Periodontal tissue generation using autologous dental ligament micro-grafts: case report with 6 months follow-up

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Abstract

Introduction
In the previous years, stem cell research has found several problems in transferring the scientific models into clinical practice. For this, it has devised a new clinical process of tissue regeneration, through autologous micro-graft named Rigenera. Using Rigenera, the patient is, at the same time, the donor and the acceptor of calibrated micro-graft rich of stem cells. This system permits an increase in the stem cell number in the surgical site that needs to be regenerated. This case report was performed to analyse the clinical outcomes of an innovative treatment protocol of deep intra-osseous periodontal defects using dental ligament micro-grafts loaded onto collagen sponges as filling biomaterial.

Case report
We selected a healthy female patient with two intra-osseous defects distal to second lower molars, due to mesio-impact of both vital third molar included. We used the two defects, one as a test site (cell suspension + collagen sponge scaffold) and the other as a control site (only collagen sponge scaffold). Clinical and radiographic examination was performed at baseline and at 1 week, 1, 3 and 6 months after surgery. The x-rays performed at 3 and 6 months show significant differences between the control site and test site: the latter presented an increased mineralisation rate and the complete filling in the coronal component of the defect with respect to the control site.

Conclusion
The results indicate that the autologous stem cell obtained from connective tissue could be used to provide the basis for bone regenerative surgery, with limited sacrifice of tissue, low morbidity at the collection site and significant reduction in time needed for clinical recovery.

Introduction
The increasing research on autologous stem cells and their abilities gave an input to the study of self regenerative potential of the injured tissues—the presence of autologous cells reservoir able to provide elements to restore the periodontal tissues is extremely intriguing and enforces the concept at the base of conservative and minimally invasive surgery. The problem of these cells reservoir is the surgical management, because often we do not have tools and procedures to use them for clinical application. In this study we present a split-mouth case report, where autologous dental ligament was used to generate micro-graft through a new technology called Rigenera Protocol. This Protocol is made by a machine, the Rigenera Machine, and disposable medical devices, the Rigeneracons, able to mechanically break-up small tissue samples preserving cell viability.

From the 1960s periodontal research has been interested in regenerating the periodontium, moving on a wide spectrum of hypotheses as the use of biomaterials and biomolecules. In the previous years, the approach has pointed on increasing the self regenerative potentials of the injured tissues, in order to reduce complications and make the results more predictable1-3.

In the recent years, it has been supposed to collect stem cells from a rich zone and use these cells to treat diseases or trauma of tissues with a limited self regenerative potential thanks to their particular plasticity4; therapeutic protocols for homologous and autologous human transplantation have been conducted with different results5. Actually the surgical access to these potential collection sites often is a limiting point and the ratio between the tissue collected and stem cells isolated is often disadvantageous6.

The dental ligament has been shown to own stem and progenitor cells able to restore the periodontium7. We used the dental ligament of an extracted impacted wisdom tooth to restore the periodontal defect of another molar of the same patient. Intra-osseous pockets are bony defects of the periodontal complex, occurring as a result of infection of bacteria and exiting in a bone resorption. After an adequate therapy it is possible to obtain the resolution of the infection but not the restitution ad integrum of the tissue injured and these defects can represent niches for a new infection or limit the functional activity of the tooth affected.

The aim of this study was to apply dental ligament micro-graft, rich with progenitor cells, to the tissue engineering for periodontal regeneration8-11. This approach has never been used before in this field and their success could open new
strategies to clinical therapies of the chronic periodontal disease that currently affects up to 40% of the adults in western countries.

Case report

We performed a split-mouth study. The patient: MC, female, 32 years old; she had two intra-osseous defects distal to the second lower molars, due to mesio-impact of both vital third molar included. The patient was healthy from systemic diseases and was a non-smoker. The hygiene phase of the periodontal therapy included oral hygiene instructions, supra- and sub-gingival scaling and root-planning under local anaesthesia and limited occlusal adjustment. The selected teeth presenting the intra-osseous defect were vital and had at least a 2 mm zone of buccal keratinised gingiva. The patient had a medical history making her qualified for dento-alveolar surgery (i.e. ASA 1). The patient had to sign an informed consent form prior to surgery. She agreed to carry out all study procedures and follow all instructions.

