Influence of bone marrow-derived mesenchymal stem cells pre-implantation differentiation approach on periodontal regeneration in vivo


Abstract

Aim: The implantation of bone marrow-derived mesenchymal stem cells (MSCs) has previously been shown successful to achieve periodontal regeneration. However, the preferred pre-implantation differentiation strategy (e.g. maintenance of stemness, osteogenic or chondrogenic induction) to obtain optimal periodontal regeneration is still unknown. This in vivo study explored which differentiation approach is most suitable for periodontal regeneration.

Materials and Methods: Mesenchymal stem cells were obtained from Fischer rats and seeded onto poly(lactic-co-glycolic acid)/poly(ε-caprolactone) electrospun scaffolds, and then pre-cultured under different in vitro conditions: (i) retention of multilineage differentiation potential; (ii) osteogenic differentiation approach; and (iii) chondrogenic differentiation approach. Subsequently, the cell-scaffold constructs were implanted into experimental periodontal defects of Fischer rats, with empty scaffolds as controls. After 6 weeks of implantation, histomorphometrical analyses were applied to evaluate the regenerated periodontal tissues.

Results: The chondrogenic differentiation approach showed regeneration of alveolar bone and ligament tissues. The retention of multilineage differentiation potential supported only ligament regeneration, while the osteogenic differentiation approach boosted alveolar bone regeneration.

Conclusion: Chondrogenic differentiation of MSCs before implantation is a useful strategy for regeneration of alveolar bone and periodontal ligament, in the currently used rat model.

Key words: cell differentiation; in vivo; mesenchymal stem cells; periodontal regeneration; tissue engineering

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Conflict of interest and source of funding statement

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Destructed periodontal tissues have limited potential to regenerate. The use of guided tissue regeneration (GTR) leads to minor, and highly variable clinical improvements (Needleman et al. 2006). Therefore, several recent studies have focused on...
the use of cell-based tissue engineering to achieve periodontal regeneration (Kawaguchi et al. 2004, Hasegawa et al. 2005, Yang et al. 2010, Yu et al. 2013). The principle of tissue engineering, is to expand or condition primary derived cells seeded on a biomaterial scaffold. Such a scaffold can subsequently be implanted into a periodontal defect to regenerate the lost tissue.

Although cell transplantation has mainly been pursued with periodontal ligament fibroblast (PDL cells), also bone marrow-derived mesenchymal stem cells (MSCs) were studied. Bone marrow-derived MSCs show multilineage differentiation potential, are easily accessible, and exhibit a higher growth capacity than PDL cells (Huang et al. 2009, Hynes et al. 2012). Bone marrow-derived MSCs are capable of regenerating not only bone tissue, but also periodontal tissue in various animal models (Li et al. 2009, Lin et al. 2009). Still, so far, no consensus regarding the optimal pre-culture conditions exists. In literature, several main approaches are distinguished, that is the retention of multilineage differentiation potential (Hasegawa et al. 2006, Yang et al. 2010), the osteogenic differentiation approach (Tsumanuma et al. 2011), or a chondrogenic differentiation strategy (Scotti et al. 2010, Harada et al. 2014).

The first approach is based on the notion that stem cells can participate directly to regeneration by differentiating into the progenitor cells of different tissues under the influence of the local microenvironment; or alternatively can regulate regeneration via secretion of trophic factors (Mooney & Vandenburgh 2008). In the presence of fibroblast growth factor-2 (FGF-2), MSCs can maintain their multilineage differentiation potential (Tsutsumi et al. 2001, Bianchi et al. 2003). After transplantation, the local factors present in the host tissue can influence transplanted MSCs to differentiate into various connective tissue cells (Liechty et al. 2000). Recent data suggest that bone marrow progenitor cells can communicate with dental tissues and become tissue-specific mesenchymal progenitor cells to maintain tissue homeostasis (Zhou et al. 2011).

Ultimately, this strategy could result in periodontal regeneration (Kawaguchi et al. 2004, Hasegawa et al. 2006, Yang et al. 2010).

