

# Periodontal regeneration *via* chemoattractive constructs

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## Abstract

**Aim:** Chemoattractants, such as stromal cell-derived factor-1 $\alpha$  (SDF-1 $\alpha$ ), can offer an advantage for periodontal regeneration by recruiting the patient's own stem cells to stimulate self-repair. We here developed a chemoattractive construct for periodontal regeneration using SDF-1 $\alpha$  and evaluated its efficacy *in vivo*.

**Materials and Methods:** SDF-1 $\alpha$  was loaded on gelatin sponge and tested *in vitro* for SDF-1 $\alpha$  release. Subsequently, SDF-1 $\alpha$  constructs were implanted into rat periodontal defects for 1 and 6 weeks, with unloaded materials and empty defects as controls. The regenerative efficacy was evaluated by micro-CT, histological and histomorphometrical analyses.

**Results:** *In vitro* results showed limited SDF-1 $\alpha$  release up to 35 days. In contrast, SDF-1 $\alpha$  constructs significantly improved periodontal defect regeneration in terms of alveolar bone height, new bone area and functional ligament length. Additionally, SDF-1 $\alpha$  constructs decreased the inflammatory response at Week 6.

**Conclusion:** Chemoattractive constructs significantly improved periodontal regeneration in terms of alveolar bone height, new bone area and functional ligament length.

## KEYWORDS

cell homing, *in vivo*, periodontal regeneration, SDF-1 $\alpha$ , tissue engineering

## 1 | INTRODUCTION

Periodontal regeneration has been attempted over the last 30 years. Despite the evolution in treatment, the clinical results of periodontal regenerative techniques are not optimal and still unpredictable. Currently, the successful application of guided tissue regeneration (GTR) with most predictable clinical results is limited to certain defect morphology (e.g., two and three-wall intrabony defects, class II mandibular furcations), patient's characteristics (good oral hygiene, nonsmokers) and surgeons' experience (skills, decision-making) (Villar & Cochran, 2010). The concept of endogenous cell recruitment can be considered as very suitable and appealing for this purpose, particularly, in view of modifying the early

phase of wound healing, which has been proposed to be the main target of future periodontal regenerative technique (Dickinson et al., 2013; Susin & Wikesjo, 2013). Increasing evidence in the field of regenerative medicine suggests that cell recruitment stimulates latent self-repair mechanisms in patients and harnesses the innate capacity for tissue regeneration (Chen, Wu, Zhang, Zhang, & Sun, 2011). This strategy has been explored as an alternative to cell-based therapies for *in situ* tissue regeneration of heart, cartilage and bone tissue (French, Somasuntharam, & Davis, 2016; Ji et al., 2013; Zhao, Jin, Li, Qiu, & Li, 2017). Instead of introducing *ex vivo* expanded stem cells, recruiting endogenous cells relies on active attraction of regenerative cells to a site of tissue damage or injury.

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For the periodontium, such a strategy seems very appropriate considering that resident mesenchymal stromal cells (MSCs) are regarded as the key for periodontal regeneration (Hynes, Menicanin, Gronthos, & Bartold, 2012; Susin & Wikesjo, 2013). Additionally, development and clinical application of periodontal cell homing technique will mean elimination of constraining factors associated with clinical management of periodontal wounds, specific patient and site characteristics. As a result, optimal conditions for wound healing that will favour periodontal regeneration could become feasible in most clinical settings.

For cell recruitment approaches toward periodontal regeneration, the chemoattractant stromal cell-derived factor-1 $\alpha$  (SDF-1 $\alpha$ ) is the primary candidate chemokine. SDF-1 $\alpha$  plays a major role in cell trafficking and recruitment of CD34<sup>+</sup> stem cells (Aiuti, Webb, Bleul, Springer, & Gutierrez-Ramos, 1997), and several recent periodontal studies have shown the potential of SDF-1 $\alpha$  for periodontal regeneration (Du, Yang, & Ge, 2012; Kaku et al., 2017; Wang, Du, & Ge, 2016). As the mechanism underlying this enhanced periodontal regeneration, Zhou et al. have found that bone marrow-derived stem cells migrated preferentially to the periodontium over other organs to get involved in periodontal regeneration (Zhou et al., 2011). In addition to this cell recruitment efficacy, SDF-1 $\alpha$  is anticipated to modify the inflammatory response and early wound healing (Chen et al., 2015; Dickinson et al., 2013; Zhao et al., 2017).

Both clinically and experimentally, the haemostatic gelatin-based material Spongostan<sup>®</sup> has been widely used for different applications and as a carrier for cells and proteins, respectively (Arias-Gallo, Chamorro-Pons, Avendano, & Gimenez-Gallego, 2013; Cai et al., 2015; Yu et al., 2013). Using this material, we here developed a chemoattractive construct and evaluated its feasibility and biological performance in a rat periodontal defect model. We hypothesized that this chemoattractive construct could recruit regenerative cells to the periodontal defect site and hence would improve periodontal regeneration (i.e., formation of new alveolar bone, new cementum and new periodontal ligament) compared with defects treated with virgin constructs (i.e., without SDF-1 $\alpha$ ) and empty defects.

## 2 | MATERIALS AND METHODS

### 2.1 | Material preparation

Commercially available gelatin sponge (Spongostan<sup>®</sup>; Ferrosan Medical Devices, Denmark) was resized to the defect size ( $2 \times 2 \times 2 \text{ mm}^3$ ,  $W \times L \times D$ ) and loaded *via* absorption with 20  $\mu\text{l}$  PBS solution containing three different doses of recombinant human SDF-1 $\alpha$  (R&D systems, Abingdon, UK): 100, 200 and 400 ng SDF-1 $\alpha$  (Cao et al., 2013; Du et al., 2012). All procedure was performed under aseptic conditions.

