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Research article

Application of a collagen scaffold saturated with platelet-rich plasma in prevention of bisphosphonate-related osteonecrosis of the jaw in the rat animal model

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ABSTRACT

Background: Among the myriad adverse events of drugs in the oral cavity, Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is one of the most detrimental drug reactions that have ever been known.
Objective: This study was aimed to investigate the success of applying collagen scaffold alone and platelet-rich plasma (PRP)+collagen scaffold in prevention of zoledronic acid-induced BRONJ in the rat.
Methods: A total of 17 male Wistar-rats were treated with 4 weekly doses of zoledronic acid. All rats were undergone bilateral tooth extraction of mandibular first molars and divided into three groups of scaffold + PRP + suture, scaffold + suture, and suture only. All rats were scarified and clinical, radiological, histological and histomorphomerical evaluations were made on week 8 post-treatment. The soft tissue healing, bone mineralized density (BMD), number of osteoclasts and osteoblasts, necrotic bone (NB), intensity of inflammation and new bone formation (NBF) were analyzed.
Results: BMD, number of osteoblasts and NBF variables proved to be statistically were higher in the treatment groups than the control group. In addition, the PRP + scaffold group showed the better results in terms of BMD, number of osteoblasts and NBF than that of the scaffold alone group. Number of osteoclasts, inflammation intensity and osteonecrosis were also significantly different in the PRP + scaffold group compared to the scaffold alone and the control groups.
Conclusion: Application of a PRP-enriched collagen scaffold appeared to be a successful preventive treatment for BRONJ by effecting of the number of osteoblasts and osteoclasts, BMD, NBF, inflammation, and osteonecrosis.

1. Introduction

Among the myriad adverse events of drugs in the oral cavity, BRONJ is one of the most detrimental drug reactions that have ever been known. Bisphosphonates, as pyrophosphate analogues, avidly bind to calcium crystals of the bone, impede osteoclastic bone resorption by their toxic effect on osteoclasts and interference with some intracellular pathways of these cells (Soares et al., 2016). Therefore, BPs are suitable medications for decreasing osteoclast-mediated bone resorption in medical conditions such as osteoporosis, Paget's disease, multiple myeloma, osteogenesis

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imperfecta, hypercalcemia inhibition and modulation of bone metastasis progression in osteoprotic malignant tumors (Drake et al., 2008). BRONJ is characterized as an exposed necrotic bone area in the maxillofacial region persisting for at least 8 weeks, in the patients with previous or current treatment of BPs and without any history of radiotherapy of the head and neck region (Kuhl et al., 2012). Type and route of administrated BP, cumulative dose of BP, duration of treatment, concomitant therapies and diseases, patient habits, gender and age are systemic risk factors of BRONJ (Ruggiero, 2009).

The etiopathogenesis of BRONJ is still a matter of conjecture. BRONJ is most probably a multifactorial phenomenon. As a consequence, inhibition of keratinocytes proliferation by BPs, poor vascularity, and persistent bacterial infection lead to an ominous cycle of impaired soft tissue healing, osteonecrosis and suppression of bone turnover (Gavaldá and Bagán, 2016).

