Study of the involvement of allogeneic MSCs in bone formation using the model of transgenic mice

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ABSTRACT
Mesenchymal stem cells (MSCs) are thought to be the most attractive type of cells for bone repair. However, much still remains unknown about MSCs and needs to be clarified before this treatment can be widely applied in the clinical practice. The purpose of this study was to establish the involvement of allogeneic MSCs in the bone formation in vivo, using a model of transgenic mice and genetically labeled cells. Polylactide scaffolds with hydroxyapatite obtained by surface selective laser sintering were used. The scaffolds were sterilized and individually seeded with MSCs from the bone marrow of 5-week-old GFP(+) transgenic C57/Bl6 or GFP(−)C57/Bl6 mice. 4-mm-diameter critical-size defects were created on the calvarial bone of mice using a dental bur. Immediately after the generation of the cranial bone defects, the scaffolds with or without seeded cells were implanted into the injury sites. The cranial bones were harvested at either 6 or 12 weeks after the implantation. GFP(+) transgenic mice having scaffolds with unlabeled MSCs were used for the observation of the host cell migration into the scaffold. GFP(−) mice having scaffolds with GFP(+) MSCs were used to assess the functioning of the seeded MSCs. The obtained data demonstrated that allogeneic MSCs were found on the scaffolds 6 and 12 weeks post-implantation. By week 12, a newly formed bone tissue from the seeded cells was observed, without an osteogenic pre-differentiation. The host cells did not appear, and the control scaffolds without seeded cells remained empty. Besides, a possibility of vessel formation from seeded MSCs was shown, without a preliminary cell cultivation under controlled conditions.

KEYWORDS bone formation; fluorescence imaging; GFP; Mesenchymal stem cells; transgenic mice

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Introduction
The repair of architecture, function and retention of the normal shape of bone tissues continues to be a special problem for tissue engineering. Bone defects in the head and face due to trauma, tumor removal or congenital abnormalities leave patients with reduced tissue function and also render them psychologically scarred. Currently, the standard approach for the treatment of critical size bone defects is to use an autologous graft. However, this approach is limited by the amount of available donor tissue and inevitably entails a second injury site, resulting in additional trauma and associated risks. There is an alternative method to treat bone defects, based on the so-called tissue engineering triad: cells, scaffolds and growth factors. This cell approach consists in the preliminary seeding of cells onto scaffolds before these matrices are implanted in the defect area. Several cell types can potentially be used as a cellular material, but mesenchymal stem cells (MSCs) have been considered the most attractive type for such a bone replacement. Mesenchymal stem cells, also known as multipotent mesenchymal stromal cells, are self-renewing cells which can be easily isolated from a small aspirate of bone marrow or adipose tissue and then expanded to clinical scales in vitro. However, the bone marrow and adipose tissue are not the only sources of MSCs. The large number of sources of MSC-like cells in the adult body is one of the reasons why these cells are so attractive. MSC-like cells have been identified in the dental pulp, skin, umbilical cord blood, placenta, liver and lungs. Under certain in vitro conditions MSCs are capable of differentiating into osteoblasts, adipose cells, cartilage cells, skeletal muscle cells and even into neuron-like...
cells. MSCs lack expression of the major histocompatibility complex I and of costimulatory molecules like CD40, CD80, and CD86, which makes them largely non-immunogenic. Furthermore, these cells are capable of suppressing the immune system via still unknown mechanisms, thus, the treatment with such allogeneic cells is largely immunoinert. Unlike the case with embryonic stem cells the MSC treatment presents a lower risk of the development of malignancy and also allows avoiding the ethical issues. Currently, several approaches to the MSC treatment have been used to promote bone regeneration in animal models. However, an enormous amount of knowledge about MSCs is still missing, and this needs to be resolved before such a treatment can be widely applied in clinical situations. In particular, the opinions concerning the roles of seeded MSCs on the scaffolds remain very controversial. On the one hand, it has been suggested that seeded MSCs may be capable of releasing major growth factors, reducing the innate immune response and mobilizing the host’s cells. On the other hand, since these cells can differentiate into osteoblasts, they may contribute directly to the bone formation. Nevertheless, the in vivo results are still unclear despite many in vitro experiments that show excellent results concerning the expansion, proliferation, migration, viability and osteogenic differentiation of MSCs on different types of scaffolds. The involvement of seeded cells in the stimulation and promotion of bone regeneration in vivo remains one of great uncertainties in the MSCs studies. It is not completely understood what happens to MSCs seeded onto scaffolds when they are implanted into a bone defect, and whether they are involved directly in the bone formation. The purpose of the present study was to establish the involvement of seeded allogeneic MSCs in the bone formation in vivo, using a model of transgenic mice and genetically labeled cells.