### 2.2 | In vitro release experiment

Stromal cell-derived factor-1 $\alpha$  (100, 200 and 400 ng) loaded gelatin sponges were incubated in 1 ml PBS solution in an Eppendorf tube

### Clinical Relevance

*Scientific rationale for the study:* Chemoattractants can stimulate latent self-repair mechanisms in patients and harness the innate capacity for tissue regeneration. However, the efficacy of applying this cell homing strategy in periodontal regeneration is still uncertain.

*Principal findings:* SDF-1 $\alpha$  constructs (without the application of barrier membrane) significantly improved periodontal regeneration at 6 weeks in terms of alveolar bone height, new bone area and functional ligament length.

*Practical implications:* Chemoattractive constructs based on SDF-1 $\alpha$  were proven effective and should be considered for further development towards clinical use, in the treatment of periodontology.

at 37°C. At each time point, the complete volume was taken and refreshed with 1 ml PBS ( $n = 4$ ). The measurements were performed using an ELISA kit (RayBiotech Inc., Norcross GA, USA) with a sensitivity of 80 pg/ml according to manufacturer's instructions.

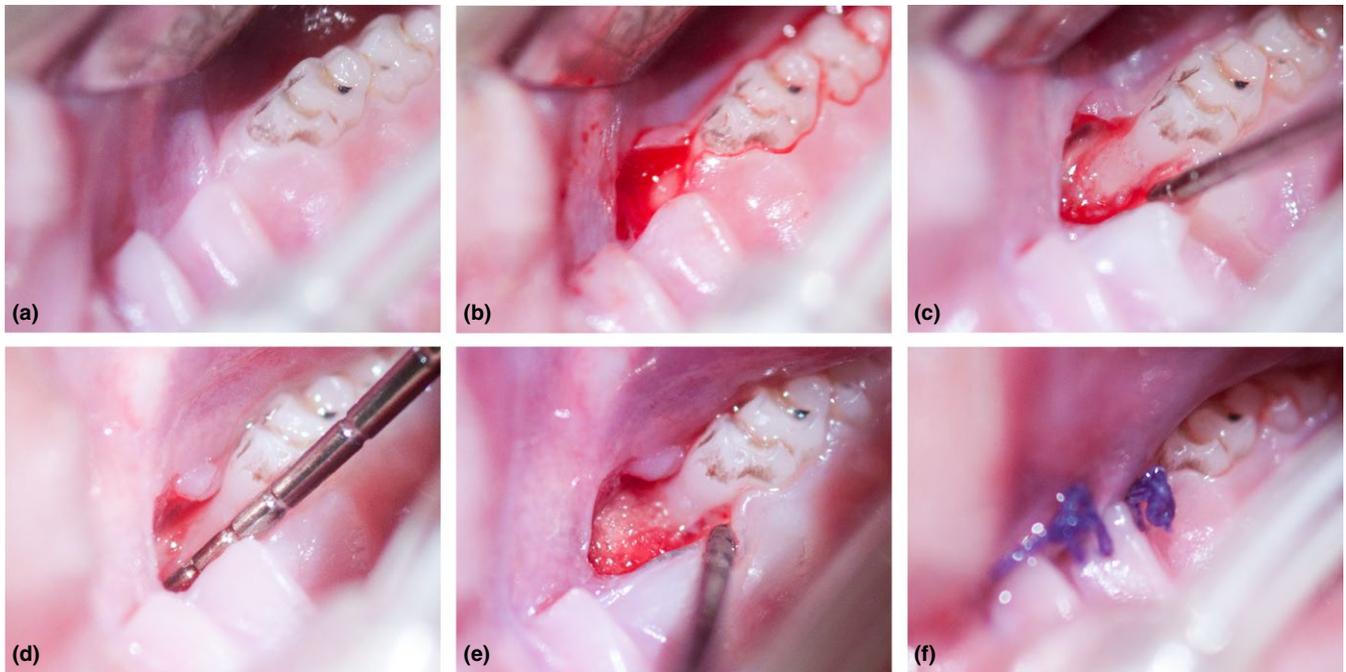
### 2.3 | Cell culture

Isolation of GFP cells from 12-week-old GFP-transgenic Sprague-Dawley rats was done as described in previous studies by our group (Ji et al., 2013; Yu et al., 2013). It was approved by the Animal Ethical Committee of Radboud University (Approval number: RU-DEC 2014-101). This study complied with the ARRIVE guidelines for preclinical animal studies. Briefly, two femora of each rat were extracted, epiphyses were cut off, and diaphyses were flushed out with 15 ml proliferation medium ( $\alpha$ -MEM supplemented with 10% FBS and 50  $\mu\text{g/ml}$  gentamicin). The flush-out of bone marrow from different rats was pooled and cultured for 2 days in a humidified incubator (37°C, 5% CO<sub>2</sub>), after which the medium was refreshed to remove nonadherent cells.

### 2.4 | Animals & surgery

Forty-eight adult male athymic nude rats (CrI: NIH Foxn1<sup>nu</sup>, Charles River, Germany) were used as experimental animals and recipients of the GFP-transgenic rat BMSCs cells to evaluate the biological response to the implants.