Antibiotic prophylaxis pre and post-oral surgery has been proposed as an effective tool for prevention of BRONJ and promotes healing of the extraction socket (Ikebe, 2013). Some researchers encourage discontinuation of BPs at least 2 months prior to the oral surgery according to the pharmacokinetics of BPs (Bermúdez-Bejarano et al., 2017; Ruggiero et al., 2014), whereas some other didn't approve the cessation of BPs as a successful tool for prevention of BRONJ (Damm and Jones, 2013). Further complementary methods are being evolved in conservative prophylactic and therapeutic fields of BRONJ such as ozone therapy, teriparatide, hyperbaric oxygen, low-level laser therapy (LLLT), bone morphogenic protein, mesanchymal stem cells (MSCs) and PRP (Mücke et al., 2016; Lopez-Jornet et al., 2016). Among the wide variety of newly discovered therapies, PRP has attracted attentions for their possibly successful application in both prevention and treatment of BRONJ. Although few observational and experimental studies have been implemented to scrutinize their efficacy in prevention or treatment of BRONJ, favorable results are provided probably due to healing stimulatory growth factors (GFs) and cytokines secretion such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-B), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), platelet-derived angiogenesis factor (PDAF) insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF), IL-1, IL-6, tumor necrosis factor-α (TNF-α), serotonin, histamine, dopamine, calcium and adenosine (Fliefel et al., 2015). By the virtue of abundant growth factors and cytokines, PRP is assumed to have a crucial role for increasing the forseeability of hard and soft tissue regeneration (Zhang et al., 2013). Being a source of GFs and possessing consequent mitogenic, angiogenic and chemotactic properties, PRP is a fascinating treatment of choice for recalcitrant wounds. In fact, PRP leads four main activities in the local environment of application such as proliferation, differentiation, migration and angiogenesis (Arora et al., 2009). As well as soft tissue regeneration, PRP also takes place in bone regeneration and together with production of pro-inflammatory agents and collagen and by controlling local inflammatory responses, this agent emerges as a suitable alternative for prevention or treatment of BRONJ (Alves and Grimalt, 2018; Oryan et al., 2014). When administered for purposes of bone regeneration, PRP in the form of gel doesn't seem to be a dependable tool solely, for it may fail to accomplish cell attachment and act as a suitable scaffold during the healing process. Inspite of a sound scientific foundation in bone regeneration, PRP appears only beneficial when combined with osteoconductive scaffolds (Malhotra et al., 2013). In addition, bone tissue engineering relies on engaging interplay between the contexts of tissue engineering triad meaning progenitor cells, regulatory signals and scaffolds (Murphy et al., 2013). Conclusively, engineering an apt method of delivery of PRP, may ultimately bring about superior success in its clinical use (Rodriguez et al., 2014). To the best of our knowledge, there is no study regarding the efficacy of a PRP-enriched scaffold model for the prevention/treatment of BRONJ to date. Moreover, lack of established preventive interventions after tooth extraction despite the significance of prevention as the key to BRONJ management, necessitated designing a new prophylactic approach for the researchers. As to appraise the success of applying PRP gel to a collagen scaffold in prevention of BRONJ, we aimed to develop a zoledronic acid-induced BRONJ model in rats and implemented the preventive intervention after tooth extraction. To evaluate the success of this approach, inflammation, necrotic bone, new bone formation, number of osteoclasts and osteoblasts, bone mineralized density and soft tissue healing between the three groups were compared.

2. Materials and methods

This study was reviewed and approved by the Tehran University of Medical Sciences Ethics Committee (Ethic No: IR.TUMS.DENTISTRY.REC.1397.020).

2.1. Study design

BRONJ was induced as previously described by Zandi et al. (2016) as it was a reliable design with a success rate of 83% that can develop BRONJ in a relatively short period of time with less suffering of the animals. More importantly, same doses as humans were administered to the rats in this protocol.

Seventeen male albino Wistar rats with the mean age of 10 weeks and average weight of 300 g, with mandibular first molars were obtained from the Laboratory Animal Research Center of the University. All the rats were undergone 4 doses of 0.06 mg/kg of zoledronic acid (Bonsta, Exir Iran, Boroujerd, Iran) intra-peritoneally every week for 4 succeeding weeks. One week after the last dose administration, all the rats were undergone bilateral first mandibular molar extraction under intraperitoneal general anesthesia using 75 mg/kg of 10% ketamine hydrochloride (Ketamine, Alfasan, Utrecht, Netherlands) and 8 mg/kg of 2% xylazine (Xylasine, Alfasan, Utrecht, Netherlands). After placing the rats in a supine position and soft tissue detachment of right and left mandibular first molars, 33 teeth were extracted (n = 33). Extraction sockets were randomly divided into three groups. Eleven sockets received a collagen-scaffold (Collacone®, Botiss, Berlin, Germany) saturated with PRP (PRP + scaffold group), 11 sockets received scaffold solely (scaffold group) and 11 remained sockets were sutured (control group). All the sockets were sutured continuously with an absorbable 5-0 silk (Supa Chromic, Supa Medical Devices, Tehran, Iran) to obtain complete mucosal coverage.