Secondly, the osteogenic differentiation approach hypotheses that establishment of a bony support is the first step in periodontal regeneration, after which functional loading from mastication may stimulate the formation of new ligament, as well as its organization into a certain preferential direction (Yu et al. 2013). Based on this hypothesis, pre-culture of cell-seeded scaffolds in osteogenic medium, can be another conditioning approach (Sikavitsas et al. 2003, Mauney et al. 2005). It has already been shown that the use of a MSCs cell sheet, which was pre-cultured in osteogenic medium for 5 days and placed in a canine one-wall periodontal defect, induced regeneration of hard as well as soft periodontal tissues (Tsumanuma et al. 2011).

Finally, several studies have been using a chondrogenic differentiation approach for bone regeneration (Scotti et al. 2010, Farrell et al. 2011, Harada et al. 2014). In our group, a subcutaneous implantation experiment showed that such constructs have osteoinductive ability (Yang et al. 2013). In this approach, bone is generated through the endochondral pathway instead of direct osteoblastic differentiation. The underlying principle behind this approach is that chondrocytes are able to survive with limited nutrition and oxygen. It has not been investigated whether this approach is also useful for periodontal regeneration.

Therefore, this study aimed to compare the efficacy of three different MSCs differentiation approaches for periodontal regeneration. In practice, a three-dimensional fibrous wet-electrospun scaffold was used as the cell delivery vehicle. MSCs were seeded onto the scaffold and pre-cultured in different conditions to achieve desired differentiation status before implantation into a previously described intrabony three-wall periodontal defect model in Fischer rats (Oortgiesen et al. 2012). Subsequently, histomorphometrical analysis was used to assess the regenerated periodontal tissues.

Materials and Methods

Fabrication and characterization of wet-electrospun scaffolds

Scaffolds were prepared using a wet-electrospinning technique as described previously (Yang et al. 2013). Poly(lactic-co-glycolic acid) (PLGA; Purasorb® PDLG 5010), poly(ε-caprolactone) (PCL; inherent viscosity 1.0–1.3 dl/g) and organic solvent 2,2,2-trifluoroethanol (TFE; purity ≥99.8%) were purchased from Purac Biomaterials BV (Gorinchem, The Netherlands), DURECT Corporation (Pelmah, AL) and Acros (Geel, Belgium), respectively.

Scaffold morphology was observed by scanning electron microscopy (SEM; JEOL6340F, Tokyo, Japan). The fibre diameter was measured from SEM micrographs that were obtained at random location (n = 90) using Image J software (National Institutes of Health, Bethesda, MD).

Cell isolation, seeding and pre-culture

Bone marrow-derived MSCs were isolated from 7-week-old male Fischer rats after approval from Radboud University Nijmegen Animal Ethics Committee (Approval number: RU-DEC 2013-100). Briefly, both femora of each rat were extracted, epiphyses were cut-off and diaphyses were flushed out using the basic cell culture medium (BM, Table 1). The flush-out of bone marrow from five rats was pooled and cultured for 2 days in a humidified incubator (37°C, 5% CO₂), after which the medium was refreshed to remove non-adherent cells. Cells from passage 2 were used for cell seeding.

Disk-shaped scaffolds (Ø 2 mm; thickness 0.6 mm) were punched out (biopsy punch; Kai medical, Gifu, Japan) from electrospun meshes and treated with an ultrasonic processor (UP50H, Hielscher Ultrasonics, Germany) in 100% ethanol for 5 min. to obtain a very loose structure. Scaffolds were subsequently sterilized in 70% ethanol for 2 h and soaked in BM overnight at 37°C. MSCs were seeded by soaking the scaffolds in a cell suspension containing 2.0 × 10⁶ cell/ml (10 scaffolds per 1 ml cell suspension) and gently rotating for
Table 1. Cell culture media used in the experiment

