Case Report

Case reports of medication-related osteonecrosis of the jaw (MRONJ) treated with uncultured stromal vascular fraction and L-PRF

C. Bouland a,b,c,* , N. Meuleman b,d , J. Widelec e , K. Keiani-Mothlagh f , C. Voisin f,g , L. Lagneaux b , P. Philippart a,e

a Medicine faculty, université Libre de Bruxelles (ULB), 808, route de Lennik, 1070 Brussels, Belgium
b Laboratory of clinical cell therapy (LCCT), campus Erasme, bâtiment de transfusion (Level +1), Jules-Bordet institute, université Libre de Bruxelles (ULB), 808, Route de Lennik, 1070 Brussels, Belgium
c Stomatological and maxillofacial surgery department, CHU de Saint-Pierre, 322, rue Haute, 1000 Brussels, Belgium
d Hematology department, université Libre de Bruxelles (ULB), 121, boulevard de Waterloo, 1000 Brussels, Belgium
e Radiology department, hôpitaux IRIS SUD, site Molère, 142, Rue Marconi, 1190 Brussels, Belgium
f Stomatological and maxillofacial surgery department, hôpitaux IRIS SUD, site Brucops, 79, Rue Docteur-Huet, 1070 Brussels, Belgium
g Private practice maxillofacial close du parnasse Local 3F, 1050 Brussels, Belgium

ARTICLE INFO

Historique de l'article :
Received 26 January 2020
Accepted 25 May 2020

Keywords:
Medication-related osteonecrosis of the jaw
Stromal vascular fraction
L-platelet-rich fibrin
Bone regeneration
Tissue engineering

ABSTRACT

Medication-related osteonecrosis of the jaw (MRONJ) is a challenging affection, considering the absence of a “Gold Standard” treatment. Cell therapy and tissue engineering, using adipose-tissue stromal vascular fraction (SVF) containing mesenchymal stromal cells (MSC) and endothelial progenitor cells (EPC); and a scaffold with healing properties, L-platelet-rich fibrin (L-PRF), could be a therapeutic option. Two cases of MRONJ were treated by tissue engineering. The patients presented respectively a stage-II and a stage-III MRONJ. The protocol consists of SVF injection in the L-PRF applied on the cleaned bone. Patients are followed clinically and by medical imaging (MI) for 18 months. The buccal mucosa was closed within a month. No recurrence was observed during the follow-up. The MI highlighted bone formation. MSC and EPC presence, in the SVF, were confirmed by immunophenotyping. We report the preliminary results of MRONJ patients successfully treated with the association of autologous fresh L-PRF-SVF.

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1. Introduction

Medication-related osteonecrosis of the jaw (MRONJ) is a condition affecting the patient’s quality of life, described, first, by Marx in 2003 [1]. The incidence ranges from 0.028% to 18.6%, depending on the treatment indication, the study population and the sample size [2]. The American Association of Oral and Maxillofacial Surgery (AAOMS) defines MRONJ by the association of the three following criteria [1]: a current or previous treatment with antiresorptive or antiangiogenic agents; an exposed bone or a probed bone through an intraoral or extra-oral fistula in the maxillofacial region for at least eight weeks; no history of radiotherapy or evident metastatic disease of the jaws. Nowadays, there is no “Gold Standard” treatment [2]. Different recommendations have been made depending on the MRONJ stage [1]. Among those, the surgical procedure seems to offer better results for all the stages taken together [3]. Its goal is to reduce the procedure aggressiveness and to improve the long-term control of the disease [3].

Cell therapy and tissue engineering could be a therapeutic option [4,5]. Cell therapy, in the form of autologous platelet concentrates (APC), has been suggested to treat MRONJ. The leukocyte-platelet-rich fibrin (L-PRF), a second-generation APC, is an autologous three-dimensional fibrin scaffold obtained by whole blood centrifugation without the addition of any component. It
relates growth factors, for at least seven days. The growth factors released play an essential role in cell proliferation and bone cells differentiation [6]. The platelet-derived growth factor, epidermal growth factor, transforming growth factor-beta, vascular endothelial growth factor will stimulate the regenerative and healing potential of the soft and hard tissues locally. Different in vitro and animal model studies also highlighted an antibacterial effect through the antimicrobial protein release [5].