2.2. PRP preparation

After general anesthesia and before the initiation of the surgical procedure of each rat, 3.15 ml of blood samples were taken from the tail vein of rats in PRP + scaffold group by using a 5ml disposable syringe containing 0.35 ml of 10% sodium citrate as an anticoagulant agent. The blood samples were kept in 5ml silicone vacuum tubes (Serum, FarTest, Isfahan, Iran). The blood samples were firstly centrifuged (Universal 320R, Hettich Co, Tuttlingen, Germany) at 160 G for 20 min in the room temperature (22 °C). Then, a point was marked at 1.4 mm below the line dividing the two fractions. All the content above this point was pipetted and transferred to other 5 ml vacuum tube. The samples were then centrifuged at 400G for 15 min resulting in a two-layered sample. The lower layer or PRP was pipetted and transferred to sterile dappen dishes and activated by 0.05 ml of 10% calcium chloride solution to each 1 ml of PRP. Afterwards, PRP was applied to extraction sockets of PRP + scaffold group within 10 min. All the rats were scarified by overdose of anesthetic drugs on eight weeks post-tooth extraction. All the rats were undergone mandibulectomy, dissected to hemimandibles, and harvested for further histologic and radiologic studies. Samples were then kept in 10% formaldehyde in plastic receptacles for 4 weeks prior to the imaging procedure.

2.3. Macroscopic assessment

Soft tissue healing was assessed after the mandibulectomy procedure of the rats and scored from 1 to 3:

1: no soft tissue coverage of the extraction socket
2: partial coverage of the extraction socket
3: complete coverage of the extraction socket

2.4. Radiologic assessment of micro-computed tomography

The Micro-CT imaging of the samples were implemented by Micro-CT unit (LOTUS inVivo, BehinNegareh Co,Tehran, Iran), operating at 50 kV, 0.2 mA with a pixel size of 7.8 μm. Image reconstruction was performed with SaniVis reconstructor software. The mean bone mineralized density values of each slice were measured by ImageJ software (Wayne Rasband, National Institute of Health, USA) after being converted into 8-bit data (256 grayscale levels).

2.5. Histological assessment

After imaging analysis, the half-jaws were decalcified by 10% aqueous solution of ethylenediaminetetracetic acid (EDTA). A total of 5μm-thickness histological sections were obtained from tissue-embedded paraffin blocks and stained with hematoxylin-eosin (HE). Histological analysis was performed by two pathologists blinded to the identity of specimens with a light microscope (Leica Microsystems, Wetzlar, Germany) and the averages of the counts were obtained. Three histological HE sections of each animal were analyzed. All the three groups of the study were compared based on 4 histological parameters: number of osteoclasts, number of osteoblasts, inflammation intensity and necrotic bone.

1. Number of osteoclasts: arithmic mean of osteoclasts count in five different fields (×40), in three random cuts of each specimen.
2. Number of osteoblasts: arithmic mean of osteoblasts count in five different fields (×40), in three random cuts of each specimen.
3. Inflammation intensity: inflammation was scored from 0 to 3 based on the intensity of inflammatory cells in the defect area:

    0: no inflammation
    1: mild inflammation
    2: moderate inflammation
    3: severe inflammation