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full name</th>
<th>Constituents</th>
<th>Origin of constituents</th>
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<tr>
<td>BM</td>
<td>Basic cell culture medium</td>
<td>10% fetal bovine serum (FBS)</td>
<td>Gibco</td>
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<td></td>
<td></td>
<td>100 U/ml penicillin</td>
<td>Sigma</td>
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<td></td>
<td></td>
<td>100 μg/ml streptomycin</td>
<td>Gibco</td>
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<tr>
<td>FM</td>
<td>FGF-2 medium</td>
<td>3 ng/ml FGF-2</td>
<td>R&amp;D systems</td>
</tr>
<tr>
<td>PM</td>
<td>Proliferation medium</td>
<td>BM</td>
<td></td>
</tr>
<tr>
<td>OM</td>
<td>Osteogenic medium</td>
<td>10 mM sodium</td>
<td>Sigma</td>
</tr>
<tr>
<td>CM</td>
<td>Chondrogenic medium</td>
<td>High-glucose Dulbecco’s modified Eagle’s medium</td>
<td>Gibco</td>
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<tr>
<td></td>
<td></td>
<td>1% FBS</td>
<td>Sigma</td>
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<tr>
<td></td>
<td></td>
<td>100 U/ml penicillin</td>
<td>Gibco</td>
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<td></td>
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<td>100 μg/ml streptomycin</td>
<td>Sigma</td>
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<td></td>
<td></td>
<td>50 μg/ml L-ascorbic acid</td>
<td>Gibco</td>
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<td></td>
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<td>100 mM sodium pyruvate</td>
<td>Sigma</td>
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<td></td>
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<td>1:100 insulin-transferrin-selenium</td>
<td>BD biosciences</td>
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<td></td>
<td></td>
<td>100 nM dexamethasone</td>
<td>Sigma</td>
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<tr>
<td></td>
<td></td>
<td>10 ng/ml transforming growth factor beta-2 (TGF-β2)</td>
<td>R&amp;D systems</td>
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<tr>
<td></td>
<td></td>
<td>100 ng/ml bone morphogenetic protein 2 (BMP-2)</td>
<td>BD biosciences</td>
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2 h (Stuart® SB3, Jencons Scientific Ltd., Bedfordshire, UK). Then the non-attached cells from the suspension were collected, centrifuged and reseeded equally onto each scaffold to ensure highest cell loading efficiency. Culture medium was added to each well after 1 h. As a negative control, empty scaffolds without cells were prepared simultaneously. Finally, the constructs were pre-cultured under different conditions:

- For the retention of multilineage differentiation potential (FGF2) group, the constructs were cultured in FGF-2 medium (FM, Table 1) for 10 days;
- For the osteogenic differentiation (O+) group, the constructs were cultured in osteogenic medium (OM, Table 1) for 10 days;
- For the chondrogenic differentiation (C+) group, the constructs were cultured for 1 week in proliferation medium (PM, Table 1) and then transferred to chondrogenic medium (CM, Table 1) for 4 weeks according to our previous study (Yang et al. 2013);
- For the empty (EMP) group, empty scaffolds were kept in BM for 10 days.

In vitro assays
For in vitro assays, haematoxylin and eosin (HE), Safranin O and Von Kossa staining were performed to evaluate cell distribution, cartilage formation and in vitro calcification ability, respectively.

After pre-culture, samples were fixed in 10% phosphate-buffered formalin, dehydrated in graded ethanol and embedded in paraffin. Following deparaffinization in xylene and rehydration through graded ethanol, 6-μm-thick sections were cut with a microtome (Leica RM2165, Nussloch, Germany), and stained with HE or Safranin O (n = 5).

For Von Kossa staining, samples were further cultured for 21 days (FGF2 and C+ group) or 14 days (O+ group) in OM. Thereafter, samples were fixed in 10% phosphate-buffered formalin, decaledified in 10% EDTA, dehydrated in graded ethanol and embedded in paraffin. Micromtome sections of 6-μm thick were stained with Von Kossa reagent to visualize the mineralized matrix, and counterstained with Nuclear Fast Red (n = 5).

Histological preparation
Six weeks after implantation, rats were deeply anesthetized prior to 10% formalin cardiovascular perfusion. Complete maxillae were dissected and excess tissues were removed. After fixing in buffered 10% formaldehyde for 24 h, the specimens were decalledified in 10% EDTA at room temperature, with X-ray examination to confirm the decalcification process. Subsequently, the specimens were dehydrated in...
Fig. 1. Overview of the surgical procedure and new tissue measurement in the rat periodontal defect model: (A) pre-operative general observation; (B) a 3-mm full thickness incision was made to expose the root surface and alveolar bone; (C) the created defect; (D) a periodontal probe was used to confirm the defect size; (E) implant placement in the defect; (F) flaps were closed with 5-0 resorbable sutures; (G) Schematic drawing illustrating the histomorphometrical analyses of periodontal tissue regeneration: reference line 1 is drawn based on the cemento-enamel junction (CEJ) at the distal portion of first molar; reference line 2 is drawn based on the alveolar level of the intact bone; Relative alveolar bone height = $L_{ac}/L_{bg}$, where $L_{ac}$ is the length of the mesial molar from apex to cusp, and $L_{bg}$ is the length of bone gap, that is the distance from the regenerated bone level to the normal alveolar bone height level; Relative new bone area = $A_{nb}/A_{df}$, where $A_{nb}$ is the area of new bone (within yellow frames), and $A_{df}$ is the area of defect (within blue frame); Relative epithelial down-growth = $L_{je}/L_{ca}$, where $L_{je}$ is the length of junctional epithelial from the CEJ to the apical extent; Relative functional ligament = $L_{fl}/L_{ca}$, where $L_{fl}$ is the length of functional ligament, and $L_{ca}$ is the distance from the CEJ to the apical end of the defect.