The healing criteria of MRONJ are mucosal coverage, symptoms/pain, signs of infection/inflammation and radiographic signs observed in the bone [7]. According to Caplan hypothesis, bone formation requires not only a scaffold and growth factors such as L-PRF but also a cell source [8]. The stromal vascular fraction (SVF) is a heterogeneous cell population, containing mesenchymal stromal cells (MSC) and endothelial progenitor cells (EPC), isolated by adipose tissue (AT) enzymatic digestion and centrifugation [9]. Both populations present attractive properties for bone regeneration. MSC display proliferation, differentiation, immunomodulatory and trophic properties [10] and EPC enhance angiogenesis and indirectly new bone formation [11]. Together MSC and EPC present synergistic interactions fostering bone regeneration [11]. In 2018, Kuroshima et al. presented, in an animal model, the first cases of osteonecrosis of the jaw (ONJ) -like lesion successfully treated with uncultured SVF [9].

This article presents the two first cases of MRONJ successfully treated with the combination of uncultured SVF and L-PRF.

2. Materials and methods

This study followed the Declaration of Helsinki on medical protocol and ethics. It was approved by the ethics committee of Hôpital Erasme – Cliniques universitaires de Bruxelles (No. P2015/547/B406201526869). After written informed consent, MRONJ patients benefited from the protocol. Before the surgical procedure, the patient had to undergo several medical examinations: an orthopantomogram (OPG), tridimensional imaging and a preoperative anaesthetic consultation. All the cell analyses were performed at the Laboratory of clinical cell therapy (LCT-T-ULB721) of the Jules-Bordet Institute (JBI) – Université libre de Bruxelles (ULB).

2.1. Surgical procedure and follow-up

The procedure consists of a one-step surgery under general anaesthesia. No local anaesthesia was performed during at the operating site. The local anaesthetic would limit the AT-MSC proliferation [12] of the injected SVF locally. It includes an AT harvest and its enzymatic treatment to obtain SVF, a blood sample to prepare L-PRF, and surgical debridement of the infected area, depending on the preoperative imaging and the vitality of the bone determined by bleeding. The procedure ends with the application of the L-PRF scaffold containing the uncultured SVF and the site closure with a mucoperiosteal flap. The granulation tissue and the necrotic bone were collected for anatomopathological analysis. After the operation, the patient received painkillers and amoxicillin 1 g 3x/day for a week. During the follow-up, the patient was seen weekly the first month and subsequently monthly for 17 months. Three jaw CBCT were planned at 6, 12 and 18 months after the operation in order to evaluate a potential bone formation.

2.2. AT sample and SVF preparation

A sample of 30 mL of AT is collected from the inner face of the knee by liposorption, using a standard multi-hole infusion 3 mm cannula. Fat is harvested with a gentle mechanic aspiration. No vasoconstrictors have been used, to avoid the cell cytotoxicity from the local anaesthetic. Later on, digested by Liberase® MTF C/T, GMP grade kit (Roche Diagnostics GmbH, Mannheim Germany, lot 11674320) during 30 minutes at room temperature and centrifuged at 800 g for 5 minutes. The SVF pellet is harvested and washed with saline solution. A fraction of SVF is characterised at the LCCT. The rest is reserved for the patient’s treatment.

2.3. L-PRF preparation

A blood sample is taken by a peripheral puncture, in 9-mL glass collection tubes, without the addition of any anticoagulants. The blood was immediately centrifuged (Intra-Spin EBA 200, Intra-Lock System, FL, The United States of America) for 12 minutes at 400 g (±2700 rpm) in order to obtain the L-PRF [6].

2.4. Preparation of the L-PRF scaffold containing the SVF

The SVF is injected inside the L-PRF, taking advantage of its three-dimensional properties.

2.5. SVF Characterization

The analysis aims to determine the distribution of SVF cell subpopulations analysed by flow cytometry for cellular expression of CD31, CD34 and CD45. This allowed to determine the respective percentages of hematopoietic (CD45+), stromal (CD31+/CD34+/CD45−) and endothelial cells (CD31+/CD34+/CD45−). The cell phenotyping is immediately evaluated after the SVF’s harvest. The cells are incubated with the appropriate fluorescent monoclonal antibodies for 30 minutes at room temperature in the dark. Data acquisition is performed on a MACSQuant analyzer (Miltenyi Biotec MACS) and analysis with FCS Express 4 software (DeNovo Software). The number of mesenchymal progenitors (MP) in the SVF sample is evaluated by CFU-F assay.

