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Effect of temporal onsets of mechanical loading on bone formation inside a tissue engineering scaffold combined with cell therapy

T.C. Hausherr[a], K. Nuss[b], E. Thein[c], S. Krähenbühl[d], L.A. Applegate[d], D.P. Pioletti[a,*]

[a] Laboratory of Biomechanical Orthopedics, Ecole Polytechnique Fédérale de Lausanne, Switzerland
[b] Musculoskeletal Research Unit, Vetsuisse Faculty, University of Zürich, Switzerland
[c] Orthopedic and Traumatology Department, University Hospital of Lausanne (CHUV), Switzerland
[d] Regenerative Therapy Unit, Plastic and Reconstructive Surgery, University Hospital of Lausanne (CHUV), Switzerland

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ABSTRACT

Several approaches to combine bone substitutes with biomolecules, cells or mechanical loading have been explored as an alternative to the limitation and risk-related bone auto- and allo-grafts. In particular, human bone progenitor cells seeded in porous poly(L-lactic acid)/tricalcium phosphate scaffolds have shown promising results. Furthermore, the application of mechanical loading has long been known to be a key player in the regulation of bone architecture and mechanical properties. Several in vivo studies have pointed out the importance of its temporal offset. When an early mechanical loading was applied a few days after scaffold implantation, it was ineffective on bone formation, whereas a delayed mechanical loading of several weeks was beneficial for bone tissue regeneration. No information is reported to date on the effectiveness of applying a mechanical loading in vivo on cell-seeded scaffold with respect to bone formation in a bone site. In our study, we were interested in human bone progenitor cells due to their low immunogenicity, sensitivity to mechanical loading and capacity to differentiate into osteogenic human bone progenitor cells. The latest capacity allowed us to test two different bone cell fates originating from the same cell type. Therefore, the general aim of this study was to assess the outcome on bone formation when human bone progenitor cells or pre-differentiated osteogenic human bone progenitor cells are combined with early and delayed mechanical loading inside bone tissue engineering scaffolds. Scaffolds without cells, named cell-free scaffold, were used as control. Surprisingly, we found that (1) the optimal solution for bone formation is the combination of cell-free scaffolds and delayed mechanical loading and that (2) the timing of the mechanical application is crucial and dependent on the cell type inside the implanted scaffolds.

1. Introduction

Cells such as mesenchymal stem cells (MSCs), human bone progenitor cells (hBPCs), and bone marrow-derived MSCs, have shown their potential for bone tissue engineering (BTE) in several in vitro studies, by producing mineralized extra cellular matrix under osteogenic conditions (Krattinger et al., 2011; Krebsbach et al., 1999; Montjovent et al., 2004; Owen et al., 1987; Phinney et al., 1999; Pittenger et al., 1999; Wu et al., 2015). In different experimental and in vivo implantation conditions, those cells have demonstrated their capacity to induce bone formation when implanted with BTE scaffolds. In citing a few studies, Serafini et al. have highlighted the ability of bone marrow-derived MSCs to form bone marrow and hematopoietic niches when implanted in heterotopic sites (Serafini et al., 2014), whereas other studies have shown an increase in bone formation when implanted in bone sites (Corre et al., 2015; Dupont et al., 2010; Jäger et al., 2007; Liu et al., 2013; Montjovent et al., 2008; Srouji and Livne, 2005; Xu et al., 2010; Yasko et al., 1992).

In parallel, it has long been known that mechanical loading plays an important role in the regulation of bone architecture and properties (Carter et al., 1989; Huiskes et al., 2000). Capitalizing on this phenomenon for applications, several studies demonstrated in vivo that the temporal onset of mechanical loading on bone formation in scaffolds was crucial (Boerckel et al., 2012; Roshan-Ghias et al., 2010, 2011). The application of early mechanical loading, applied a few days post-implantation, was seen to be ineffective or moderate compared to delayed mechanical loading, applied several weeks post-implantation.

Therefore, in the present work, based on longitudinal microCT images and histological analysis we investigated the effect of the combination between mechanical loading and cell therapy in the

* Corresponding author at: Laboratory of Biomechanical Orthopedics, Ecole Polytechnique Fédérale de Lausanne, Station 9, CH-1015 Lausanne, Switzerland.
E-mail address: dominique.pioletti@epfl.ch (D.P. Pioletti).

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outcome of a tissue engineering scaffold implanted in a rat model. We were able to score the effects of the different bone tissue engineering treatments in scaffold bone formation under these experimental conditions.

2. Materials and methods

2.1. Cell culture and scaffold seeding

The interest in using hBPCs arose from their low immunogenicity (Montjovent et al., 2009) and capacity for osteogenic differentiation into mature osteoblasts (Montjovent et al., 2004), referred in the present work to as osteogenic hBPCs (hOBPCs). This differentiation capacity allows us to test in vivo two different bone cell fates originally derived from the same type of cell.

