular anteriors and the pocket epithelium was removed.

Granulation tissue residing in the defect areas was carefully excised to expose severe horizontal bone defect and the root surface was planed. Subsequently, the isolated cells (45 x 10^6 BMMNCs) were seeded in 4 ml of the TGP and the construct was allowed to solidify (figure 2). The cell-construct was grafted on to osseous defects (figure 3). The flaps were replaced and retained with sutures. Post surgical oral hygiene instructions were given. After 2 weeks, the sutures were removed and supportive professional care was performed for every three months.

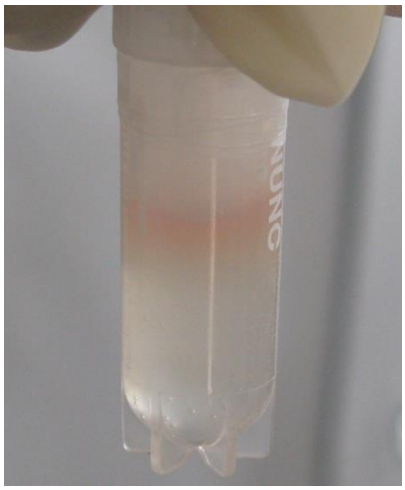

Figure 2. BMMNCs seeded in TGP and allowed to solidify.

Clinical Outcome

The patient had an uneventful postoperative period. She was regularly followed up for PPD, CAL, GR, and radiographic assessments at 6, 12, 24 and 36 months (table 1). The intraoral periapical radiograph (Figure 4) of the site after 36 months showed stable bone height in evidence of the horizontal bone regeneration.

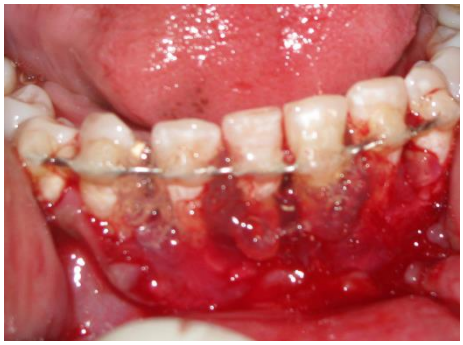

Figure 3. BMMNCs-TGP construct is applied on osseous defects and adjacent root surface.

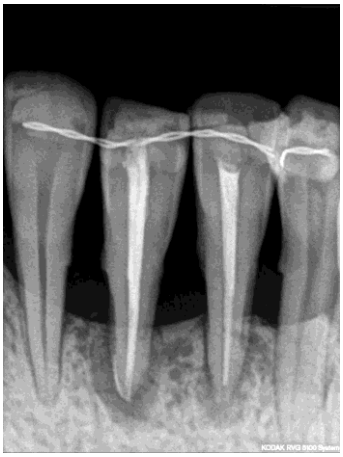

Figure 4. Postoperative cropped panoramic radiograph after 36 months showing mandibular anteriors. Note the significant trabecular bone formation with increase in vertical and horizontal bone height.

Table 1. Clinical data from pre-treatment (Baseline) to 36 months post-treatment

Measurements at #24 and 25	Baseline	6 months	12 months	24 months	36 months
PPD (mm)	6	2	2	2	2
CAL (mm)	13	7	7	7	7
GR (mm)	7	5	5	5	5

Values represent deepest measured data at the mandibular four incisors at different sites.

PPD, probing pocket depth; CAL, clinical attachment level; GR, gingival recession.

Discussion

The BMMNCs contain CD34+ hematopoietic stem cells and Mesenchymal stem cells (MSCs) population in addition to other subpopulations including the endothelial progenitors. This is beneficial as tissue regeneration can be complete only if the regeneration of the matrix component is supported by adequate angiogenesis. There are studies, which have proven that the application of whole BMMNCs is more successful than methods, which use sub fractionated cell preparations like MSCs or other cell populations [11, 13]. Hence, BMMNCs were utilized in the present study. Secondly, use of autologous BMMNCs excludes the issues of immunorejection and transmission of infection. Further it has been shown that transplantation of cells in scaffolds yield better results than when the cells are implanted alone [14].

Osanai *et al* [14] demonstrated that in cerebral infarct, transplantation of MSCs with TGP scaffold showed higher cells compared with the group without the scaffold. TGP has shown to maintain stem cells in an undifferentiated state for longer period of time and it can release growth factors incorporated into it in a controlled manner which are additional benefits [15].

Further, TGP is purely inert synthetic material which does not alter the gene expression profiles or other characteristics of the cells cultured in it. It is free from prions of bovine spongiform encephalopathy which is a drawback with biological scaffolds. Various animal studies have proven TGP to be safe for implantation with bone marrow stem cells [14], chondrocytes [15], hepatocytes [16], and corneal limbal stem cells [17]. All these justify the use of TGP as a scaffold for the present study.

Six months post operatively, PPD had reduced to 2 mm and the CAL had improved by 6 mm. The clinical parameters were stable even after 36 months of surgical treatment with professional debridement at regular intervals. This may be attributed to the conventional surgical treatment and BMMNCs-TGP therapy, which are effective in arresting the disease progression and bone regeneration. Radiographic assessment was consistent clinically with good prognosis of teeth.

Conclusion

This three-year follow-up study proves the safety and efficacy of transplantation of autologous BMMNC in horizontal alveolar bony defect. However, studies with larger sample size are necessary to statistically confirm the efficacy of this procedure in the future.

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Potential Conflict Of Interests

None.

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