3. Results

3.1. Case report 1

A 77-year-old woman developed a Stage-III MRONJ after one year of monthly intravenous Zoledronic Acid (4 mg) intake. The patient discontinued the antiresorptive agent after MRONJ onset. The patient presented a non-secreting multiple myeloma (MM) Stage IIIa (Durie and Salmon) and stage 1 (International Staging System (ISS)), in remission after eight cycles of polychemotherapy (Melphalan-Prednisone-Bortezomib (MPV)) between February and November 2014. The patient’s medical history included a Parkinson disease, an operated breast cancer, a hysterectomy, and a knee prosthesis. MRONJ was affecting the patients’ life with chronic pain, trouble eating and social embarrassment. Conservative treatments (antibiotics and mouthwash) had been tried without success, for a year. The patient had persistent pain, and the clinical examination showed a suppurating area, in the right maxilla, with a mobile bony sequestrum removed at the consultation department. Steroid intake during chemotherapy was the only identified risk factor. The patient was not smoking, nor drinking alcohol, and had no history of tooth extraction. The OPG and the facial computerised tomography scan (CT-scan) showed the bony sequestrum (8 × 8 mm) (Fig. 1). An oro-antral communication was observed through the CT-scan. The results of the histological analysis showed a bony necrotic sequestrum with granulation tissue and actinomy- cotic colonies. The SVF was obtained from AT harvesting and its analysis, by flow cytometry, confirmed the presence of a heterogeneous cell population containing endothelial cells, EPC, MSC, hematopoietic cell lineage,… (Fig. 2). In total, we have harvested
and injected $4.81 \times 10^6$ viable cells in the L-PRF scaffold, which contained 5550 MP. A palatal splint was designed for maintenance and protection. After the surgery, the patient felt discomfort at the operated site for one week. Two weeks after the procedure, the buccal mucosa was closed. The three CBCT, performed respectively at 6, 12 and 18 months after the surgical procedure highlighted a centripetal ossification through a dynamic and progressive closure of the oro-antral communication. Eighteen months after the operation, a bony bridge was observed (Fig. 3). No signs of clinical recurrence were observed since.

### 3.2. Case report 2

A 76-year-old woman, suffering from osteoporosis, developed a Stage-II MRONJ after ten years of intravenous zoledronic acid (5 mg) intake. The patient discontinued the BP after MRONJ onset. The patient did not present any comorbidities. MRONJ was affecting the patient’s life with chronic pain, trouble eating and social embarrassment. Conservative treatments (antibiotics and mouthwash) had been tried without success, for a year. The patient had persistent pain, and the clinical examination showed an exposed bone with a suppurating area, in the left part of the

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**Fig. 1.** Patient 1: preoperative cervicofacial CT scan. The preoperative facial CT scan highlights the alveolar ridge sequestrum, the oro-antral communication and the radioopaque right maxillary sinus.

**Fig. 2.** Patient 1: phenotypic characterisation of SVF subpopulations. A. FACS profiles from flow cytometric analysis of the SVF with fluorochrome-conjugated antibodies to human CD31, CD34 and CD45. B. Levels of hematopoietic cells (CD45 + ), stromal cells (CD31−/CD34+/CD45−) and endothelial cells (CD31+/CD34+/CD45−) in SVF. SVF: stromal vascular fraction.

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Please cite this article in press as: Bouland C, et al. Case reports of medication-related osteonecrosis of the jaw (MRONJ) treated with uncultured stromal vascular fraction and L-PRF. J Stomatol Oral Maxillofac Surg (2020), https://doi.org/10.1016/j.jormas.2020.05.024
mandible. The patient was smoking but not drinking. MRONJ started just after one tooth removal. An OPG and a CBCT showed an empty alveolar socket and an area of osteocondensation (Fig. 4). The results of the histological analysis showed necrotic bone with granulation tissue and actinomyces colonies. The SVF analysis, by flow cytometry, confirmed that it is a heterogeneous cell population containing endothelial cells, EPC, MSC, hematopoietic cell lineage (Fig. 5). In total, we have injected $20.8 \times 10^6$ viable cells in the L-PRF scaffold, which contained 1349 MP. After the surgery, the patient felt discomfort at the operated site for one week. Four weeks after the procedure, the buccal mucosa was closed. The three consecutive CBCT showed increasing signs of bone formation with osteocondensation from the basis of the alveolar socket (Fig. 6). No signs of clinical recurrence were observed since.