The culture conditions and scaffold seeding were performed as thoroughly reported elsewhere (Hausherr et al., 2017). In short, hBPCs were harvested from fetal bone tissue of 15 weeks gestational age following a voluntary interruption of pregnancy (Biobank, CHUV, Switzerland, Protocols 51/10). hBPCs were first expanded in standard culture medium composed of DMEM basal culture medium high glucose (Invitrogen, USA), supplemented with 10% (v/v) FBS (Thermo Fisher Scientific, USA) and 1% (v/v) L-Glutamine (200 mM, Invitrogen, USA). We showed in a previous study that under these culture conditions, hBPCs kept their osteoblastic phenotype (Hausherr et al., 2017). At passage 4, the cells were seeded into scaffolds composed of poly(L-lactic acid) (PLA, Boehringher Ingelheim, Germany) and 5% β-tricalcium phosphate (5% β-TCP, Fluka, Sigma-Aldrich, ST Louis, USA) (Mathieu et al., 2006). Before seeding hBPCs at a concentration of 0.5×10^6 cells per PLA/5% β-TCP scaffold using a pressure-driven technique, the scaffolds were sterilized by ethylene oxide, perfused with 0.9% NaCl solutions (B. Braun, Germany) and sonicated to avoid micro-air bubbles inside the scaffolds. Three different scaffold conditions were prepared: cell-free scaffolds (CF), scaffolds seeded with hBPCs (CS) and scaffolds seeded with hOBPCs (OCS). For CS scaffolds, hBPCs were cultured in standard culture medium and were seeded three days before implantation. In the case of OCS scaffolds, hBPCs were seeded two weeks before implantation. To induce osteogenesis, the medium of OCS scaffolds was changed three times a week with osteogenic differentiation medium, composed of α-MEM (Gibco, USA), 10% (v/v) FBS (Thermo FisherScientific, USA), 1% (v/v) L-Glutamine (200 mM, Invitrogen, USA), 1% (v/v) Vitamin C (5 mg/mL, Sigma-Aldrich, USA), 1% (v/v) β-glycerophosphate (500 mM, Sigma-Aldrich, USA) and 1% (v/v) dexamethasone (1 mM, Sigma-Aldrich, USA). Just before implantation, the scaffolds were washed three times with sterile 0.9% NaCl solution (B. Braun).

2.2. Animal study design

Tissue engineering PLA/5% β-TCP scaffolds were implanted in a pre-drilled hole in both femoral condyles of female rats. The bone trauma site in the femoral condyle was situated under the growth plate, therefore corresponding to a metaphysis location. The study included 5 experimental groups with 5 to 6 animals assigned to each group. Each experimental group corresponded to one scaffold condition and two loading cases. For scaffold conditions, we implanted either CF, CS or OCS scaffolds bilaterally, while for the mechanical loading, we defined three cases: early, delayed and no external mechanical loadings. In each experimental group, one leg of each rat was subjected to either an external early or an external delayed mechanical loading while the other leg received no specific external mechanical loading. In the early mechanical loading case, the application of the mechanical loading started 2 days post-implantation, while in the delayed mechanical loading case the mechanical loading started 14 days post-implantation. A longitudinal in vivo micro-computed tomography (microCT) imaging follow-up was performed to evaluate the bone formation inside the different scaffold conditions and loading cases.

2.3. Animal model and surgical procedure

The animal model and surgical procedure were used as described elsewhere (Hausherr et al., 2017; Kettenberger et al., 2014). Briefly, female Wistar rats (280–300 g, licence N° 2631.0, EXPANIM, SCAV, Epalinges, Switzerland, provided by Janvier Labs, Saint-Berthevin, France) were anesthetized with Isoflurane (Piramal Entreprise Ltd., Bombay, India) and their legs shaved. Before the surgery, they were injected subcutaneously with Buprenorphine (0.03 mg/kg/day, Temgesic®, Reckitt Benckiser AG, Wallisellen, Switzerland) as analgesic and their eyes were covered with tears fluid (Viscotears®, Alcon, Forth Worth) to avoid eye drying. Prior to scaffold implantation, one leg was put in a flexed position to fix and stabilize the knee. After skin incision and muscle fascia splitting, a hole measuring 3 mm in diameter and 3 mm in depth was drilled in the lateral side of the femoral condyle using a motorized dentist's drill (DEC 100, Nobelcare, Sweden). Bone and blood remaining in the hole were rinsed with 0.9% NaCl solution (B. Braun) and removed with a surgical aspiration, followed by scaffold implantation. The scaffold (CF, CS or OCS) was press-fitted inside the drilled hole before muscles and skin were closed. The same surgical intervention was done on the contralateral femur of each animal. As post-operative care, the rats were injected with Buprenorphine (0.03 mg/kg/day, every 8 h for 48 h, Temgesic®) and paracetamol (Dafalgan 500 mg effervescent tablet, UPSA Bristol-Myer Squibb SA, Barr, Switzerland) was added to the drinking water for one week. The rats were euthanatized with an intracardiac Pentobarbital (< 200 mg/kg, Esconarkon, Streuli Pharma SA, Uznach) injection 12 weeks after scaffold implantation.