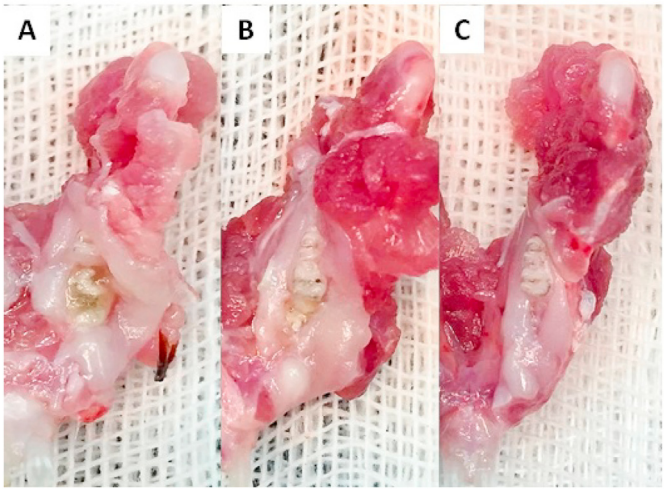

Figure 1. (A) clinical presentation of BRONJ in control group with exposed necrotic bone. (B) partial mucosal coverage of the bone in scaffold group. (C) complete mucosal coverage of the bone in PRP + scaffold group.

4. Necrotic bone: the score of osteonecrosis was determined by scoring from 0 to 3:

    0: osteonecrosis not seen
    1: necrotic bone between 0 to 1 mm^2
    2: necrotic bone between 1.1 to 2.5 mm^2
    3: necrotic bone between 2.6 to 4 mm^2

2.6. Histomorphometric assessment

To perform the histomorphometric analysis, one millimeter grid was placed on the 20 × 25-cm amplifications of the obtained images in Image J software. By counting the filled box from each target, the proportion of osseous neo-formation related areas characterized by the marker was quantified (Chopard, 2004).

The percentage of the new bone formation was evaluated by the following relation (eq. (1)):

$$\%\ new\ \ bone\ \ formation = \frac{total\ \ area\ \ of\ \ marker \times 100}{total\ \ bone\ \ area\ \ of\ \ the\ \ section} \quad (1)$$

2.7. Statistical analysis

Data analysis was performed with SPSS program v. 25.0.0.0 (SPSS inc., Chicago, IL, USA). The normality of data was tested by One-sample Kolmogorov-Smirnov. Respectively, the parametric and non-parametric variables were compared using One-way ANOVA and Kruskal-Wallis tests followed by Post Hoc Tukey and Mann–Whitney tests. The statistical significance level was set at $p < 0.05$ in all tests.

3. Results

3.1. Macroscopic results

Clinical assessment of the specimens revealed no significant difference in relation to soft tissue healing between the groups of study ($p = 0.27$) (Figure 1, Table 1).

3.2. Radiologic results

The average bone mineralized density demonstrated a statistically significance between the three groups of the study (Figure 2, Table 2).

3.3. Histological results

Histopathological investigation showed that the mean number of osteoclasts in PRP + scaffold group (12.41 osteoclasts per ×40 field) were increased statistically significant compared to the scaffold (5.36 osteoclasts per ×40 field) and control groups (3.85 osteoclasts per ×40 field). However, there was no significant difference between the control and scaffold groups regarding the number of osteoclasts ($p = 0.27$) (Figures 3, 4, and 5, Table 2).

The mean number of osteoblasts was significantly different between all groups of study, with 3.50, 5.72 and 14.56 osteoblasts per ×40 field in control, scaffold and scaffold + PRP groups, respectively (Table 2).

The intensity of inflammation was significantly lower in PRP + scaffold group than those of scaffold and control groups ($p = 0.012$ and 0.001, respectively). However, there was no significant difference between the control and scaffold groups regarding the intensity of inflammation ($p = 1.00$) (Table 1).

A significant difference was detected in the PRP + scaffold group compared to scaffold and control group in term of osteonecrosis. However, there was no significant difference between the scaffold and control groups in term of osteonecrosis ($p = 0.76$) (Table 1, Figure 4).

Table 1. Quantitative parameters.

Parameters and respective score	Number of specimens		
	groups		
	n = 11	n = 11	n = 11
	control	scaffold	Scaffold + PRP
Intensity of local inflammatory response			
(0)Absence of inflammation	0	0	3
(1) Mild inflammation	1	2	6
(2) Moderate inflammation	6	7	2
(3) Severe inflammation	4	2	0
Osteonecrosis			
(1) Not seen	0	0	6
(2) 0–1 mm^2	0	3	3
(3) 1.1–2.5mm^2	6	5	2
(4) 2.6–4 mm^2	5	3	0
Soft tissue coverage of the extraction socket			
(1) no mucosal coverage	5	3	2
(2) Partial soft tissue coverage	4	5	4
(3) Complete soft tissue coverage	2	3	5