For the creation of standardized and validated rat periodontal defects, a previously described surgical protocol was followed (Cai et al., 2015; Yu et al., 2013). As shown in Figure 1, under general anaesthesia, a unilateral intrabony defect was created on the mesial side of the first maxillary molar. Afterwards, the defect was filled with one of the experimental groups in a randomized manner: (a) empty defect (EMP); (b) unloaded sponge (M); (c) SDF-1 $\alpha$  loaded sponge (SDF). The flaps were closed with resorbable sutures (Vicryl<sup>®</sup>)



**FIGURE 1** Overview of the surgical procedure: (a) preoperative surgical site; (b) the root surface and alveolar bone were exposed via a 3-mm full thickness incision; (c) a piezoelectric device (Piezosurgery<sup>®</sup>, Mectron, Carasco, Italy; loaded with OT5 B-tip; Ø1.7 mm) was used to create an unilateral intrabony defect; the residual bone, periodontal ligament and root cementum were carefully removed from the root surface, to create the defect ( $W \times L \times D$ ;  $2 \times 2 \times 1.7 \text{ mm}^3$ ), with constant size monitoring by a clinical periodontal probe; (d) the defect size was confirmed by a periodontal probe; (e) implant placement in the defect; (f) the flap was closed with 5-0 resorbable sutures

5-0; Ethicon Products, Amersfoort, The Netherlands). The three different experimental groups ( $n = 8$ ) were assigned for evaluation of the biological response after 1 and 6 weeks.

At 2 days after implantation, 1 ml PBS solution containing  $1.5 \times 10^6$  GFP-transgenic rat BMSCs was injected into the tail vein of the rats. During the first 10 postoperative days, the animals were fed with powdered food to minimize wound disturbance and visual wound inspection was performed on a daily basis.

## 2.5 | Histological preparation and microcomputed tomography (micro-CT) analysis

After 1 and 6 weeks of implantation, the animals were sacrificed using CO<sub>2</sub> suffocation. The maxillas were harvested and split into two parts through the palatal median line. After fixation in buffered 10% formaldehyde for 24 hr, samples were decalcified in 4% EDTA at 4°C for 6 weeks, dehydrated with graded series of ethanol and embedded in paraffin. Mesiodistal sections (thickness 6 µm) were cut with a microtome (Leica RM2165, Nussloch, Germany) and every 10th section was stained using haematoxylin and eosin (HE) for general tissue survey. For epithelial, ligament and bone tissue observation, sections were stained with Azan and Elastica-van Gieson (EVG) staining. For visualization of the GFP-positive cells in the defect area, anti-GFP immunohistochemical staining was applied (rabbit anti IgG fraction, 1:400; Molecular Probes, Eugene, OR, USA). Anti-CD68 staining was used to evaluate the inflammatory response.

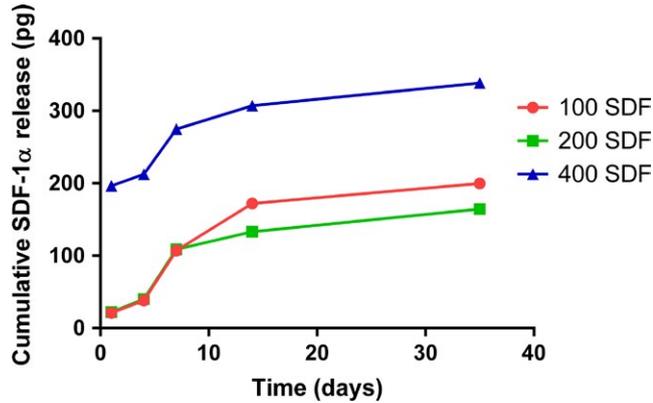
After being fixed in 10% buffered formaldehyde for 48 hr, randomly chosen complete maxillae ( $n = 4$ ) were analysed by micro-CT; 3D-reconstruction of the defect areas and volumetric analysis of the newly formed bone was performed as described previously (Oortgiesen et al., 2012) (SkyScan 1072; SkyScan N.V., Aartselaar, Belgium). A subregion of the originally measured data was selected on both sagittal and transversal slices. This selected region of interest (ROI) included the entire defect area with the newly formed bone inside. By auto-interpolation of manually determined ROIs from the resliced images, each specimen yielded a volume of interest (VOI), which served as the essential basis for all quantitative analyses. Landmarks, such as intact alveolar bone height of the un-operated side and root apex were used for the manual drawing the ROI.

## 2.6 | Histomorphometry

For histomorphometrical analysis, three sections per specimen for each of the stainings (HE, Azan, EVG, anti-CD68) were evaluated. Measurements were performed using Image J software. All measurements were done as described previously for the same periodontal defect model (Cai et al., 2015). The relative alveolar bone height, new bone area, epithelial down-growth and functional ligament length were analysed. The inflammatory response (macrophages) was detected with anti-CD68 staining and subsequently digitalized by a computer programmer in the ROI (defect area). The areas with positive anti-CD68 staining were given as percentage (%) of the total ROI.

## 2.7 | Statistical analysis

Data were expressed as mean  $\pm$  standard deviation. Statistical analysis was carried out using GraphPad (GraphPad Inc, San Diego, CA, USA) by one-way ANOVA and post hoc Tukey testing, for which differences were considered significant at  $p < 0.05$ .