2.4. In vivo mechanical loading

After the surgery, either external early or delayed mechanical loading protocols were applied depending on the experimental group. In both cases, one leg of each rat received an external controlled mechanical loading for 5 min (10 N at 4 Hz, every two days over a period of 9 days) using a machine design based on previous studies (De Souza et al., 2005; Fritton et al., 2005; Stadelmann et al., 2009). The contralateral leg of the rat was used as a control (no loading during the 5 min sessions). The loading parameters were based on studies described elsewhere (Roshan-Ghias et al., 2010, 2011). For both loading cases, the rats were kept under anaesthesia during the loading sessions and were free to move between the sessions.

2.5. In vivo microCT imaging and data analysis

A longitudinal in vivo microCT imagings of both femurs at 6 time points (2, 4, 6, 8, 10 and 12 weeks after scaffold implantation) was performed using a SkyScan 1076 scanner (Bruker microCT, Kontich, Belgium), except for the OCS scaffold experimental group at week 6 due to a source breakdown of the microCT. Each leg was scanned separately and introduced in a plastic tube to stretch and fix the leg during scanning. The scanning parameters were the same for all scans (pixel size: 18 μm, filter: 0.5 mm aluminium, voltage: 80 kV, current: 120 μA, exposure time: 360 ms, rotation step: 0.5°). The chosen scan frequency had no impact on the structural bone parameters as described elsewhere (Brouwers et al., 2007).

The 2D reconstruction (ring artefact: 4, beam hardening: 20%, no smoothing) was done using NRecon software (Brukuer microCT), followed by the selection of the volume of interest (VOI) on 3D reconstructed datasets of each leg on Amira® (FEI Visualization Sciences Group, Burlington, USA). As the scaffold was not visible in microCT images because of its low absorption values, the VOI was selected as a cylinder with the same dimension than the scaffold (3 mm diameter and 3 mm high). In the present study, we were interested to compare the

different conditions in the process of bone healing. We have then taken as a reference point, the initial situation in the scaffold. We found this approach more contrasting than taking as a reference, a region in the uninjured femora. Accordingly, the datasets of the first time point (week 2) of each leg were reconstructed in Amira®, then manually placed around the VOI and its new coordinate system was saved to the one of the VOI. Using a custom script adapted from a published script (Kettenberger et al., 2014), the following datasets (weeks 4, 6, 8, 10 and 12 of the same leg) were then loaded to Amira® for registration and segmentation. The segmented images were finally analysed by CTan software (Bruker microCT) to quantify bone volume fraction (BV/TV),

2.6. Histology

The implanted femoral condyles were harvested and dehydrated in a graded ethanol (EtOH, VWR, Dietikon, Switzerland) series, cleared by two subsequent toluene (VWR) baths and embedded by infiltration with methylmethacrylate (MMA, 100 mL, Sigma-Aldrich, ST Louis, USA) and 0.5% bis(tert-butylcyclohexyl) peroxydicarbonate (Perkadox 16, Dr. Grogg Chemie AG, Deisswil, Switzerland) as described elsewhere (Hausherr et al., 2017; Kettenberger et al., 2014). The embedded samples were then sliced in the sagittal plane into sections of 180 µm thickness, attached to custom-made opaque OMMA microscope slides (Semadeni, Ostermundingen, Switzerland) and ground to approximately 80–100 µm thickness. The sections were stained with 0.1% toluidine blue (VWR) used to perform semi-quantitative and qualitative evaluations.

2.7. Statistical analysis

Statistical analysis within the different scaffold groups (CF, CS and OCS) and loading cases (early, delayed and no external mechanical loading) were performed using a series of flexible and powerful statistical models called Generalized Additive Mixed Models (GAMMs) as described elsewhere (Roshan-Ghias et al., 2011; Wood, 2007). The GAMMs, based on original values, use a semi-parametric model to capture the highly non-linear trend of the response variable in time. Outliers were excluded from the analysis when their value was outside of an interval of 1.5 times the quartile range. For each condition (e.g. CF scaffold with early loading), a curve is adjusted to the response variable to obtain a flexible model, capable of capturing the trend of the response variable in time. Based on the obtained predicted curves and at each time point, the difference between predicted values for two conditions (e.g. CF under an early mechanical loading vs CF without mechanical loading) were assessed using the confidence interval of predicted values of adjusted curves. The confidence interval was constructed to have coverage of 95% (equivalent to $\alpha = 0.05$). The grey zone on the graphics represents the regions in which two predicted values are well separated from each other. As the analysed datasets are from a longitudinal study, the advantage of GAMM, compared to standard statistical tests, is that the significant difference can be detected for a time frame and not only for individual time points, with an error margin of 5%. Concerning the statistical analysis of the histology slides, a student *t*-test was carried out on the semi-quantitative evaluation. A p-value of < 0.05 was considered as significant. All statistical analysis were done in R (R Development Code Team, 2010).