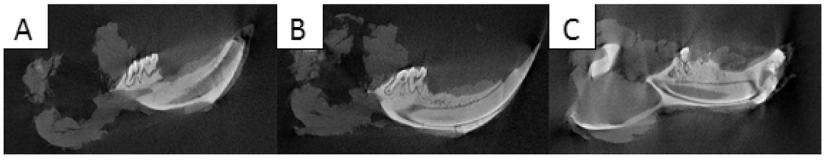

Figure 2. Micro-CT images of the specimens. (A) Undesirable mineralized bone density of the extraction socket in control group. (B) Proportionate bone formation in extraction socket of the scaffold group. (C) High bone mineralized density and almost complete bone repair in PRP + scaffold group.

Table 2. Mean values of quantitative parameters.

Groups	BMD	Number of osteoclasts in ×40 field	Number of osteoblasts in ×40 field	New bone formation
Control	94.17 ± 6.54	3.08 ± 1.52	3.50 ± 0.86	2.35 ± 1.41
Scaffold	109.02 ± 7.63	5.36 ± 2.58	5.72 ± 2.63	19.07 ± 4.42
Scaffold + PRP	135.73 ± 6.50	12.14 ± 2.52	14.56 ± 2.05	74.29 ± 4.68

3.4. Histomorphometric results

The highest average percentage of new bone formation was seen in the PRP + scaffold group with the mean value of 74.29% that was significantly higher than those the scaffold and control groups (19.07 and 2.35, respectively). Bone formation depicted a statistically significant difference between all the groups of the study (Table 2).

4. Discussion

The prescription of BPs has notably increased over recent years, which can be attributable to increasing proportion of aging population who benefit from the antiresoptive characteristics of these drugs for treatment of their common diseases. This leads to increasing number of such patients seeking for dental care procedures that may possibly induce BRONJ (Kim et al., 2017). Moreover, general practitioners as prescribers

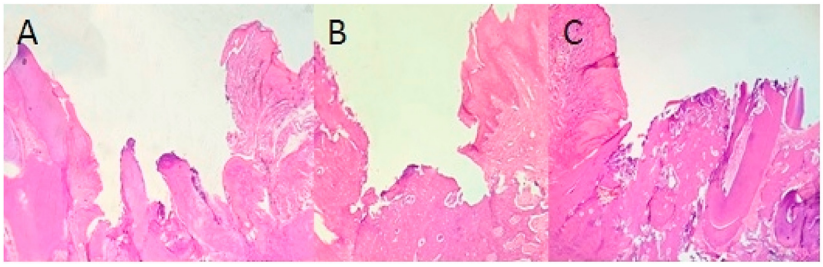

Figure 3. Histological findings of the extraction socket after eight weeks. (A) No considerable bone formation in the extraction socket of the control group is observed. (B) Note the partial, immature bone formation of extraction socket in scaffold group. (C) Mature and vital bone formation of the extraction socket in the PRP + scaffold group, (HE staining, ×4 magnification).