**FIGURE 2** Cumulative release profiles of SDF-1 $\alpha$  from different groups in 1 ml PBS

## 3 | RESULTS

### 3.1 | In vitro SDF-1 $\alpha$ release

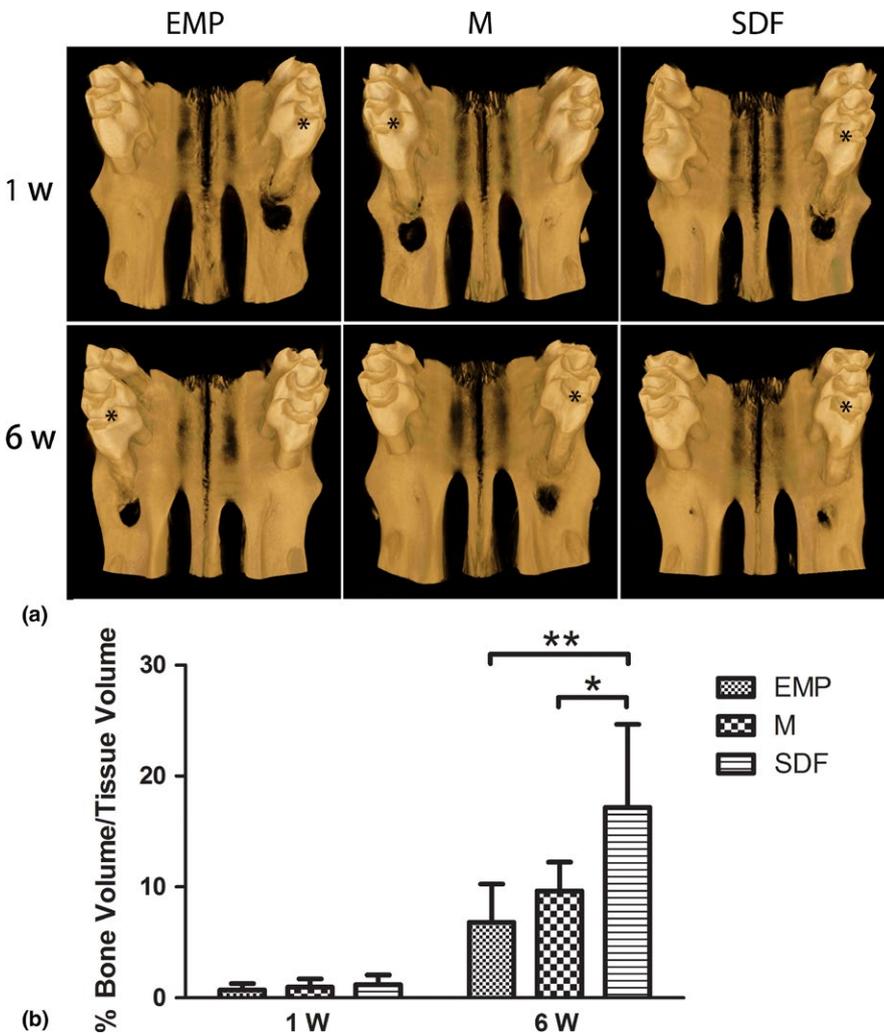
Figure 2 shows the release profiles for the constructs loaded with different amounts of SDF-1 $\alpha$ . For all three loading amounts, a similar release pattern was observed. The cumulative released amount of SDF-1 $\alpha$  increased gradually up to 35 days, where the 400 SDF group showed the highest release (338.6 pg). Nevertheless, for all three groups, the release of SDF-1 $\alpha$  over time was limited in pg compared with loading in ng.

### 3.2 | General in vivo observations

All animals had uneventful recovery after surgery and gained weight during the experimental period. No signs of infection or clinical inflammation were observed. At euthanasia, visual inspection of the surgical sites showed an undisturbed wound healing.

### 3.3 | Micro-CT analysis

Data on new bone formation and 3D reconstructed images of the periodontal defects are presented in Figure 3. After 1 week, defect areas were easily detected, and minor bone formation was observed



**FIGURE 3** (a) 3D reconstructed micro-CT images of periodontal defects with different groups after 1 and 6 weeks: empty control (EMP); unloaded material (M); SDF-1 $\alpha$  loaded gelatin sponge (SDF). Black asterisk indicates the position of the molar. (b) The histogram below represents the percentage of bone volume to tissue volume of different groups at Weeks 1 and 6

predominantly at the defect edges in all experimental groups. After 6 weeks, EMP showed limited new bone formation, whereas more newly formed bone was observed for M and SDF.

The volumes of the newly formed bone at Week 1 were similar (1%) for all groups ( $p > 0.05$ ). After 6 weeks, all groups presented a significant increase in the amount of new bone formation (6.8%, 9.6% and 17.1%, respectively); SDF showed significantly higher new bone formation compared with EMP ( $p < 0.01$ ) and M ( $p < 0.05$ ). Only for SDF, bone ingrowth from the bottom of the defect was observed.

### 3.4 | Descriptive histology

#### 3.4.1 | One-week results

After 1 week, the defects were easily detected by the removed cementum from the root surface and absence of supportive alveolar bone. This occasionally resulted in penetration into the glands apically (Figure 4a–c). For M and SDF, the implanted material was found in place with hardly any signs of degradation and surrounded by thin fibrous tissue capsule. Above them subepithelial inflammatory infiltrate was found (Figure 4d–f). For EMP, the defects were filled with connective tissue and immense inflammatory infiltrate. Besides the presence of extensive inflammation, other common findings observed in all groups were epithelial down-growth (to the level to which the cementum was removed), minor start of bone formation from the defect edges and no signs of PDL formation or cementum formation.