Materials and methods

3D scaffolds

3D scaffolds made from poly(D,L)-lactic acid with hydroxyapatite (HA), obtained by surface selective laser sintering (SSLS), were used. One of the main reasons for the choice of these scaffolds was the absence of their autofluorescence. SSLS is a technique of rapid prototyping that allows fabrication of 3-dimensional bioactive and biodegradable scaffolds with precise dimensions and intricate structures (the spatial resolution is ~200 μm). Fabrication of scaffolds using this method is based on a layer-by-layer sintering in accordance with a pre-set program. An important step in the process is the sintering that results from the laser-induced melting of the interface layers between the 2 types of free polymer particles. The details of the processing methodology can be found in refs. In brief, powdered polylactide (with the particle size of ~200 μm) and nanoscale HA (20%) were mixed and sintered using an SLS-100 selective laser sintering device (Institute of Laser and Information Technologies, Russia). A single-mode fiber laser (IRE-Polyus, Russia) was used as a source of irradiation. The emission wavelength was 1.06 μm, the laser power was ≤10 W. As a sensitizing agent, carbon (0.1 % by weight, with the particle size of ~100 nm) had also been added to the polylactide powder. The matrices appeared as discs with the diameter of 4 mm, the height of 0.6 mm, and the porosity of 60%. A uniform distribution of HA inside the scaffolds was confirmed by Raman spectroscopy. The internal structure of the scaffolds was observed by scanning electron microscopy (Fig. 1).

Cell cultures

MSCs were isolated from the tibial and femoral bone marrow of male 5-week-old GFP(+) transgenic C57/Bl6

Figure 1. Internal structure of SSLS-scaffolds (scanning electron microscopy, LEO 1450, Carl Zeiss, Germany); (A) bar 1 mm; (B) bar 200 μm.
and normal GFP(−) male C57/Bl6 mice. The cell suspensions were centrifuged, mixed with a full growth medium (MesenCult™ MSC Basal Medium (Mouse) with MesenCult™ Stimulatory Supplements Mouse) supplemented with 0.58 mg/mL L-glutamine (PanEco, Moscow, Russia), and 40 U/mL gentamicin, and plated on culture flasks. After 2 days, non-adherent cells were removed by washing with phosphate buffered saline (PBS), and the monolayers of adherent cells were cultured until they reached confluence. Then, the MSCs were detached (ACCUTASE™ cell detachment solution) and subcultured. Culturing was conducted under the standard conditions (37°C, 5% CO2, saturation humidity). The cells were immunophenotypically characterized by flow cytometry with a Cell Lab Quanta SC instrument (Beckman Coulter, Brea, CA, USA) for the markers typical for the murine bone marrow MSCs (CD44, CD45, CD90). Besides, to demonstrate the ability for osteogenic differentiation, the cells were cultivated in an osteogenic medium (MesenCult™ Osteogenic Stimulatory Kit Mouse) for 21 days and stained with Alizarin Red S (Sigma Aldrich, USA) to detect calcium deposits.

In vitro pre-implantation scaffold analysis
The scaffolds were sterilized, individually seeded (2 × 10^5 cells/scaffold) with MSCs in the third passage and cultured for 3 days. The GFP(+)MSC fluorescence was detected by fluorescence microscopy to confirm that the cells had become attached. To demonstrate the cells’ viability before implantation into the mice, several scaffolds with MSCs were stained with the specific fluorescent dyes: Calcein/Propidium Iodide (Live/Dead Cell Double Staining Kit, Sigma Aldrich, USA). First, an assay solution was prepared according to the Sigma Aldrich protocol. Then the scaffolds carrying MSCs were washed with PBS, placed into the assay solution and incubated at 37°C for 20 min. After the staining, the fluorescence of the cells was detected using a fluorescence microscope.

Surgical procedure
All in vivo experiments were approved by the Ethics Committee of the Nizhny Novgorod State Medical Academy (Nizhny Novgorod, Russia). The experiments were performed on male GFP(+) transgenic C57/Bl6 mice and normal GFP(−) male C57/Bl6 mice. The mice were anesthetized with Zoletil at a concentration of 80 mg/kg. 4-mm-diameter critical-size defects were created on the calvarial bone of each animal using a dental bur. Immediately after the generation of the cranial bone defects, the scaffolds with, or without seeded cells were implanted into the injury sites. The skin was then closed.