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graded ethanol. Before paraffin embedding, the molars were stained with black ink to ensure that sections were made in the area of interest. Sections of 6 μm thick were cut in mesio-distal direction with a microtome. Every 15th slide was stained with HE for general tissue assessment. For ligament, epithelial and bone tissue observation, adjacent sections were stained with Azan staining and Elastica-van Gieson (EVG) staining.

**Histomorphometrical analysis**

For the quantitative analysis, three sections from the middle third of the defect area for each specimen were evaluated. Sections were photographed using a Zeiss Imager Z1 equipped with an AxioCam MRc5 camera operated with AxioVision 4.6.3 software (Carl Zeiss Microimaging GmbH, Göttingen, Germany). Measurements were performed using Image J software, as previously described (Yu et al. 2013) (Fig. 1G).

**Statistical analysis**

Data were expressed as mean ± 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.

**Results**

**Scaffold characterization**

The wet-electrospun mesh showed a loose, fluffy structure, with a diameter of 3–4 cm and a thickness of 2–3 mm (Fig. 2A). SEM images (Fig. 2B) showed a porous fibrous structure with randomly dispersed fibres. The pores between fibres were about 3–30 μm and the average fibre diameter was 2.25 ± 0.31 μm.

**In vitro assays**

Haematoxylin and eosin stained sections revealed that samples from the FGF2 and O+ groups were fragile and upon handling did not retain the original shape. The seeded cells were mostly found in the middle and edge of the scaffold (Fig. 3A–D). In contrast, samples from the C+ group were mostly intact, and cells were distributed evenly through the scaffold (Fig. 3E, F). In particular, calcified deposits (Fig. 3D) and a cartilage-like structure (Fig. 3F) could be observed in the O+ and C+ scaffolds, respectively. No accumulation of cells or matrix was detected in the EMP scaffold (Fig. 3G, H).

Safranin O staining was used to further confirm the cartilage-like structure of the C+ group (Fig. 4), and showed an abundant glycosaminoglycan content, representing the cartilage matrix. Typical chondrocytes were embedded in large lacunae and formed isogenous groups (Fig. 4B, arrow).

Von Kossa staining was used to test the calcification ability of the cell-scaffold constructs after prolonged culture in OM. All samples from the experimental groups showed calcified deposits, indicated by black/brown colour (Fig. 3I–N), while empty scaffolds showed no calcification (Fig. 3O, P). Among these groups, the C+ group showed the largest positively stained area by visual inspection.

**Descriptive histology**

Two rats died during the surgery, which resulted in the loss of one sample from the FGF2 group, one sample from the C+ group, and two samples from the EMP group. Postoperatively, all animals showed uneventful recovery and gained weight during the 6 week experiment period. No clinical signs of infection or inflammation were detected. At euthanasia, visual inspection of the surgical sites showed an undisturbed wound healing. After histological processing, 24 out of 28 retrieved samples from surgery could be analysed in this study.

Light microscopical examination of the sections revealed that the periodontal defects could still be distinguished in all samples, as none of the specimens showed complete periodontal regeneration. On the apical area of the root surface, a sharp dark-stained line between newly formed tissue and old tissue could be observed in all groups, indicating the apical end of cementum/ligament removed by the surgery. In the defect site, newly formed alveolar bone and ligament could be observed, cementum formation was limited to the apical root surface. The fibrous scaffold could still be detected, surrounded by multinucleated giant cells, showing an inflammatory response to the scaffold remnants. Minor root resorption, dentinoclast-like cells and ankylosis could be observed in some specimens.