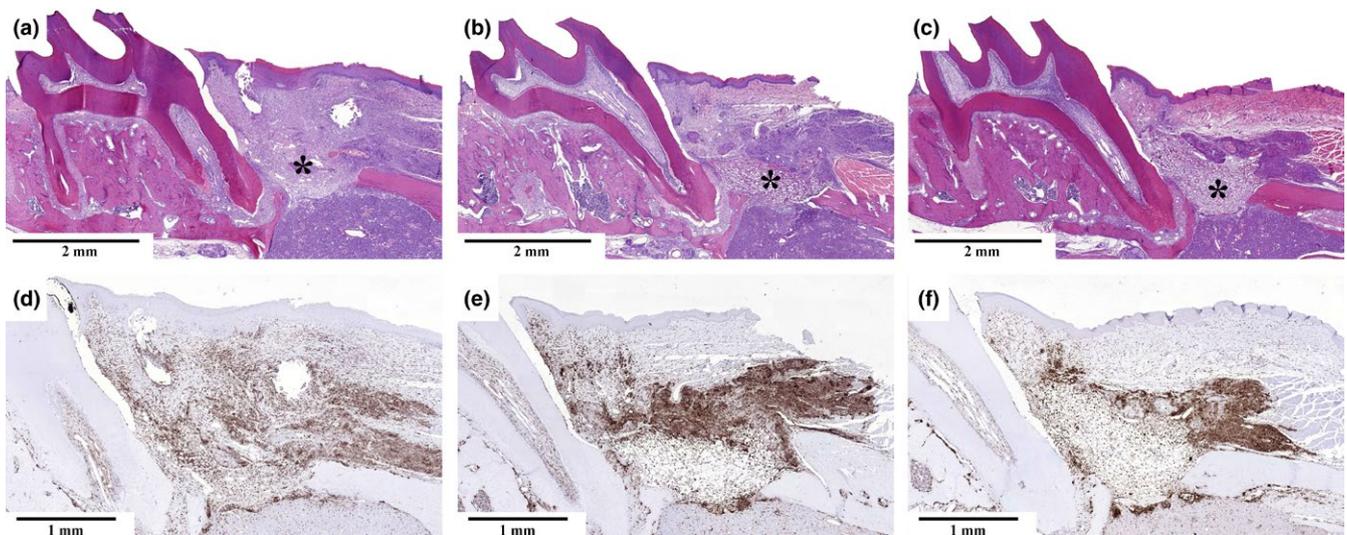
#### 3.4.2 | Six-week results

After 6 weeks, the edges of the periodontal defects were still visible. All materials were found completely degraded, and the

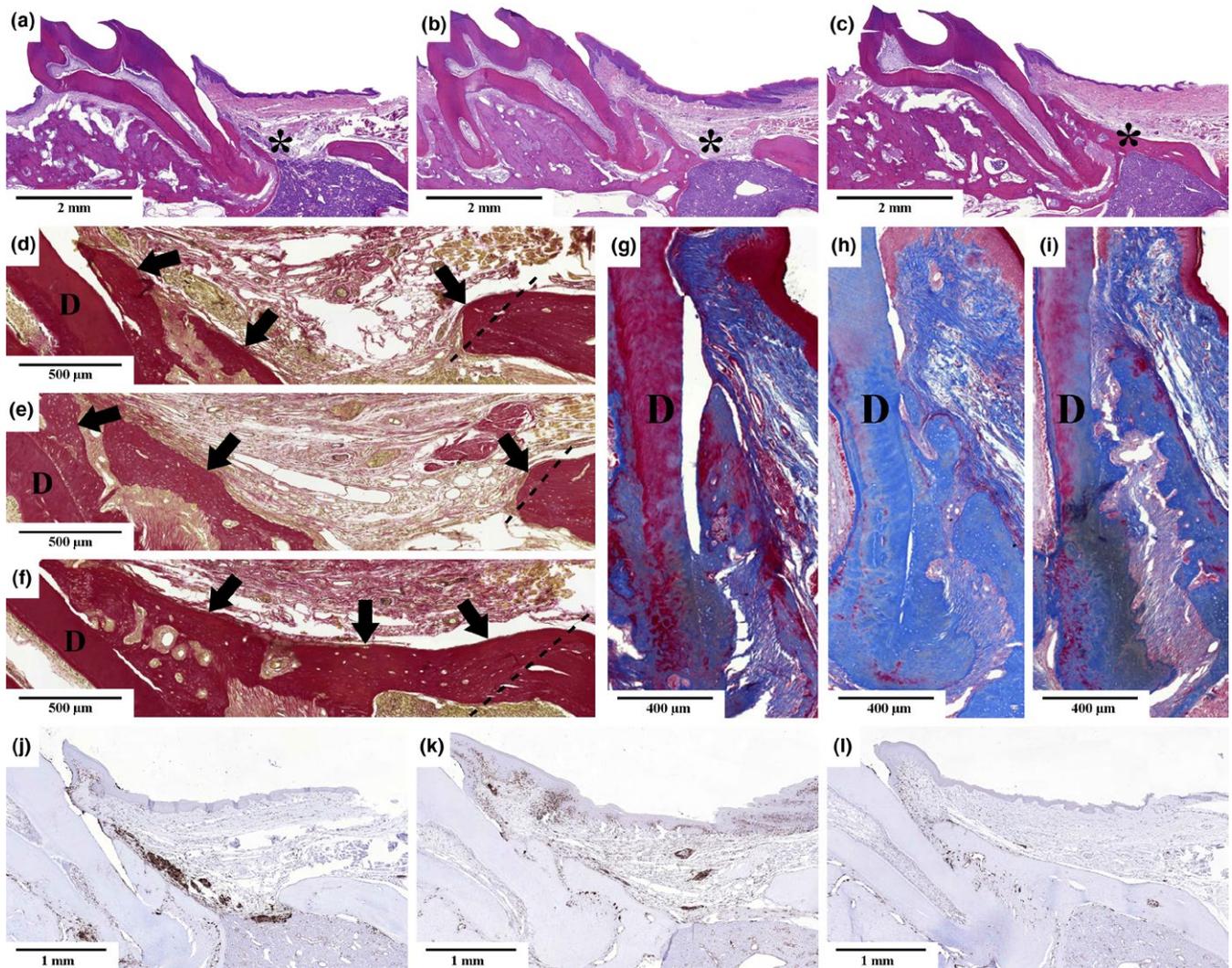
inflammatory infiltrate in the defect region was considerably reduced (Figure 5a–c). For SDF, bone formation was more pronounced than for EMP and M, and in 3/8 of samples, the alveolar bone in the defect area was completely regenerated (Figure 5d–f). Epithelial down-growth remained at the same level as at Week 1, with no obvious differences among the experimental groups, which proves that a barrier membrane is not required to prevent soft tissue ingrowth. As such, we opted to leave out a barrier membrane in the current study. Limited formation of periodontal ligament with new cementum and new bone was observed, which was significant in SDF compared with the other two groups (Figure 5g–i). For the pan-macrophage anti-CD68 staining, an evident decrease in inflammatory response was observed for all experimental groups compared with Week 1. SDF demonstrated a significantly lower level of inflammatory response compared with EMP and M (Figure 5j–l). In addition, anti-GFP staining was performed to track the recruitment of GFP-positive rat BMSCs to the periodontal defect in vivo, but no positive result could be obtained for all groups (data not shown).