Experimental design
To study the involvement of the seeded MSCs in the bone repair, 2 models were used (Fig. 2). GFP(+) transgenic mice having scaffolds with unlabeled MSCs were used for observation of the host cell migration into the scaffold. GFP(−) mice having scaffolds with GFP(+) MSCs were used to assess the functioning of the seeded MSCs. The control group comprised GFP(+) transgenic mice having scaffolds without any cells. Each group consisted of 3 mice.

Figure 2. Experimental design and mouse models.
In vivo fluorescence imaging of bone formation

The cranial bones were harvested at either 6 or 12 weeks after the implantation. All samples were stained with the Hoechst dye and observed using fluorescence microscopes. All light and fluorescence imaging was acquired using an Axio Zoom V16 (Carl Zeiss, Germany) fluorescence stereo zoom microscope and a Leica DM IL LED Fluor (Germany) inverted fluorescence microscope. For imaging the Calcein, Propidium Iodide (PI) and GFP an I3 filter cube (Germany) was used. The Hoechst preparation was observed using an A4 filter cube (Germany).

Histology and immunohistochemistry

The cranial bones were fixed in 10% formalin solution and decalcified with nitric acid for 2 weeks. Then the samples were dehydrated in an ascending ethanol series before being embedded in paraffin wax. 5 μm sections were cut using a microtome (Leica SM 2000; Germany) and mounted on glass slides. 10 cross-sections from the middle of each implant were stained with Van Gieson picrofuchsin. The content of the endothelial cells was confirmed by immunohistochemical staining of monoclonal antibodies to CD31 (Abcam, USA). Visualization of the immune complexes was performed using the peroxidase method with a Mouse UnoVue HRT/DAB detection system (Diagnostic BioSystems).

Statistical analysis

The areas of different implant components in the histological sections after 6 and 12 weeks were evaluated with the “Area” parameter using ImageJ 1.43u software (National Institutes of Health, USA). The mean values (M) and the standard deviations (σ) were calculated for 15 fields of view in each group. The Student’s t-test and the one-way ANOVA with Fisher’s post-hoc test were used to compare the data (P ≤ 0.05 was considered statistically significant).

Results

Characterization of MSCs

It was shown that the cells isolated from the bone marrow of GFP(+) and GFP(−) mice had the features of MSCs. During their expansion into monolayers, the cells exhibited a spindle-shaped and fibroblast-like morphology (Fig. 3A). The adherent cells were positive for CD44, CD90 (>85%) and negative for CD45 (<5%). MSCs isolated from the GFP(+) transgenic mice exhibited a strong green fluorescence of GFP (Fig. 3B). The culture-expanded MSCs also maintained their ability for osteogenic differentiation. After 21 days of cultivation in the osteogenic medium, very extensive calcium deposits were detected in the plate with such cells (Fig. 3C).

3D-cultivation and viability analysis of the cells on scaffolds

The cells in the third passage were applied to both sides of the scaffolds to speed up the process of penetration and then cultured for 3 days. Before their implantation into the mice all scaffolds were checked as follows. The fluorescence of GFP(+)MSCs on a scaffold was confirmed (Fig. 4A and B). To avoid overlapping of the emission spectra of GFP and Calcein, 3 randomly selected scaffolds with GFP(−)MSCs were stained with Calcein/PtdIns. This viability analysis demonstrated the

![Figure 3. Characterization of MSCs: (A) MSCs attached to a culture flask (light microscopy); (B) GFP(+)MSCs (fluorescence microscopy, excitation filter: BP 450–490, dichromatic mirror: 510, suppression filter: LP 515); (C) calcium deposits in MSC culture (light microscopy). Bar 100 μm.](image-url)
absence of necrotic zones. The stained scaffolds showed structures filled with viable cells (Fig. 4 C and D).

**Scaffold and bone formation monitoring in critical-size defects**

Non-healing, full-thickness defects of 4 mm in the diameter were made in the cranial bone and filled with the scaffolds. This type of a calvarial defect cannot heal spontaneously for the bone healing period used in our experiments. The cranial bones of the experimental and control groups were harvested and assayed at either 6 or 12 weeks after the scaffold implantation.