In the FGF2 group, more bone was found in the defect area compared to the EMP group (Fig. 5A and E). The newly formed bone was located in the scaffold or along the defect margin. Considering ligament formation, collagen fibres were attached obliquely to the coronal root surface. In total, 4/6 of the samples showed collagen fibres inserted into the cementum-like tissue layer at the apical end of the defect, while between these two parts, collagen fibres ran in parallel to the root surface (Fig. 5I).

In the O+ group, more bone was found in the defect area compared to the EMP group, mainly initiated from the bone margin of the defects (Fig. 5B and F). Along the denuded root surface, a thin layer of fibres was observed running parallel to the root surface. Only at the coronal side
Fig. 3. Representative photomicrographs from different in vitro samples. (A) Overview of a sample from the FGF2 group. Note cell accumulation along the scaffold perimeter, and in the central areas (HE staining, 5×); (B) High magnification of the framed area in (A) (20×); (C, D) overview and detail of the O+ group; (E, F) The C+ group. Note the even cell distribution and apparent formation of cartilage-like tissue in the central areas of the scaffold; (G, H) overview and detail of the empty control scaffold. Von Kossa staining for cell-scaffold constructs after in vitro osteogenic culture. (I) Overview of a sample from the FGF2 group (5×). Note that calcified deposits were indicated by black/brown colour; (J) High magnification of the framed area in (I) (20×); (K, L) overview and detail of the O+ group; (M, N) overview and detail of the C+ group. Note samples from this group showed highest positively stained area by visual inspection; (O, P) overview and detail of the empty control scaffold.

Fig. 4. In vitro Safranin O staining of C+ group. (A) Abundant GAG (orange-red colour) was secreted (5×); (B) higher magnification (20×) of the framed area in (A), typical chondrocytes were embedded in large lacunae and formed isogenous groups.
a very limited amount of fibres, obliquely oriented to the root surface, were observed (Fig. 5J).

In the C+ group, new bone formation was more pronounced compared to the EMP as well as FGF2 groups. Newly formed bone was observed at the margin of defect, and in 4/6 of the samples, new bone formation was detected as islands without direct contact with the defect edges (Fig. 5C and G). Collagen fibres were attached obliquely to the coronal root surface (Fig. 5K), and anchored in the newly formed cementum in the apical regions (in 5/6 of the samples), while in between, running mostly in parallel to the root surface. Cartilage-like tissue could be detected in all samples.

As a control, all specimens in the EMP group showed very limited new bone formation (Fig. 5D and H). Obliquely oriented collagen fibres were limited to the coronal side of denuded root surface (Fig. 5L). The defect area was mostly filled with scaffold remnants and multinuclear giant cells.

Histomorphometry and statistical analysis

As shown in Fig. 6, the C+ group demonstrated a significantly higher alveolar bone height compared to the EMP group. The results of the bone area measurement showed that the C+ group had significantly more new bone formation in the defect area than the FGF2 and EMP groups. The O+ group also had significantly more new bone formation than EMP group. For epithelial down-growth, no statistical differences were found between any of the groups, including the empty control. Finally, for functional ligament length, both the FGF2 and C+ groups had significantly higher scores than the O+ group.

Discussion

The overall objective of this study was to explore the most optimal differentiation approach for MSCs to
achieve periodontal regeneration, using an intrabony periodontal defect rat model. For this purpose, MSCs were seeded on a scaffold and pre-cultured in different conditions, that is retention of multilineage differentiation potential (FGF2), an osteogenic differentiation approach (O+) and a chondrogenic differentiation approach (C+). The in vitro histological results confirmed that cells were distributed throughout the scaffolds. Cartilage was formed in the C+ group, and in vitro calcification occurred in all experimental groups under osteogenic culture condition. The in vivo results demonstrated evident differences. Especially the C+ approach resulted in the regeneration of both alveolar bone and periodontal ligament, whereas FGF2 only contributed to periodontal ligament regeneration and O+ to alveolar bone regeneration.