3. Results

3.1. MicroCT based static histomorphometry

We observed a gradual increase of BV/TV over the entire duration of the study in all scaffold conditions and loading cases (Fig. 1). Each graph depicts a pairwise comparison between early or delayed mechanical loading and no loading cases for either CF, CS, or OCS scaffolds, namely the loading cases of each experimental group. Significant differences between mechanical loading cases of the same experimental group are highlighted in grey in the figures. Statistical analysis of BV/TV between loading cases and scaffold condition of different experimental groups are detailed in Table S1 (see Supplementary materials). The results show that BV/TV inside CF scaffolds for all loading cases, namely no loading, early and delayed loading, is significantly higher than inside CS or OCS scaffolds (Fig. 1), which has already been observed after the first scanning session at week 2. At week 12, BV/TV was 36% lower inside CS scaffolds than inside CF scaffolds under early mechanical loading, and 57% lower when no loading was applied. When comparing BV/TV inside CF scaffolds to OCS scaffolds, a decrease of 58% for early mechanical loading and a loss of 28% under no loading cases was observed. Regarding the results between CS and OCS scaffolds, we noticed an inverse effect of the early mechanical loading and the no loading cases. The negative impact of early mechanical loading on OCS was an indication to exclude it from the study of delayed loading in order to reduce the number of used animals. To further support this decision, we also observed from the group cell-seeded scaffold that the delayed loading induced a less potent reaction than the early loading. In the case of CS condition, early mechanical loading had a positive effect and started to significantly increase BV/TV from week 6 until week 12, which was improved by 48% than in the no mechanical loading case. In contrast, early mechanical loading had a negative impact inside OCS scaffolds as a significant lower BV/TV was observed starting from week 6 to week 12. Indeed, after 12 weeks of implantation, 41% less BV/TV was observed when early mechanical loading was applied. The only condition where early mechanical loading had no impact on the bone formation was in the CF scaffold condition. In the case of delayed mechanical loading, the amount of formed bone inside both CF and CS scaffolds increased significantly as shown in Fig. 1. In the case of CS scaffolds, the significant increase started from week 8, while for CF scaffolds, it began from week 5. After 12 weeks of implantation, the bone formation increased by 26% inside CS scaffolds and of 32% inside CF scaffolds when delayed loading was applied compared to no loading.

3.2. Histological analysis

Representative histological slides of CF, CS and OCS scaffolds under no and early mechanical loading are depicted in Fig. 2, whereas CF and CS scaffolds subjected to no and delayed mechanical loading are shown in Fig. 3. Some more detailed histological close-up views of the different scaffold conditions and loading cases are presented in Fig. S1–S5 (see Supplementary materials), whereas the corresponding semi-quantitative and qualitative evaluations are summarized in Tables S2 and S3 respectively (see Supplementary materials).

With respect to the maturity of the remodeled bone and bone marrow inside CF scaffolds, we noticed a degree of bone maturity like the one of surrounding bone independently of the loading cases (see Figs. 2 and 3 and Fig. S1 and S4). Concerning the bone maturity inside CS scaffolds, immature to mature bone was observed under early and delayed mechanical loading cases, whereas in the case of no loading less mature bone was observed (see Figs. 2 and 3). We noticed less active bone marrow in the early and delayed loading cases and no bone marrow formation in the no mechanical loading case (see Fig. S2 and S5 and Table S2). In OCS scaffolds, independently of the mechanical loading cases, no bone marrow activity or few fatty structures were observed (see Fig. 2). Few mature areas of bone tissue and some immature bone were noticed (see Fig. S3 and Table S2). The less mature state of the bone inside CS and OCS scaffolds compared to CF scaffolds is in accordance with the higher number of observed blood vessels and the higher number of observed active osteoblasts (Table S2). As the bone is still in a modelling phase, a higher osteoblast activity is needed as well as more nutrient and oxygen supply, represented by a higher number of blood vessels present.