4. Discussion

We present here the preliminary results of MRONJ treated by cell therapy with the association L-PRF-SVF. In both cases, the oral mucosa healed within a month. No signs of recurrence were observed during the 18-months follow-up. Bone formation was observed through medical imaging (MI). MRONJ is currently controlled. The patients' life improved drastically. The literature review did not identify any published studies using the association L-PRF-SVF to treat MRONJ. Each component presented interesting properties for cell therapy. Indeed, the L-PRF enhance soft and hard tissue healing [6]. However, in the treatment of MRONJ, the literature does not reach a consensus. According to Fortunato et al. systematic review [4], results are not sufficient to establish effectiveness of APC in the prevention and the treatment of MRONJ while the case-series presented by Mourao et al. [5] indicated the opposite. Both recommended to perform large randomized controlled trial [4,5]. Furthermore, the SVF presents osteogenic and angiogenic properties [9]. Nevertheless, only one study used SVF to improve ONJ-like lesions in the literature [9]. The authors noticed an enhancement of both osseous and soft tissue healing of tooth extraction socket and blood vessels, after systemic transplantation of uncultured SVF. Each element separately presents properties that, if put together, could lead to innovative treatment. Different biases remain under discussions. They include the contribution of the surgical management itself and the anti-biotherapy procedure. Fortunato et al. carried out a systematic review of the APC used in the treatment of MRONJ [4]. They did not observe any significative differences ($P = 0.0788$) between the surgical management with or without APC in the MRONJ. Notwithstanding, different potential biases have also been highlighted in this systematic review: the contribution of other therapies in the MRONJ care or the APC and its obtention protocol could have played a confounding effect. The different APC do not present the same properties. The PRP, first-generation APC releases mostly their growth factors the first hour. The PRF, a second-generation APC, displayed a continual and steady release of growth factors for at least a week [13]. In the current study, the two patients benefitted from the L-PRF protocol. The L-PRF is an autologous fibrin matrix releasing VEGF, EGF, BMP2, TGFβ-1, PDGF-AB for at least seven days, improving healing and promoting tissue regeneration [6], this could explain the good results obtained in our 2 cases.

Conservative treatment: antibiotics and mouth-rinse, are not sufficient to control MRONJ. Ninety-two MRONJ patients were treated with conservative measures and followed for 21.5 months (±SD 17.9 months) and only eight patients (8.7%) achieved complete mucosal coverage. Moreover, 42 benefited from surgery during the follow-up [14]. The two patients benefitted from long-term anti-biotherapy before the surgical procedure without success. Antibiotics are dispensed to control infection and for perioperative administration in MRONJ care. Akashi et al. highlighted in a systematic review that surgery combined with antibiotics presented good results in term of healing. If the antibiotic had been administered for less than 14 days postoperatively, the median healing rate was 85.9% and 94.4%
Fig. 5. Patient 2: phenotypic characterisation of SVF subpopulations. A. FACS profiles from flow cytometric analysis of the SVF with fluorochrome-conjugated antibodies to human CD31, CD34 and CD45. B. Levels of hematopoietic cells (CD45+), stromal cells (CD31−/CD34+/CD45−) and endothelial cells (CD31+/CD34−/CD45−) in SVF. SVF: stromal vascular fraction.

Fig. 6. Patient 2: postoperative jaw CBCT. The four consecutive CBCT showed increasing signs of bone regeneration remineralization, respectively 6 A; 12 B; and 18 C months after the surgical procedure. CBCT: cone beam computed tomography.

if the antibiotics were administered for a longer period [15]. Nevertheless, we highlighted complete soft tissue healing with symptoms resolution within the month with this protocol. No signs of MRONJ recurrence occurred after 18 months.

We observed, like Cella et al. assessed a concentric ossification after 15 months of follow-up [16], a progressive bone regeneration through MI. In front of MRONJ, we cannot perform any bone biopsy. It could act as a trigger for disease recurrence. We had to follow bone formation through medical imaging. The CT imaging presents the advantage of morphological evaluation and delineates the disease extension [17]. Indeed, the CT evaluation highlights lytic and/or sclerotic lesion, periosteal reaction, cortical perforation, periosteal reaction, mandibular fractures, and soft tissue inflammation. But CBCT can also assess bone formation or bone regeneration [18]. We have assessed for the first case a concentric ossification closing the oro-antral communication and the sign of bone regeneration in the alveolar socket for the second case. Moreover, we have not used any specific tools to further assess or to quantify this potential bone regeneration, Pardinas Lopez et al. have suggested one. They highlighted a 12 to 30% bone regeneration, through the CBCT, at the MRONJ operated site after surgical treatment combined with plasma rich in growth factors (PRGF) [19].