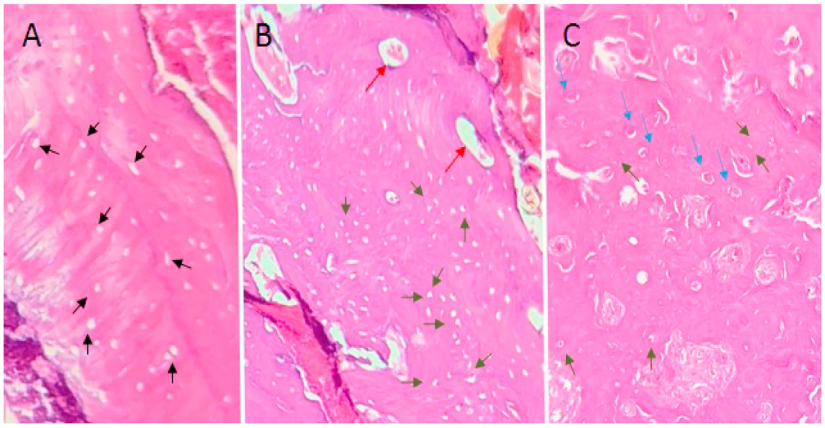

Figure 4. (A) Black arrows indicate empty lacunas and thus osteonecrosis in the control group. (B) Green arrows indicate of osteocytes inside the lacunas representative of vital bone in scaffold group. Note the angiogenesis (red arrows). (C) Blue arrows show Haversian canals and green arrows indicate osteocytes within lacunas. (HE staining, ×10 magnification).

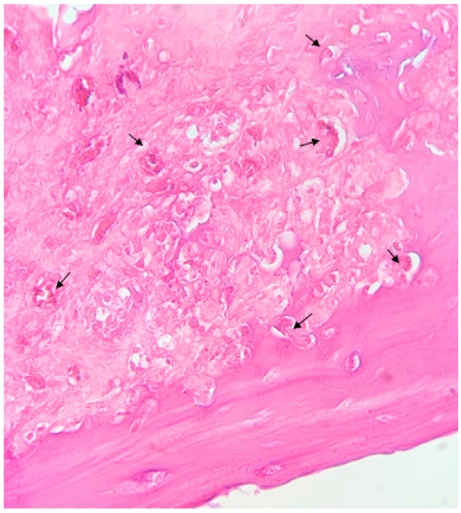

Figure 5. Black arrows are indicative of osteoclasts. Note the invasion of osteoclasts to the necrotic bone area of the PRP + scaffold group.

of bisphosphonates have demonstrated relatively limited knowledge regarding BRONJ and preventive strategies in relation to this condition (Sturrock et al., 2017). Therefore, it's of great importance to achieve a successful protocol for management of BRONJ.

Several studies have focused on the regenerative potentialities and translational applications of PRP (Cardoso et al., 2019; Inchingolo et al., 2017) associated with the choice of the best scaffold seeded with different SCs such as dental-derived SCs (Ballini et al., 2017), mesenchymal SCs (Cantore et al., 2018), and adipose-derived SCs (Barba-Recreo et al., 2015) in the regenerative medicine such as the management of medication-related osteonecrosis of the jaw (Boccaccio et al., 2018; Crincoli et al., 2015). Since the pathogenesis of BRONJ as well as its prevention and treatment has not been established, this study was aimed to investigate the success of applying PRP + collagen scaffold and collagen scaffold alone in prevention of zoledronic acid-induced BRONJ grossly, histologically, and radiographically in the rat animal model. To the best of our knowledge, the efficacy of a PRP-enriched scaffold model for the prevention/treatment of BRONJ has yet investigated.

The most important finding of the present study was that implantation of collagen + PRP reduced the negative consequences of zoledronic acid-induced BRONJ in the rat model. In the present study, histological variables such as osteoblasts and new bone formation demonstrated a significant difference between the three groups of study, which suggests that collagen scaffold could solely give rise to the number of osteoblasts and percentage of bone formation. However, the combination of PRP and collagen scaffold illustrated significant superior results not only in regard to the number of osteoblasts and new bone formation but also concerning the number of osteoclasts, inflammation intensity and osteonecrosis, where the collagen scaffold alone failed to depict a notable result compared to the control group. The highest number of osteoblasts in scaffold and scaffold + PRP groups is probably due to the capacity of each element (scaffold and PRP) in promoting mitogenesis, proliferation, and differentiation of osteoblasts.

It is assumable that collagen scaffold may have provided a bedrock for the gradual release of growth factors (GFs) and therefore leaded to an even higher amount of osteoblasts and bone formation. Osteoclasts are the main target cells of BPs. Nitrogen containing BPs (NBPs) are potent inhibitors of the farnesyl-phosohate synthase enzyme in mevalonate pathway, which decreases the GTPase activity in cytoskeletal arrangement and vesicular trafficking within osteoclasts. Therefore, BPs are capable of inhibiting maturation, function and survival of the osteoclasts (Lombard et al., 2016). In fact, the reduction in number and activity of osteoclasts decreases bone resorption which is a favorable function in treatment of osteolytic diseases but detrimental to bone remodeling (Paulo et al., 2014).