Furthermore, in between different scaffold conditions and

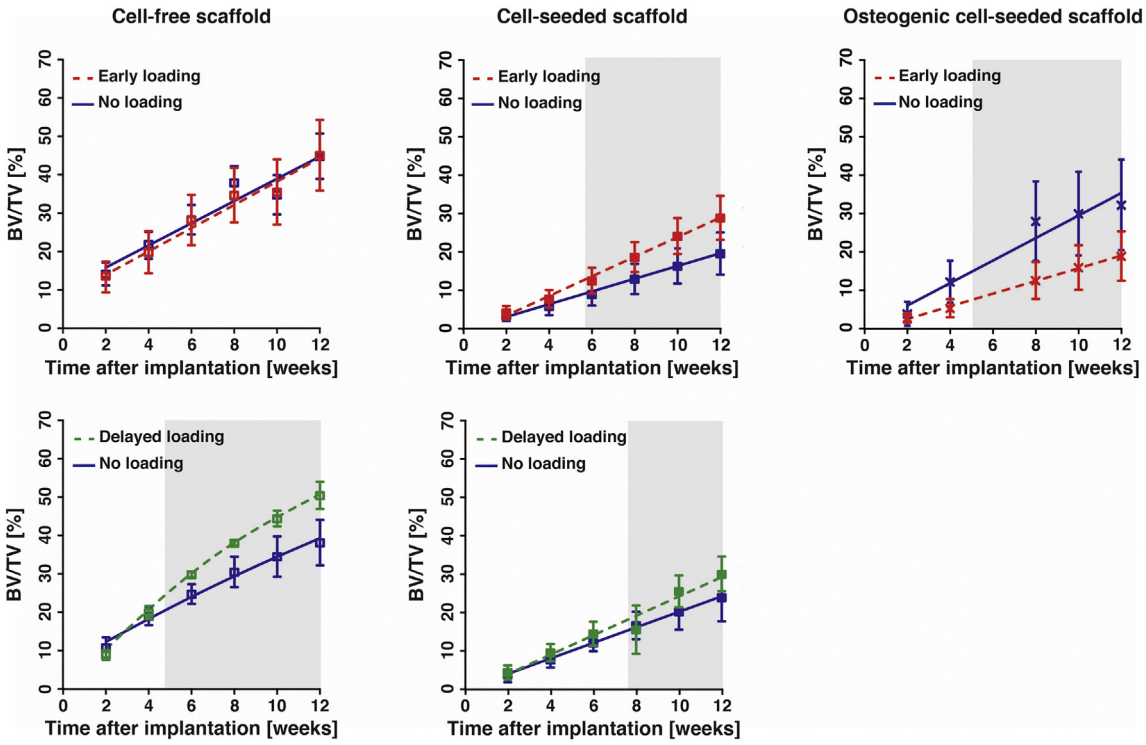

Fig. 1. Bone volume over tissue volume (BV/TV) evolution inside different scaffold conditions and loading cases over 12 weeks. CF scaffolds are represented by empty squares, CS scaffolds are shown by filled squares, and OCS scaffolds by crosses. The lines represent the fitted GAMM model. The no loading groups are illustrated by solid blue line while the early and delayed mechanical loading groups are drawn in dashed red and dashed green lines respectively. The grey zone on the graphics shows the time span where a significant difference exists between two groups (n = 5–6). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