The SVF osteogenic potential was already observed in 2013, in an animal model [20]. Jurgens et al. highlighted that cultured adipose stem cell (ASC) and uncultured SVF possess similar bone regeneration properties. Nevertheless, after one month, bone regeneration was better with cultured ASC than SVF, but after four months, SVF showed more bone formation. One of the hypotheses proposed therein suggested a synergy between the different cell populations. Liang et al. have reported synergistic interactions of MSC and EPC to favour bone regeneration [11]. They pointed out
that bone neo-formation is significantly higher in MSC-EPC co-culture compared to MSC mono-culture in an animal study and in vitro [21]. EPC would not have a role in osteogenic differentiation, but rather on osteoblastogenesis through angiogenesis. In their study, neovascularization and bone regeneration were significantly increased. Moreover, a more mature bone was detected in MSC-EPC co-culture. In another study, Wen et al., have demonstrated the properties of EPC in the bone regeneration in an MSC-EPC co-culture in an animal study and in vitro. Both MSC and EPC had been harvested from bone marrow (BM). Thus, by indirect cell-cell interaction, EPC plays a dynamic role in maintaining MSC stemness and pluripotency capacities [22].

Beforehand, Cella et al. treated a Stage-III bisphosphate-related osteonecrosis of the jaw (BRONJ) refractory to treatment in 2011 [16]. A two-step treatment with cured autologous BM heterogeneous solution containing MSC, EPC... led to bone reconstruction. The patient had severe osteoporosis, treated with IV and Per Os (PO) BP for almost three years. Unfortunately, this protocol does not apply to every clinical situation. The BM, potential cell source for cell therapy, is invaded by medullary clonal plasma cells in MM. Hence, direct and indirect interactions between malignant cells and BM microenvironment contribute to abnormalities in BM-MSC, such as IL-6 and DKK1 overexpression and early senescence [23]. Interestingly, Lin et al. showed that in the case of MM, AT-MSC present no abnormality and can be used as an alternative source [24].

Osteonecrosis is considered as an interruption of the vascular supply [1]; it presents no regeneration potential. The literature suggests that in L-PRF-SVF association, only the SVF contains cells with proliferation and bone differentiation properties [22]. MI is rarely displayed in the articles. MI could help to determine if L-PRF is stimulating osteoblastogenesis or is a simple dressing. No significant differences have been demonstrated by using PRF or not on the osteoblastic activity after third molar surgery in the systematic review and meta-analysis published by Xiang et al. [25]. Farré-Guasch et al. have observed, the strong SVF angiogenic properties in a clinical setting, with bone and blood vessel formation [26]. Vascularization is a crucial aspect of bone regeneration and tissue engineering [11,26]. L-PRF releases growth factors and therefore is also involved in the neovascularization. Moreover, the fibrin matrix appears to be a relevant scaffold to support osteoblastic growth and differentiation [6]. The association could counteract the deleterious osteonecrosis effects on the local vasculature. Even if we cannot yet understand the specific role of each component, the preliminary results described, in the cases using the L-PRF-SVF association could be a step towards MRONJ treatment.

Funding
This research did not receive any specific grant from funding agencies from public, commercial, or not-for-profit sectors. Disclosure: the preliminary results of this two cases were presented in different congresses: 2016 Enhanced in natural healing (ENHD) (oral presentation); 2016 Société royale belge de stomatologie et de chirurgie orale et maxillo-faciale-SRBSOM (oral presentation); 2017 Société belge d’hémato-logie-BHS (poster); 2018 Société française de Printemps-SFSCMFCO (oral presentation). The final results of these two cases were presented in the annual meeting of IFATS in Marseille in 2019 (oral presentation).

Disclosure of interest
The authors declare that they have no competing interest.

Acknowledgements
Authors’ roles: study design: BC, MN, LL, PP; study conduct: BC, VC, KK, LL; data collection: BC, WJ, VC; data analysis: BC; WJ; data interpretation: BC, LL; drafting manuscript: BC, LL; revising manuscript content: BC, WJ, KK, VC, LL, PP; approval manuscript content: BC, MN, LL, PP. PP takes responsibility for the integrity of the data analysis.

Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jormas.2020.05.024.

References