Although collagen scaffold could solely result in higher bone formation, BMD and number of osteoblasts in this study, it failed to have a significant impact on BRONJ-inducing parameters such as osteonecrosis, inflammation and number of osteoclasts. However PRP + scaffold could notably increase the number of osteoclasts and reduce osteonecrosis and inflammation compared with scaffold and control groups. Effects of PRP on osteoclasts are controversial. Mokhtari et al. (2018) revealed that PRP does not cause any significant increase in osteoclastic differentiation.

Cenni et al. (2010) have proposed that thrombin –activated PRP at 10% interferes with the complete differentiation of osteoclasts precursors. However, some GFs in PRP are able to take control of osteoclast-mediated remodeling of the bone.

Collin-Osdoby et al. (2002) believed that local osteoclast-mediated bone resorption coincides with angiogenesis both in normal bone development and pathological disorders such as avascular necrosis. They suggested that basic fibroblast growth factor (bFGF) causes recruitment, activation, adhesion, transmigration and differentiation of hematopoietic cells which may correspondingly enable greater numbers of osteoclasts. They demonstrated that bFGF sensitively regulates local bone remodeling and develops the formation, recruitment and differentiation and activates bone pit resorption in osteoclasts (Collin-Osdoby et al., 2002). It can be understood that delivering PRP with a suitable scaffold may result in an average of osteoclasts.

The efficacy of combined use of PRP and collagen scaffold as a bio-enhanced repair therapy in treatment of partial cruciate ligament rupture as well as increasing capability of controlled releasing of growth factors have been recently demonstrated (Sample et al., 2018; Zhang et al., 2016). Application of PRP and collagen scaffold alone has been carried out for BRONJ preventive/therapeutic approaches (Toro et al., 2019; Oh and Kim, 2017). Particularly, as collagen type I is the most abundant component of the extracellular matrix of bone, it can be utilized as a scaffolding material for bone tissue engineering that provides innate biological information for cell proliferation, adhesion and orientation, and encourages chemotactic responses (Stevens, 2008).

The effect of collagen scaffolds for adhesion, differentiation and proliferation of bone marrow-derived SCs into osteoblasts in a rat model has been shown (George et al., 2006). Collagen content of scaffolds has been also proved to affect the viability, proliferation and spatial distribution of osteoblasts within the scaffold (Tierney et al., 2009). The use of a collagen sponge with/without BMP-2 is assessed for prevention of BRONJ. The results revealed that the collagen sponge with/without BMP had a potential for a positive effect in reducing the incidence of BRONJ in the rats. Additionally, Micro-CT analysis demonstrated almost complete bone regeneration and significant higher BMD values in collagen sponge treated extraction sites with/without BMP comparing to the control group (Oh and Kim, 2017).

It has been recently reported that the use of autologous PRP is a favorable therapy for preventing the occurrence of BRONJ after tooth extraction (Toro et al., 2019). PRP has been demonstrated the prospect to modify natural bone regeneration by the virtue of growth factors (GFs) and bioactive proteins secreted by activated platelets (Sharma and Maffulli, 2005). Among the copious GFs of PRP, PDGF, IGF and TGF-β have been identified to have a pivotal role in bone regeneration (Arora et al., 2009). PDGF acts as a chemoactrant and mitogen for mesanchymal SCs and osteoblasts and is the first GF that initiates vacularization, collagen synthesis and bone regeneration (Wrotniak et al., 2007). TGF-β involves in long term bone remodeling and primarily work on fibroblasts, pre-osteoblasts and undifferentiated marrow cells. IGF is also thought to be a mitogen inducing preosteoblast differentiation and osteoblasts accumulation (Floege et al., 2008).