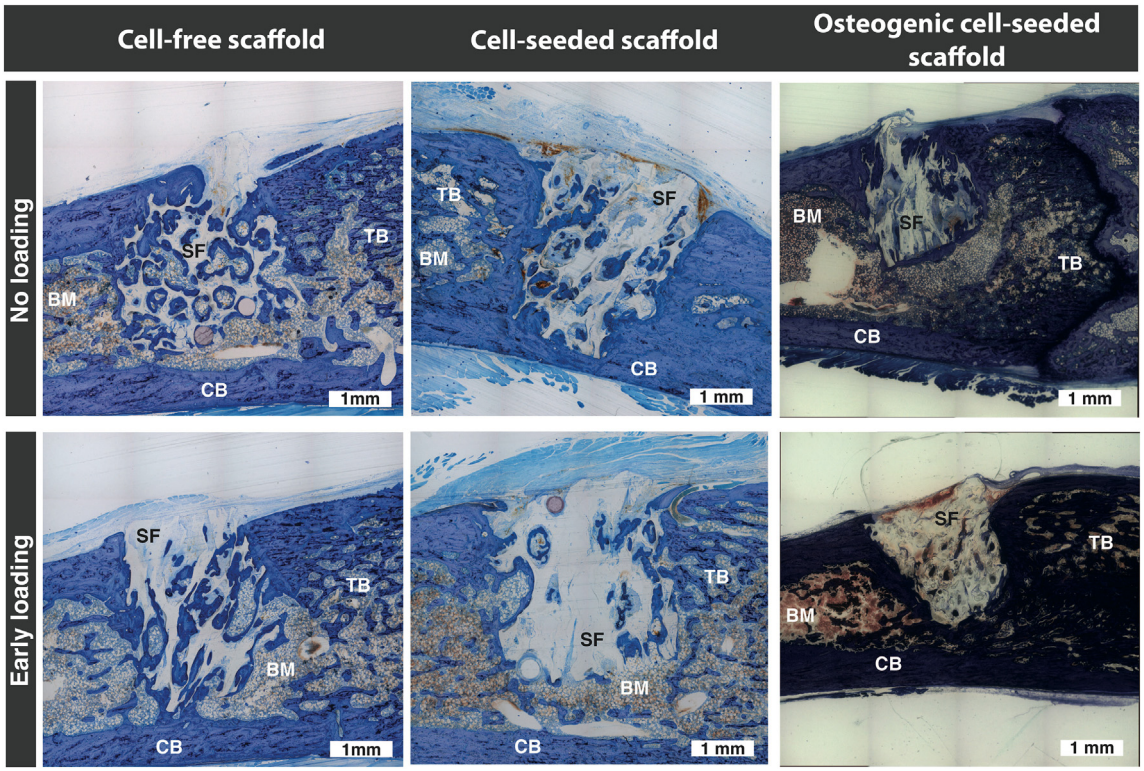

Fig. 2. Toluidine blue stained sections of CF, CS and OCS scaffolds in no and early mechanical loading cases after 12 weeks of implantation. No loading case shown on the top of the figure, early mechanical loading case illustrated at the bottom for the three scaffold conditions. The different tissues and the scaffolds are shown using the following abbreviations: trabecular bone (TB), cortical bone (CB), bone marrow (BM) and scaffold (SF). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

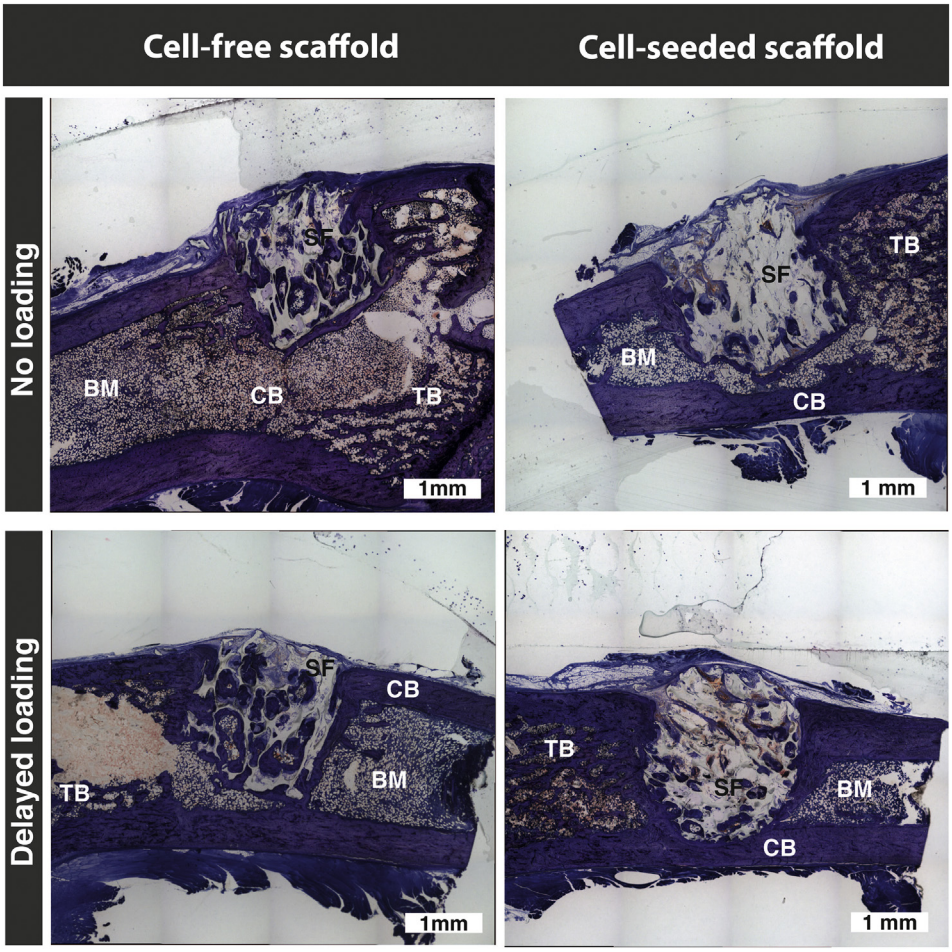

Fig. 3. Toluidine blue stained sections of CF and CS scaffolds in no and delayed mechanical loading cases after 12 weeks of implantation. No loading case shown on the top of the figure, delayed mechanical loading case illustrated at the bottom for both scaffold conditions. The different tissues and the scaffolds are shown using the following abbreviations: trabecular bone (TB), cortical bone (CB), bone marrow (BM) and scaffold (SF). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mechanical loading cases, no difference was found for bone-scaffold interface, bone reaction and the qualitative evaluation of the interstitial tissue (Table S3). A direct and clear bone-scaffold contact was noticed in the three scaffold conditions. The trend for both semi-qualitative and quantitative evaluation confirms the results obtained by microCT scanning.