Fluorescence microscopy showed that, after 6 weeks, seeded GFP(+) and GFP(−) MSCs were represented in the scaffolds (Fig. 5A and B). Moreover, the scaffolds had only allogeneic cells. This was indicated by the lack of GFP(+) cells on the Hoechst-stained scaffolds with GFP(−) MSCs in the GFP(+) transgenic mice (Fig. 5B). The histological images of the scaffolds with seeded GFP (+) and GFP(−) cells indicated that the scaffolds retained their porous structure and the small black particles of the carbon sensitizing agent. No significant amount of the polymeric material could be detected. There was a large amount of a coarse-fibered connective tissue (about 50%) in the scaffolds of all groups (Table 1). A thin layer of a newly formed bone was found at the bottom of the scaffolds with GFP(−)MSCs (Fig. 5E).

The control scaffold (without seeded cells) in the GFP(+) transgenic mice remained empty (Fig. 5C), the host cells could not be detected. The histological images of the scaffolds without seeded cells showed that a large proportion of the scaffold material remained unchanged. A coarse-fibered connective tissue was present in large amounts. A newly formed bone was almost absent (Fig. 5F, Table 1).

After 12 weeks, fluorescence microscopy indicated that the scaffolds originally seeded with GFP(+) MSCs contained green fluorescent cells (Fig. 6A). The scaffolds originally seeded with GFP(−) MSCs did not have fluorescent cells, furthermore, no host GFP(+) cells appeared on these scaffolds (Fig. 6B). Thus, the scaffolds with seeded MSCs had only allogeneic cells. The shape of MSCs had changed, which might be related to MSCs’ osteogenic differentiation and bone formation, as indicated by the histological analysis. The histological images

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**Figure 4.** *In vitro* scaffold analysis: fluorescence of GFP(+)MSCs on the scaffolds (A) - bar 1 mm, (B) - bar 250 μm; GFP(−)MSCs on scaffolds stained with Calcein/PI (C) - bar 1 mm, (D) - bar 250 μm; fluorescence microscopy.
of the scaffolds with seeded cells indicated that most of the scaffold material had degraded. Only 8% of the scaffold material was present in the scaffolds with seeded MSCs (Table 1). A thin layer of the scaffold material and the black particles of carbon remained at the edges of the scaffolds. The region of the main part of the scaffolds with seeded MSCs had become filled with a coarse-fibered connective tissue and a newly formed bone (Fig. 6D and E; Table 1).

By contrast, the control scaffold, without seeded cells, had no further changes after 12 weeks as compared to the 6 weeks time point (Fig. 6C). The host cells could not be observed, so these scaffolds without seeded MSCs remained empty. The histological images indicated that the defects containing such scaffolds had only the polymer material, black particles of carbon and a coarse-fibered connective tissue. Bone formation was not found (Fig. 6F, Table 1). No inflammatory cell infiltration around any of the scaffolds was present in the histological images.

Vessel growth on the scaffolds

The early results of fluorescence imaging indicated that newly-formed vessels could be found on the scaffolds by week 12. A vascular network could be seen in the center of the scaffold and at the border of the defect. Fluorescence microscopy for this transgenic mouse model enables us to determine a possible source of these new vessels. In GFP(−) mice, the center of the scaffolds seeded with GFP(+) MSCs had fluorescent vessels (Fig. 7C, D, E). The presence of vessels was confirmed in the light microscopy images and by CD31 immunohistochemical staining specific for endothelial cells (Fig. 7A and B). In GFP(+) transgenic mice the centers of scaffolds seeded with GFP(−) MSCs had non-fluorescent

Table 1. Assessment of the area of different implant components after 6 and 12 weeks.

| Implant components | Area, %   | 6 weeks | 12 weeks |
|--------------------|----------|---------|----------|
| GFP(+)MSCs         |          |         |          |
| bone               | 8.9 ± 1.8**| 65.1 ± 2.9**|
| scaffold           | 33.1 ± 3.1**| 8.4 ± 2.2**|
| coarse-fibered     | 57.8 ± 3.8| 25.8 ± 1.8**|
| connective tissue  |          |         |          |
| GFP(−)MSCs         |          |         |          |
| bone               | 17.2 ± 2.2**| 72.9 ± 3.7**|
| scaffold           | 31.5 ± 3.9**| 8.6 ± 1.7**|
| coarse-fibered     | 49.9 ± 4.0| 18.2 ± 2.6**|
| connective tissue  |          |         |          |
| without seeded     |          |         |          |
| MSCs               |          |         |          |
| bone               | 2.3 ± 0.7| 9.2 ± 2.3|
| scaffold           | 42.5 ± 3.7| 25.8 ± 3.2|
| coarse-fibered     | 53.6 ± 3.3| 63.3 ± 3.5|
| connective tissue  |          |         |          |