### 3.5 | Histomorphometry

For alveolar bone height, a substantial increase with time was observed in all groups (Figure 6). The SDF demonstrated a significantly higher alveolar bone height compared with the EMP and M at Week 6 ( $p < 0.01$ ), with no statistical difference among all groups at Week 1 ( $p > 0.05$ ), which was in accordance with the micro-CT results. Similar results could also be found in the bone area measurement, where the SDF had significantly more new bone formation in the defect area than the M at Week 6 ( $p < 0.05$ ). For the epithelial down-growth, no statistical difference could be



**FIGURE 4** Histological overview after 1 week of implantation. (a) EMP group (HE staining; original magnification  $10\times$ ), no obvious periodontal regeneration could be observed; (b) M group, scaffold remnants could be found in the defect site; (c) SDF group, scaffold remnants could also be found; black asterisk indicates the defect site. (d) Higher magnification of the defect site in (a), positive staining could be found in the whole defect area (anti-CD68 staining; original magnification  $10\times$ ); (e) higher magnification of the defect site in (b); (f) higher magnification of the defect site in (c), relatively less positive staining could be found in the defect area



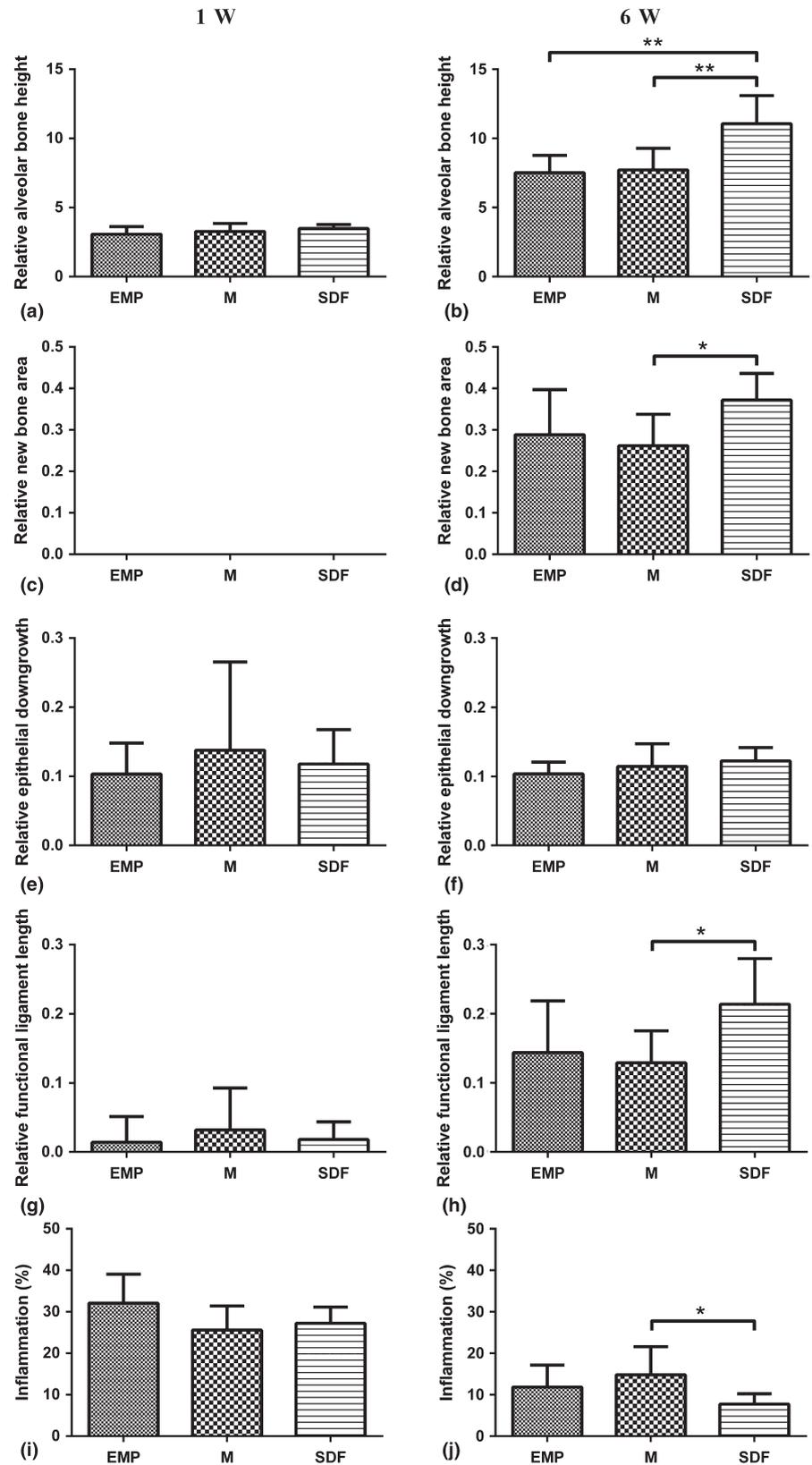
**FIGURE 5** Histological overview after 6 weeks of implantation. (a) EMP group (HE staining; original magnification  $10\times$ ); (b) M group; (c) SDF group; (d) higher magnification of the defect area in (a), new bone formation in the defect was marked with arrows, and the dashed line presents the boundary between new bone and old bone (EVG staining; original magnification  $10\times$ ); (e) higher magnification of the defect area in (b), new bone formation deriving from the bone margin of the defect (arrow); (f) higher magnification of the defect area in (c), the defect site was fully recovered by new bone formation (arrow); (g) higher magnification of the root surface in (a), collagen fibres were attached obliquely to the denuded root surface (Azan staining; original magnification  $10\times$ ); (h) higher magnification of the root surface in (b), very limited amount of fibres obliquely oriented to the root surface on the coronal side; (i) higher magnification of the root surface in (c), collagen fibres obliquely oriented to the root surface; (j) higher magnification of the defect area in (a), positive staining could be found in the defect area (anti-CD68 staining; original magnification  $10\times$ ); (k) higher magnification of the defect area in (b); (l) higher magnification of the defect area in (c), almost no positive staining could be found in the defect area. Black asterisk indicates the defect site; D: dentin of the root