Surgical intervention

Prior to the surgery facial skin was cleaned with 4% Chlorhexidine (CHX); the oral decontamination was obtained using 60 seconds of mouth rinsing with 0.2 CHX washing (Forhans). The local anaesthetic used was articaine 2% 1:100 000 epinephrine (UBISTEIN, ESPE).

Then the third molar was extracted and cleaned using CHX 0.2 for 60 seconds (Figure 1). The dental ligament attached to dental roots was gently collected using a Gracey curette and dissociated using Rigenera System with Rigeneracons sterile filters (HBW srl, Turin, Italy) in 2 ml of sterile physiologic solution (Figure 2). This tool permits the simultaneous mechanical digestion of the dental pulp and the filtering of the solution through a 50-µm strain. After 60 seconds of agitation the cellular suspension is collected from the system and used to wet a collagen sponge (Gingistat, Gaba, Milan, Italy), that has no radiopacity at all (Figure 3). After elevation of the flap all the inflammatory tissue was removed and the defect walls cleaned with hand and ultrasonic instruments (Figure 4). The root of the tooth underwent the same treatment.

In the test site the enriched collagen sponge is gently placed within the intra-osseous defect and the suture performed using non-absorbable suture (3/0 Silk, Ethicon-Johnson&Johnson, St-Stevens, Belgium) (Figures 5–6). In the control site, we used only the collagen sponge.

Post-surgical medication were prescribed as follows: antibiotic therapy, 875 mg amoxicillin + 125 mg clavulanic acid (Augmentin, Glaxo...
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Smith-Kline, Verona, Italy) twice a day for 6 days; Ibuprofen (Brufen, Abbott srl, Aprilia, Italy) 600 mg twice a day for 5 days; 0.12 CHX (Curasept, Curaden, Sarorno, Italy) rinse twice a day for 15 days. Post-surgery the patient complained of neither swelling nor pain. The post-surgical course was uneventful. The sutures were removed after 7 days. During the first week after the surgery the patient was instructed to avoid traction and chewing on the area and to limit mechanical hygiene on that side of the mouth. House hygiene was obtained only using CHX 0.12 rinses three times a day for 6 weeks. After 4 weeks the patient was advised to restore chewing and mechanical hygiene in that area using brush and interproximal tools.

All patients were subjected to supra-gingival professional tooth cleaning by means of a rubber cup with polishing paste at 1, 3 and 6 months after surgery. Sub-gingival hygiene was never performed.

Clinical examinations

Clinical examination was performed at baseline and at 1 week, 1, 3 and 6 months after surgery. Baseline measurements were performed at the day of surgery. All clinical measurements were undertaken by one experienced periodontist.

All the measures of this study were taken by the same calibrated examiner using a PCP-UNC 15 periodontal probe (Williams periodontal probe, Hu-Friedy Mfg. Inc., Chicago, IL, USA) and approximated to the nearest mm.

Presence or absence of visible dental plaque adjacent to the gingival margin was recorded at the locations of the clinical measurements. Presence or absence of marginal or papillary gingivitis, probing depth (PD), Clinical Attachment Level, gingival recession and bleeding on probing were recorded at the following six sites around each tooth. Height of keratinised gingiva at the three buccal sites was also recorded. Clinical measures were performed at baseline and at 6 months evaluation.

After the first week, clinical controls were scheduled at months 1, 3 and 6. The patient complained of symptoms neither during the first week nor during the following period after the surgery. The gingiva surrounding the defect was neither found oedematous nor ulcerated.

The PD before the surgeries was 12 mm for the test and 11 mm for the control; the surgical CAL was 6 mm for the test and 5 mm for the control; after 6 months the PPD was 3 mm for the test and 7 mm for the control.

Radiographic examination

A Tomography Computerized (TC) dental scan was taken before the surgery—the impacted molars provoked the reabsorption of the alveolar bone process distal to the second lower molars (Figure 7). Standardised periapical radiographs were obtained by a periodontist at 3 and 6 months after periodontal surgery: at 3 months both test and control showed immature bone formation (Figure 7B−C); at 6 months the radiographs (RX) in the test site, the mineralisation was higher than in the control site (Figure 7D−E).