*P ≤ 0.05, compared with the “12 weeks” group;
**P ≤ 0.05, compared with the control group (“without seeded MSCs”);
Each number represents M ± σ (n = 15)
vessels that were revealed only with the Hoechst stain (Fig. 7H, I). At the borders of the defects, the ingrowing vessels demonstrated a strong green fluorescence, like all the GFP(-) mouse tissues (Fig. 7H). However, these vessels from different sources were networked and we could detect the formation of anastomoses on the scaffolds (Fig. 7J). The presence of vessels was also confirmed in the light microscopy images and by CD31 immunohistochemical staining (Fig. 7F, G). These results are interpreted as showing the possibility of vessel formation from seeded MSCs.

Discussion

The combination of stem cell therapy and 3D-scaffolds in tissue engineering is a successful and promising strategy for bone regeneration. Many studies report specifically on the application of MSCs to treat critical-size bone defects.6,7,24-26 Grafts based on 3D-scaffolds carrying MSCs for curing bone defects have demonstrated excellent results. Good integration of an implant,27 vascularization of the grafted zone,28 extracellular matrix formation and grafts’ mineralization29 have been demonstrated using different types of scaffolds and seeded MSCs. However, this research is mostly aimed at the final result of bone repair. There are very few experiments in vivo that aim to show the destiny of the seeded MSCs after the scaffold implantation. The latter is a crucial issue, because the behavior of the seeded MSCs in new niches, their proliferation and migration can provide an essential information about the role of this cell type in bone regeneration and in their interactions inside the tissues. We focused directly on the seeded MSCs. There were 2 key points: how long these cells remain on the scaffolds, and what role they have. Many works report that the seeded MSCs do not participate directly in osteogenesis - that they are inducers rather than effectors of bone formation. Seeded MSCs were shown to release some principal osteogenic, vasculogenic and other growth factors at the early stages after implantation and to mobilize the host’s cells.14,15 Seeded MSCs were also found to enable gathering of the host’s monocytes at the implantation site where they later differentiate into osteoclasts, but disappear themselves from the implantation site after 1–2 weeks.16,17 In this work, a model of transgenic mice and genetically labeled cells with a double control (GFP(+)mice/scaffolds with GFP(-)MSCs; GFP(−)mice/scaffolds with GFP(+))MSCs; GFP(+) mice/scaffolds without any MSCs) has been used to study the involvement of allogeneic MSCs in tissue regeneration. This control setup allows separating the seeded and host cells on the scaffolds. Our data have demonstrated that allogeneic MSCs were found on the scaffolds 6 and 12 weeks later. Moreover, by week 12...
there was a newly formed bone tissue from the seeded cells without requiring an osteogenic pre-differentiation. What is more important, the host cells did not appear, and the control scaffolds without seeded cells remained empty. This information correlates with a massive amount of data indicating the ability of seeded MSCs to undergo osteogenic differentiation on scaffolds in vitro and to induce bone formation in vivo. For a deeper understanding of this phenomenon it may be necessary to revisit the structure of the scaffolds. The scaffold design for bone tissue engineering involves many parameters that directly affect adhesion, proliferation, and osteogenic differentiation of the seeded MSCs. It has been shown that the nanoscale topographical features, mechanical properties and micro-/macroscale gradient structures, in addition to the biological domains, are the most important elements in successful bone regeneration. Moreover, nanoscale particles, like HA, in the scaffold structure can significantly modulate the behavior of the seeded cells, including cell adhesion, differentiation and proliferation. Thereby, bone formation from the seeded allogeneic MSCs, as well as the absence of the host cells, may be determined by the scaffold structure.

The difference was also found between the percentage of the newly formed bone in mice with seeded GFP(–) and GFP(+)MSCs. After 6 and 12 weeks the implants with seeded unlabeled MSCs had a higher percentage of the newly formed bone than the implants with

Figure 7. Vessel growth on the scaffolds with seeded GFP(+)MSCs (A, B, C, D, E) and GFP(–)MSCs (F, G, H, I, J): (A, F) vessel growth on the scaffolds (stereo light microscopy); (B, G) corresponding histology section stained for CD31 (light microscopy); (C, H) GFP imaging (fluorescence microscopy); (D, I) Hoechst imaging (fluorescence microscopy); (E, J) combination of GFP and Hoechst channels (fluorescence microscopy). v – vessel. Bar 300 μm.
fluorescent MSCs. The differences were statistically significant. This fact may be potentially attributed to the GFP own properties. GFP is a useful biomarker, widely used in the biomedical research to track stem cells after transplantation. However, measuring the potential GFP impact on the cell engraftment and thus optimizing the strategy for GFP-based tracking in vivo in disease still remains a problem. It has been found that GFP itself may be immunogenic in immunocompetent mice. Thus the fact of a greater bone production by GFP(−) MSCs comparing to GFP(+)MSCs may be directly related to the immunogenic properties of GFP and its impact on the cell behavior and viability.