found among all groups at both Week 1 and 6 ( $p > 0.05$ ). As to the functional ligament length, the SDF had significantly higher score than the M at Week 6 ( $p < 0.05$ ), with no statistical difference among all groups at Week 1 ( $p > 0.05$ ). Although the SDF had higher scores than the EMP in the new bone area and functional ligament measurements at Week 6, no statistical difference had been obtained between them ( $p > 0.05$ ). Finally, for the inflammatory response, it was around 28% for all groups at Week 1, with no statistical difference among them ( $p > 0.05$ ). At Week 6, the SDF (7.7%) showed significantly lower level of inflammatory response than the M (14.8%,  $p < 0.05$ ), with no statistical difference to the EMP (11.8%,  $p > 0.05$ ).

## 4 | DISCUSSION

The objective of this study was to develop a construct for periodontal regeneration based on cell recruitment using SDF-1 $\alpha$  and to evaluate the feasibility and biological performance of this construct. The results showed that SDF-1 $\alpha$  constructs significantly improved periodontal wound healing at Week 6 in terms of alveolar bone height, new bone area and functional ligament length. However, in vitro release of SDF from the scaffold was suboptimal.

Our in vitro release results corroborate those of Takayama et al., whose used a collagen membrane as a carrier for SDF-1 $\alpha$  with only 10% SDF-1 $\alpha$  release after 3 weeks (Takayama et al., 2017). In our



**FIGURE 6** Histomorphometrical measurement at Weeks 1 and 6: (a, b) relative alveolar height; (c, d) relative new bone area; (e, f) relative epithelial down-growth; (g, h) relative functional ligament length; (i, j) relative amount of inflammation in percentage for all evaluation periods. \* $p < 0.05$ , \*\* $p < 0.01$ ; error bars represent standard error of the mean ( $n = 8$ )

study, also limited amount of SDF-1 $\alpha$  was released in vitro up to 35 days. The most part of SDF-1 $\alpha$  remained absorbed on the gelatin sponge, or deactivated during the test. It seems that the bonding force between SDF-1 $\alpha$  and gelatin/collagen might be too strong, and/

or physical adsorption of SDF-1 $\alpha$  on the gelatin sponge (Spongostan<sup>®</sup>) is not an efficient approach for SDF-1 $\alpha$  delivery. It has to be noticed that, Spongostan<sup>®</sup> is a commercial product, and besides gelatin, the rest of its components are unknown. Other carriers, such as a

hydrogel (Kimura & Tabata, 2010), PLGA (Thevenot et al., 2010), alginate (Rabbany et al., 2010) and radially oriented collagen scaffold (Chen et al., 2015) have been utilized with various success to continuously deliver SDF-1 $\alpha$  to accelerate blood vessel, cartilage and bone regeneration. More extensive research with other carriers (such as polymer scaffolds) for SDF-1 $\alpha$  delivery, or using gene transduction approaches to up-regulate SDF-1 $\alpha$  expression should be explored in the future.