Apart from osteogenic potentials, the angiogenic capacity and inflammatory cells recruitment of PRP leads to bone regeneration promotion as well (Arora et al., 2009). Although PRP has a great potential in bone tissue regeneration theoretically, there are some drawbacks regarding its mechanical and biological aspects that may question the application of sole PRP as a bone regenerative medicine. Despite having a fibrin structure that supports healing as a standby release mechanism, PRP as a gel and fibrin as a lone scaffold does not appear to be authentic for bone regeneration purposes (Malhotra et al., 2013). Platelets release 95% of their GFs within an hour of their activation and have an average life span of 7–10 days (Alves and Grimalt, 2018).

In fact, activation mode of the platelets leads to consequential GFs release rate. Thrombin results in a rapid activation of platelets (70% within 10 min) and thereby clears before having a stimulatory effect on cells, whereas $CaCl_2$-activated platelets tend to implement a gradual release of factors for 7 days, due to a loose fibrin matrix formation (Foster et al., 2009; Lu et al., 2008). Activated platelets perform a constant efflux of their factors until the end of their life cycle (Miron et al., 2017). However, bone healing/regeneration is a lengthy process that takes 3–6 months to restore adequate strength. As a result, development of an appropriate delivery system for PRP seems to be mandatory for sustaining a prolonged release of platelet-derived factors to maximize its regenerative potential (Rodriguez et al., 2014). With regard to the necessitation of a suitable system for PRP delivery, an absorbable collagen sponge (Collacone ®) with native collagen (type I) was employed as the material of choice to efficiently adsorb GFs due to its 3-D collagen structure scaffold (Lesclous et al., 2009). Additionally, in order to induce gradual delivery of platelet releasants, PRP was activated by $CaCl_2$ in this study.

The potential key role of inflammation resulted in osteonecrosis and clinical onset of BRONJ has been shown (Aggour and Gamil, 2017). Altogether, the ominous cycle of infection, inflammation and osteonecrosis may be a cardinal mechanism of BRONJ. On the other hands, PRP can also obstruct the progression of each of these factors. Aggour and Gamil (2017) have assessed antimicrobial effects of PRP against selected oral pathogens. El-Sharkawy et al. (2007) have assessed the anti-inflammatory potential of PRP and stated that PRP significantly suppresses Monocyte Chemotactic Pretein-1 (MCP-1) and therefore promotes changes in monocyte-mediated proinflammatory cytokine and chemokine release which leads to restraint of inflammation (El-Sharkawy et al., 2007). PRP proved to have an anti-inflammatory effect in our study as it reduced the intensity of inflammation significantly to a mild level compared to the other groups of the study. Osteonecrosis, parallel to inflammation, reduced significantly in the PRP + scaffold group, probably due to controlled inflammatory response and higher number of osteoclasts, absorbing necrotic bone areas. As a result, PRP is capable of inducing soft tissue healing which is a principal concern in management of BRONJ. However our study did not manifest any statistically significant difference regarding soft tissue healing between the groups of the study. PRP-enriched collagen scaffold depicted favorable results in prevention of BRONJ, possibly due to the fact that GFs of PRP could be gradually released and therefore, a prolonged contact between the cells and GFs resulted in better osteogenic and osteoinductive performance of PRP which was enhanced by the osteoconductive properties of the collagen scaffold. It's assumable that higher numbers of osteoclasts, induced by PRP, were capable of resorbing the necrotic bone areas and thus lowered the incidence of inflammation and clinical incidence of BRONJ consequently.

5. Conclusion

In conclusion, the developed PRP-enriched collagen scaffold may offer a fast, easy and effective alternative method for the treatment of medication-related osteonecrosis of the jaw such as BRONJ patients. Application of a PRP-enriched collagen scaffold appeared to be a successful preventive treatment for BRONJ by effecting on the number of osteoblasts and osteoclasts, bone mineralized density, new bone formation, inflammation and osteonecrosis.

Declarations

Author contribution statement

Farnoosh Razmara: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mohammad Bayat, Ghazal Shabankare, Abdolreza Mohamadnia, Mostafa Mortazavi, Mohammad-Reza Alijani: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Sadegh Shirian: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Naghmeh Bahrami: Conceived and designed the experiments; Performed the experiments.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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