4. Discussion

In our study, we first observed a significantly lower bone formation inside CS and OCS scaffolds than inside CF scaffolds, independently of the loading cases. This difference began already after 2 weeks of implantation, which resulted in a delay of bone formation in CS and OCS scaffolds. Indeed, the equivalent amount of formed bone after 6 weeks in CF scaffolds was reached after 12 weeks post-implantation inside CS scaffolds. Furthermore, the histological evaluation confirmed the observed delay and also showed an advanced maturation of newly formed bone and bone marrow inside CF scaffolds compared to CS and OCS scaffolds. A similar difference in maturity status was noticed between CS and OCS scaffolds; less bone marrow activity and more immature bone were observed in OCS scaffolds compared to CS scaffolds.

In general, a higher positive effect on bone formation has been demonstrated when bone affiliated cells were used (Boerckel et al., 2012; Corre et al., 2015; Dupont et al., 2010; Jäger et al., 2007; Liu et al., 2013; Montjovent et al., 2008; Srouji and Livne, 2005; Xu et al., 2010; Yasko et al., 1992), which is in contradiction with our results. What may be responsible for these differences in the final outcomes on bone formation are the variations in technical and experimental designs. The major contrasts between the described aforementioned studies and the one presented here are the different scaffolds used (PLA/5% β-TCP, hydrogels, porcine collagen combined with TCP, to name but a few), cell fate (BPCs, amniotic fluid stem cells, stromal MSCs), cell origin (human, allogeneic or syngeneic), defect type (partial or large), implantation site (femoral, tibial or cranial), immune status of the animals (immuno-competent or immuno-deficient) or the use of growth factors such as BMP-2 (lack or addition). Finally, it is also possible that the lower bone formation observed in cell-seeded scaffolds compared to cell free scaffolds is only transient as the initial immature bone formed in the seeded-scaffold could be remodeled. We observed this dynamical situation of bone formation and resorption in scaffold (Roshan-Ghias et al., 2011).

It should be noted that in one of our previous studies (Montjovent et al., 2008), we investigated the PLA/TCP implant resorption in cranial and femoral sites. We measured in the similar femoral site as in the present study, slight alterations of the implant structures at 2 months. The effect of scaffold degradation should then not play a critical role within the time frame of the present study.

We also noticed in our study different bone formation behaviours inside CF, CS and OCS scaffolds when subjected to early or delayed mechanical loading instead of no loading. In the case of CF scaffolds, no difference was observed between early mechanical loading and no loading cases, for which the amount of newly formed bone reached

45%. In the same scaffold conditions and loading cases, Roshan-Ghias et al. (Roshan-Ghias et al., 2010) have shown a significant but moderate increase in the amount of formed bone after 13 weeks of implantation, when early mechanical loading was applied instead of no loading. Our results did not suggest such a trend after 12 weeks of implantation. This difference could be explained by the improvement of surgical techniques (fixation of leg, automated drilling system, etc.) and different surgical operators. In the present study only one loading regime was used (but applied either early or delayed after scaffold implantation) as it has been demonstrated in previous studies to favourably increase bone formation in scaffold (Roshan-Ghias et al., 2010, 2011). We cannot exclude that other loading regimes would have been more favourable for bone formation in the different tested scaffold conditions. For the sake of reducing the number of animals and complexity in the present study, we did not investigate the effect of regime loading.

When early mechanical loading was applied to CS scaffolds, it increased the amount of formed bone, which was expected. Due to their pluripotency, hBPCs have similar capacities to differentiate into osteoblasts under certain mechanical and environmental conditions as that of MSCs. It has been demonstrated several times that MSCs or bone marrow-derived MSCs appeared to be osteogenic when they were grown on firm gels that mimic pre-calcified bone (Discher et al., 2009; Engler et al., 2006). These mechanical sensitive cells have also shown expression of early osteogenic markers with an increased mineralized matrix deposition when subjected to cyclic tensile or compressive loading (Delaine-Smith and Reilly, 2012; Haudenschild et al., 2009; Mauney et al., 2004; Sumanasinghe et al., 2006). Early mechanical loading surely induced the secretion of these osteogenic markers by hBPCs in vivo, which resulted in an increase of bone formation.