Despite the suboptimal *in vitro* release of SDF-1 $\alpha$  from the scaffold, the SDF-1 $\alpha$  construct significantly increased new bone formation in rat periodontal defect after 6 weeks of implantation. This effect was not found after 1 week of implantation, and this could be explained with the retention of SDF-1 $\alpha$  within the scaffold. After 6 weeks the gelatin was found completely degraded within our defect, and obviously during this degradation process, SDF-1 $\alpha$  was released and managed to exert its effect. Our histomorphometric and micro-CT results are in accordance with a previous study made by our group (Ji et al., 2013), in which a sixfold increase in bone formation was observed in a SDF-1 $\alpha$  mediated GBR procedure. Takayama et al. applied a SDF-1 $\alpha$  loaded collagen membrane in a rat mandibular bone defect model and got similar results to our findings on the positive effect of SDF-1 $\alpha$  on bone formation (Takayama et al., 2017). Their micro-CT analysis showed similar amounts of new formed bone (i.e., volume and area) as in our study. Although they used a model for bone augmentation, different scaffold (i.e., collagen membrane), higher doses of SDF-1 $\alpha$  (3 and 6  $\mu$ g) and implantation period of 4 weeks, the results were still comparable.

Similar to the new bone formation, the SDF-1 $\alpha$  group also demonstrated significantly longer functional ligament length than the unloaded group after 6 weeks of implantation. This finding is in accordance with other studies that utilize SDF-1 $\alpha$  incorporated collagen membrane (Wang et al., 2016), endochondral approach (Cai et al., 2015) or PDL cells implantation with gelatin sponge (Yu et al., 2013) to regenerate periodontium. In these studies, the regeneration of periodontal ligament as well as alveolar bone could be detected. It can even be hypothesized that the processes of bone and ligament formation are related to each other, similar to the development of the natural periodontium during teeth eruption.

Regarding our experimental design, no barrier membrane was included, which was widely used in the traditional GTR procedure. Based on our previous work (Cai et al., 2015; Yu et al., 2013), gelatin sponge and electrospun scaffolds have been successfully applied in rat periodontal defect without the use of barrier membranes and showing evident periodontal regeneration. Moreover, no significant differences in epithelial down-growth were observed among experimental groups at different time points, which proves that a barrier membrane is not required to prevent soft tissue ingrowth. As such, we opted to leave out a barrier membrane in the current study.

For the *in vivo* rat BMSCs recruitment test, no GFP-positive rat BMSCs could be found in the periodontal defect area. This might be explained by the slow degradation of gelatin sponge *in vivo*, which resulted in the very delayed release of SDF-1 $\alpha$ , and/or by the low

dose of SDF-1 $\alpha$  used. This mismatched timeline might result in the immobilization of GFP-positive cell in the other organs rather than the periodontal defect. Similar result had also been reported by another research group (Huang, Gronthos, & Shi, 2009). However, other studies using constructs with burst release of SDF-1 $\alpha$  in the early stage demonstrated successfully the recruitment of BMSCs (Dashnyam et al., 2014; He, Ma, & Jabbari, 2010; Ji et al., 2013). Therefore, further investigation is needed to confirm the recruitment of GFP-positive rat BMSCs in the periodontal defect, and higher dose of SDF-1 $\alpha$  might be considered.

The enhanced periodontal regeneration in response to local SDF-1 $\alpha$  stimuli could be explained such as follows. Firstly, the bone marrow-derived osteoblast progenitor cells existed in the circulating blood, might be recruited to the defect site in response to local release of SDF-1 $\alpha$  via SDF-1 $\alpha$ /CXCR4 axis (Ji et al., 2013; Otsuru, Tamai, Yamazaki, Yoshikawa, & Kaneda, 2008). Those recruited cells contributed to the periodontal healing process by not only exerting their multilineage differentiation capacity in the defect site, but also secreting a number of cytokines and growth factors to promote tissue regeneration (Bryan, Walker, Ferguson, & Thorpe, 2005). Secondly, the local release of SDF-1 $\alpha$  might also generate proangiogenic environment in the defect site by mobilizing other progenitor cells resident in bone marrow, such as hematopoietic stem cell and endothelial progenitor cell, to the defect site through SDF-1 $\alpha$ /CXCR4 axis (Hattori, Heissig, & Rafii, 2003; Petit, Jin, & Rafii, 2007). Those cells could have enhanced angiogenesis in the defect area, hence indirectly enhancing periodontal regeneration. The effect of SDF-1 $\alpha$  on angiogenesis was not investigated in our study as a haemostatic material (i.e., Spongostan) was used for its delivery into the defect. However, this mechanism should be explored when SDF-1 $\alpha$  is further investigated with a more suitable scaffold for periodontal regeneration.

Regarding the inflammatory response, the SDF showed significantly lower inflammatory response than the M at Week 6. Inhibitory effect on inflammation of SDF-1 $\alpha$  was also seen in other studies. Thevenot et al. reported that SDF-1 $\alpha$  could reduce but not reverse the inflammatory responses (Thevenot et al., 2010). There was a substantial decrease in density and activation of mast cells and inflammatory (CD11b+) cells in the space surrounding the SDF-1-loaded PLGA scaffold in mice. Meanwhile, in a rat mandibular bone defect, SDF-1 $\alpha$  significantly reduced the CD11b+ inflammatory cell response (Liu, Li, Du, Yang, & Ge, 2015).

## 5 | CONCLUSION

Based on our findings, and within the limitations of this study, it can be concluded that the chemoattractive constructs significantly improve periodontal wound healing at 6 weeks in terms of alveolar bone height, new bone area and functional ligament length. Chemoattractive constructs based on SDF-1 $\alpha$  were proven effective and should be considered for further development towards clinical use, in the treatment of periodontology.

## CONFLICT OF INTEREST

The authors declare that there are no conflict of interests in this study.

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