Discussion

This study was performed to analyse the clinical outcomes of an innovative treatment protocol of deep intra-osseous periodontal defects using dental ligament micro-grafts loaded onto collagen sponges as filling biomaterial. This surgical approach was made possible by the Rigenera System.

Carinci showed advantages of the use of stem-derived osteoblasts versus the primary cultured osteoblast. The in vitro and in vivo studies, performed...
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in animal models showed that the stem cell fraction was constituted by cells under the 70 µ diameter; under this cut-off dimension the percentage of cells expressing stem antigens grows dramatically, avoiding a magnetic or flow cytometry sorting. The efficacy of this approach of cell selection has been recently demonstrated in clinical protocol to regenerate human mandibular defects. In this experimental study on 17 patients, the enzymatic digestion of the pulpal tissue was substituted by mechanical digestion, guaranteeing in this way a close flow of the manipulation passages leading to stem cells isolation. The cell suspension was then used after loading on collagen sponges and the results after 6 months of follow-up was very encouraging when compared to the control of a non-treated bone defect resulting after impacted third molars extraction.

Although it remains well established that currently available regenerative technologies, such as membranes and EMD, may lead to a true regeneration of the periodontal tissues lost due to periodontal diseases, we designed our protocol for periodontal regeneration using the dental pulp stem cells, cells that have shown significant bone regeneration activity in clinical protocol. Although the bone engineering is the main application field for these cells, their antigenic profile makes them suitable for tissue engineering. Their behaviour in the presence of scaffold has been investigated and they showed better performance when compared with osteogenic primary culture.

In this study we investigated the possibility of the dental ligament to regenerate the entire periodontal complex, constituted by ligament, cementum and bone. All the periodontal tissues come from progenitor cells that, in origin, come from the neural crest-derived mesenchymal cells. These cells originate, during the embryonic period, in the neural crests and then migrate into the cranial region of the embryo, originating from the dental buds and surrounding tissue. After development, some of these cells remain embedded within the tissues, although in a very low percentage. A niche where it is possible to isolate these stem cells, after the birth, is the dental pulp. As demonstrated in several papers this anatomical site is an affordable collection place characterised by low morbidity for the region and the patients itself. The mechanical digestion of the dental ligament brings to a significant cell amount able to regenerate bone chips. The same protocol in this study has been used to obtain a cellular suspension to mix with a collagen III scaffold, chosen for its mechanical and biological properties. At first we needed a scaffold able to sustain cells during their differentiation and to ensure mechanical stability to the coagulum that occurs in the regenerating site—actually the stability is a key factor for cell differentiation, crucial when the committed tissue is a mineralised tissue like the root cementum or bone. On the biological side, we chose collagen III sponge because collagen III is the main component of the organic structure of an undifferentiated mesenchymal tissue, like the surrounding tissues of the dental buds during embryological period into the jaws. Wetting the collagen III sponge with the micro-graft suspension after tissue dissociation, we reconstitute an activated undifferentiated tissue, able to produce by itself BMP-2 and VEGF for vascular net claiming and cell bridging. Then this cell suspension was grafted in the osseous defect with a mini-invasive surgical technique to avoid the depletion of the residual regenerating activity of the surrounding tissues that can occur after an extensive surgical trauma. For the first 3 months we avoided performing an x-ray, although the emission dose is very low, in order to exclude any x-rays damaging the proliferating cells. The x-rays performed at 6 and 12 months confirmed the increase of the mineralisation rate, probably due to the remodelling of the woven bone into lamellar bone. Our technique provides a mini-invasive procedure for stem cell application in periodontal surgery acting as a ‘biotechnological graft’ for these types of defects. Taken together these data are very encouraging, although a much bigger number of cases must be treated and analysed to confirm the efficacy of this technique.

Conclusion

From the x-ray analysis, the more interesting data are the complete filling in the coronal component of the defect—this pattern of regeneration is more similar to those obtained with membranes than with molecular factors.

The limit of this approach is that it can be used only in selected patients with a hopeless but vital tooth that can act as a donor site of dental ligament. Nevertheless, this situation is often present in patients with chronic periodontitis, which need the extraction of the wisdom tooth or other hopeless teeth during osseous resective surgery. This therapy has never been used before for this purpose and it seems a very promising tool for the treatment of these defects.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

References

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