The second controversial issue is the vessel growth on the scaffolds. Vascularization and mass transfer in grafts present some of the biggest challenges in the field of tissue engineering, because insufficient vascularization and limited diffusion of oxygen, nutrients and waste products can lead to necrosis within large reconstruction zones. There have been 2 main approaches to solve this problem: 1) to implant a type of a repair element, in which the host cells and local microenvironment control in vivo vascularization, 2) to culture the cells under controlled conditions in vitro to develop a functioning vascular network before implantation. Recently one more idea combining both approaches has been suggested. It is based on the anastomosis between in vitro pre-engineered vessels and those of the host, so that the grafts do not have to rely solely on the host vessel invasion. The mechanism, by which this anastomosis occurs between the pre-engineered vessels and the host vasculature, has recently been determined. The pre-engineered vessels surround the nearby host vessels and disrupt the underlying host endothelium, leading to the links between both networks. The approach of pre-vascularization may therefore be more conducive to the survival of the implanted cells. Many different techniques have been used for in vitro pre-vascularization of the bone tissue. These techniques include: introducing endothelial cells into the tissues via 3D multicellular spheroids; simple mixing of different cultures such as fibroblasts, osteoblasts and endothelial cells; addition of angiogenic factors such as VEGF and bFGF. MSCs may also be considered as a promising source for endothelial cells capable of vascular networks creation Bone marrow-derived MSCs predifferentiated by VEGF have been shown to acquire the phenotypic and functional features of endothelial cells and form characteristic capillary-like structures in vitro. G.V. Silva et al. have shown that MSCs applied to the region of a myocardial ischemia in vivo can differentiate into smooth muscle cells and endothelial cells. But despite the great number of studies in vitro and in vivo, the angiogenic potential of MSCs remains controversial. In this study we report preliminary results indicating a possibility of vessel formation from seeded MSCs, without requiring cell cultivation under controlled conditions. This result may be explained by the fact that the differentiation potential of MSCs is variable. This variability is observed between different donors and between different colonies obtained from the same subject. Colonies from the same individual can be characterized as mono-, bi- or tri-potent, based on their ability to differentiate into one, two or three types of tissue: osteogenic, chondrogenic and adipogenic lineages. Furthermore, repeated passages were found to reduce the multilineage differentiation ability. It is possible to hypothesize that the angiogenic potential of MSCs is subjected to a similar variability that is influenced by the same factors.

In general, there are many parameters that can affect the functions and behavior of MSCs. Cultures of MSCs are very heterogeneous, even within the same colony. The morphology and differentiation potential of MSCs are dependent upon their source, the culture conditions (supplements, seeding density, number of passages, culture time) and the topographic localization within each colony. The characteristics of the 3D scaffolds, such as their chemical composition, topography, and roughness, have been also recognized as significant factors in affecting the cells’ behavior. The heterogeneity and morpho-functional variability of MSCs and their microenvironment may explain some of the conflicting data in different research works. In such data, the role of allogeneic MSCs in bone formation and their angiogenic potential should be highlighted.

**Conclusion**

Our data have demonstrated that allogeneic seeded MSCs are found on the scaffolds 6 and 12 weeks after their implantation. By week 12, a newly formed bone-like tissue from seeded cells without osteogenic pre-differentiation can be detected. The control scaffolds without seeded cells remained empty. A possibility of vessel formation from seeded MSCs was also shown, without preliminary cell cultivation under controlled conditions. Although the exact mechanisms of the involvement of the seeded allogeneic MSCs in bone formation need a further investigation, our data contribute to the understanding of the positive results of MSC transplantation.

**Abbreviations**

GFP green fluorescent protein  
HA hydroxyapatite
MSCs mesenchymal stem cells
PBS phosphate buffered saline
PI Propidium Iodide
SSLS surface selective laser sintering

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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