In contrast, we found that early mechanical loading had a negative effect on OCS scaffolds and significantly decreased bone formation. A potential explanation can be suggested by two in vitro studies (Kadow-Romacker et al., 2009, 2013). In these studies, Kadow-Romacker et al. have demonstrated that small changes in duration of frequency of mechanical stimulations had significant consequences on the behaviour of osteoblast- and osteoclast-like cells in single or co-culture conditions. In both studies, a three-point bending mechanical stimulation with different frequencies and durations was applied on osteoblast- and osteoclast-like cells either in single or in co-culture on dentin slices. Under the same stimulation condition, single culture of osteoclast-like cells did not influence the resorption activity, whereas in co-culture with osteoblast-like cells an increase of resorption activity was observed. Based on these studies, one can propose that not only the duration and frequency of mechanical loading affect cell behaviour, but also the cell type and the interaction with other cell types are as well crucial. Making the parallel between *these* in vitro studies and our in vivo study presented here, we can hypothesize that the osteogenic cell fate of hOBPCs, which received the same early mechanical loading than implanted hBPCs, interacted differently with its surrounding tissue under this specific mechanical loading case. This activity therefore resulted in a decrease of bone formation compared to the no loading case.

We also analysed the effect of delayed mechanical loading on CF and CS scaffolds. Here as well a significant higher bone volume inside CF scaffolds was observed compared to CS scaffolds, independently of the loading cases. This difference started 5 weeks post-implantation, which follows the same trend published elsewhere (Roshan-Ghias et al., 2011). As for the study with early mechanical loading, the histological analysis is in accordance with the previously described observation based on BV/TV for scaffold conditions and loading cases.

The different effects of early or delayed mechanical loading on CF, CS or OCS scaffolds could be explained by the absence or presence of different cell types or cell density as described elsewhere (Hausherr et al., 2017). In this previous work, the same scaffold conditions and implantation sites were used to study the immunological reaction triggered by hBPCs and hOBPCs early after scaffold implantation. The histological analysis showed that after 3 days of implantation, the pores of CF scaffolds were empty or filled with red blood cells, whereas the pores of CS or OCS scaffolds were partially colonized with hBPCs or hOBPCs and extracellular matrix. Furthermore, Hausherr et al. also analysed the tissue content of the scaffolds after 14 days of implantation. They showed that CF scaffolds were filled with immature and some mature bone tissue, whereas CS and OCS scaffolds were filled with fibrous tissue, immature bone tissue (Hausherr et al., 2017). One limitation of the present study is the lack of quantification of the cell fate in the scaffolds after implantation. Indeed in our previous study (Hausherr et al., 2017), we observed that both hBPCs and hOBPCs were still on site after 4 and 14 days of implantation in vivo, respectively. We considered then that the same situation arose in the present study as the cells and scaffolds were identical between the two studies.

The biological materials were therefore different between CF, CS and OCS scaffolds when subjected to early and delayed mechanical loadings. As both loading cases were transmitted by fluid flow to the cells present inside scaffolds, the different amount of formed bone inside CF, CS and OCS scaffolds might reflect the secretion of different growth factors and signalling molecules of host or xenograft cells to the surrounding tissue.

5. Conclusion

In this work, we combined for the first time human bone progenitor cells seeded within scaffolds and mechanical loading applied at different time points after implantation. Our goal was therefore to evaluate the effect on the amount of newly (or neo)bone inside scaffolds seeded either with human bone progenitor cells or osteogenic induced human bone progenitor cells compared to cell-free scaffolds when early, delayed and no externally mechanical loading cases were applied.

In the limitation of our study, we found that the cell-free scaffolds represent a relevant alternative for bone tissue engineering independently of the mechanical loading case, to cell-seeded and osteogenic cell-seeded scaffolds. We noticed that early mechanical loading had an equal effect on bone formation inside cell-free scaffolds as no loading, but it improved significantly the bone formation inside cell-seeded scaffolds, while decreasing it significantly inside osteogenic cell-seeded scaffolds. In the case of delayed mechanical loading, bone formation inside cell-free scaffolds increased significantly as well as in cell-seeded scaffolds.

To conclude, cell-free scaffold combined with delayed loading was finally the best option in terms of qualitative bone regeneration in this study.

Transparency document

The http://dx.doi.org/10.1016/j.bonr.2018.04.008 associated with this article can be found, in online version.

Authors' roles

The study was conceived by DP, LA and TH and the experiments were designed by TH. Surgical procedures were performed by ET, SW and TH. The data analysis of the microCT evaluation and statistical analysis was done by TH. The histological evaluation was performed by KN. The paper was written by DP and TH and the final manuscript was approved by all of the authors.

Conflict of interest

All authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bonr.2018.04.008.

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