Regarding our study design, several remarks can be made. A small animal model was applied, whereas a larger animal model would provide higher comparability with the human situation (e.g. metabolic rate, defect size) and thus would facilitate the translation from bench to bedside. Also, the time necessary for culture and differentiation was not equal as was inherent to the use of different protocols. As a necessary step for chondrogenic differentiation approach, cells were grown in CM for 4 weeks. This longer culturing time may influence the performance of C+ samples, although the low serum chondrogenic culture condition would principally not stimulate the cell proliferation. Still, it is the purpose of this study to compare different approaches for periodontal regeneration. A final technical remark should address the scaffold degradation, as in all groups scaffold remnants were visible. Thus, it seems necessary to further investigate the degradation capacity in vivo. The PLGA/PCL wet-electrospun scaffold is a highly porous scaffold, which on beforehand was proved to be suitable for MSC loading and (endochondral) bone formation (Yang et al. 2013). Still, compared to previous studies using gelatin cell carriers (Yu et al. 2013), the degradation rate of the PLGA-PCL scaffold was much slower, and abundant presence of multinucleated giant cells was seen, potentially interfering with new tissue formation.

Regarding our results in the FGF2 group, only periodontal ligament formation was found to be significantly higher than in the O+ group. This corroborates with another study, which also showed that bone marrow MSCs can gain characteristics of PDL cells, after co-culturing with periodontal ligament-derived cells (Kramer et al. 2004). However, for bone tissue regeneration, the induction from the microenvironment was apparently inadequate.

In the O+ group, the reverse process happened, that is only new bone formation was seen, but without evident ligament formation. In literature it has been postulated that only the establishment of bony support allows the transfer of functional loading, which subsequently is important for ligament formation. Therefore, it seems likely that the time in our current setup was too short for such a process, and longer time points for analyses should be regarded. Alternatively, the lack of ligament formation can be due to the induction protocol, using 10 days of osteogenic pre-culture. MSCs at the earlier stages have a strong growth potential, but the proliferative capacity decreases when most cells have reached osteogenic differentiation (Sikavitsas et al. 2003, Castano-Izquierdo et al. 2007). Thus, it can also be argued that most cells were already terminally differentiated. As a consequence, proliferation rates or differentiation capacity upon implantation were too low to achieve ligament formation.

The reason to investigate chondrogenic differentiation is mostly related to cell survival. Previous studies were already able to show green fluorescent protein (GFP)-positive cementoblasts, osteoblasts, osteocytes and fibroblasts in periodontal tissue regeneration studies (Hasegawa et al. 2006). On the other hand, other recent investigations, tracking GFP-positive PDL cells in rat calvarial critical-sized defects, underline that cell numbers decrease significantly after 4 weeks, with single or no positive cells detectable after 10 weeks (Tour et al. 2012). An enhanced cell survival can explain the positive effects in the current C+ group, as chondrocytes are known to be able to survive with limited nutrition and oxygen. The C+ group exhibited formation of both hard and soft periodontal tissues.
which corroborates our earlier findings upon subcutaneous implantation (Yang et al. 2013). For the effectiveness of the chondrogenic approach, also several other explanations can be found in literature. First, the in vitro results showed that C+ samples were more resilient to handling due to the large amounts of generated extracellular matrix (ECM) after pre-culture. Earlier investigations have shown that such abundant ECM also can actively sequester growth factors and cytokines, attract cells from the implant site, and thus inherently favours growth and differentiation (Benders et al. 2013).

Second, the chondrocytes produce specific matrix metalloproteinases, initiating remodelling and thus leading to mineralization of the hypertrophic cartilage. Finally, the chondrocytes are shown to attract blood vessels by releasing VEGF (Gawliita et al. 2010), which also could accelerate tissue regeneration in the periodontium.

Conclusion
Based on our findings, and within the limitations of this study, it can be concluded that the chondrogenic differentiation approach is a useful strategy for regeneration of alveolar bone and periodontal ligament, in the currently used rat model. Larger periodontal defect models and scaffold with higher degradation rate will be necessary to examine the full potential of the chondrogenic differentiation approach in periodontal regeneration in further investigations.

References
Clinical Relevance

Scientific rationale for the study: Cell-based tissue engineering strategies using MSC cells have been suggested to be suitable for periodontal regeneration. However, which pre-implantation differentiation approach leads to optimal regeneration in vivo is still unknown. Principal findings: Chondrogenic differentiation of MSCs before implantation proved to be effective to achieve regeneration of alveolar bone as well as periodontal ligament in a rat periodontal defect model. Practical implications: Chondrogenic differentiation of MSC cells and subsequent implantation favours the regeneration of the periodontium, and could eventually be developed towards a clinically feasible approach.