Effects of single and combined low frequency electromagnetic fields and simulated microgravity on gene expression of human mesenchymal stem cells during chondrogenesis

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Abstract

Introduction: Low frequency electromagnetic fields (LF-EMF) and simulated microgravity (SMG) have been observed to affect chondrogenesis. A controlled bioreactor system was developed to apply LF-EMF and SMG singly or combined during chondrogenic differentiation of human mesenchymal stem cells (hMSCs) in 3D culture.

Material and methods: An external motor gear SMG bioreactor was combined with magnetic Helmholtz coils for EMF (5 mT; 15 Hz). Pellets of hMSCs (±TGF-β3) were cultured (P5) under SMG, LF-EMF, LF-EMF/SMG and control (1 g) conditions for 3 weeks. Sections were stained with safranin-O and collagen type II. Gene expression was evaluated by microarray and real-time polymerase chain reaction analysis.

Results: Simulated microgravity application significantly changed gene expression; specifically, COLXA1 but also COL2A1, which represents the chondrogenic potential, were reduced ($p < 0.05$). Low frequency electromagnetic fields application showed no gene expression changes on a microarray basis. LF-EMF/SMG application obtained significant different expression values from cultures obtained under SMG conditions with a re-increase of COL2A1, therefore rescuing the chondrogenic potential, which had been lowered by SMG.

Conclusions: Simulated microgravity lowered hypertrophy but also the chondrogenic potential of hMSCs. Combined LF-EMF/SMG provided a rescue effect of the chondrogenic potential of hMSCs although no LF-EMF effect was observed under optimal conditions. The study provides new insights into how LF-EMF and SMG affect chondrogenesis of hMSCs and how they generate interdependent effects.

Key words: bioreactor, electromagnetic fields, simulated microgravity, chondrogenesis.

Introduction

Untreated cartilage defects in adults lead to osteoarthritis as there is no spontaneous repair mechanism which is able to completely restore physiological tissue structure and function [1, 2]. Procedures such as matrix-associated autologous chondrocyte transplantation (MACT) are used to facilitate the regenerative capacity and improve clinical outcome. As the use of chondrocytes is associated with donor site morbidity and dedifferentiation, human mesenchymal stem cells (hMSCs) might provide an opportunity to obtain a sufficient amount of cartilaginous cells. Advanced cell therapies for articular cartilage regeneration are developed using various bioreactor systems [3]. The main challenge in chondrogenic differentiation of hMSCs is to prevent terminal ossification represented by hypertrophy and at the same time maintain the chondrogenic potential of cells [4].

Adjuvant therapies such as electromagnetic fields (EMF) and simulated microgravity (SMG) have been used to improve cartilaginous tissues, but the literature data are very diverse.

Microgravity has been reported to reduce osteogenesis [5–8], and SMG has been shown to exert various effects on cartilaginous cells. Simulated microgravity has been reported by our group to reduce hypertrophy during chondrogenic differentiation of hMSCs [9]. However, SMG was also shown to decrease the chondrogenic potential of hMSCs [9–12].

Electromagnetic fields were shown to improve the chondrogenic potential of cells under certain conditions [13] and have been reported to develop multiple effects on hMSCs [14]. Under Low frequency electromagnetic fields (LF-EMF), the chondrogenic differentiation of hMSCs in 3D culture showed a significant increase of collagen type II expression at higher cell passages, although no EMF effect was obtained under optimal differentiation conditions [15]. However, EMF have also been reported to cause adverse effects [16–18], and the level of concern about EMF in the general population is high. Among general practitioners, there is no association between correct knowledge and concern [19]. Nevertheless, there is a lack of studies examining general EMF effects on chondrogenic cells. We therefore examined in this study the influence of LF-EMF during chondrogenesis by a screen of the entire gene expression level to detect beneficial and adverse gene expression changes which might occur under EMF treatment of cartilage defects. To our knowledge there has been no similar approach in the literature.

In a further step this study compared single and combined effects of LF-EMF and SMG on chondrogenesis of hMSCs in scaffold-free high-density 3D pellet constructs. In contrast to previously used SMG bioreactor systems, a controlled approach was used, which omitted any form of inadvertent EMF interference from power units of the SMG system by using solely an external motor gear. This approach made it possible to examine pure LF-EMF and pure SMG effects on chondrogenesis singly and combined for the first time.

The aim of this study was to show for the first time an entire gene expression screen, describe how LF-EMF and SMG affect chondrogenesis, and discuss whether they exert an influence on each other.

Material and methods

SMG

The LF-EMF/SMG bioreactor system consisted of an external motor gear and an internal vessel holder (Figure 1 A). Vessel rotation was transmitted by a flexible shaft to a 10 ml RCCS vessel (Rotary Cell Culture System; Synthecon Inc., Houston, USA) holder. The SMG bioreactor was combined with two magnetic Helmholtz coils (LF-EMF bioreactor) with an external cooling system and an external frequency generator. The vessel holder containing the pellet was situated within an incubator (Figure 1 A). To assure SMG conditions for each pellet, the rotation speed was adjusted to the velocity at which the pellet was maintained in free-fall condition at approximately 7 rpm. Previously, resulting shear forces were determined using dummy pellets. Simulated microgravity was applied between days 15 and 21 within the terminal chondrogenic differentiation phase, where hypertrophy is usually observed [20]. Pellets were transferred to the RCCS vessel during this phase.

LF-EMF

Two magnetic Helmholtz coils (D = 30 cm, 278 turns of enamelled copper wire, D = 1.5 mm, R = 2.5 Ω, L = 38.5 mH for one coil, distance between coils 15 cm, current = 3 A) placed in a cooling box (Neue Magnetodyn GmbH, Munich, Germany) connected to an external water bath (refrigerated circulator, DC50-F3 Haake, Vreden, Germany) produced a low frequency sinusoidal LF-EMF of 15 Hz and 5 mT magnetic flux density. The coils were driven by a frequency generator with power amplifier (M80, Neue Magnetodyn) (Figures 1 A, B). The field was applied three times a day (every 435 min) for 45 min throughout the differentiation period of 21 days. All stimulations were performed inside an incubator (CB150, Binder, Tuttlingen, Germany) under sterile conditions with a constant temperature level of 37 ±0.25°C.

Chondrogenic differentiation of hMSCs

Adult bone marrow derived hMSCs (Lonza, Walkersville, MD) were expanded in monolayer

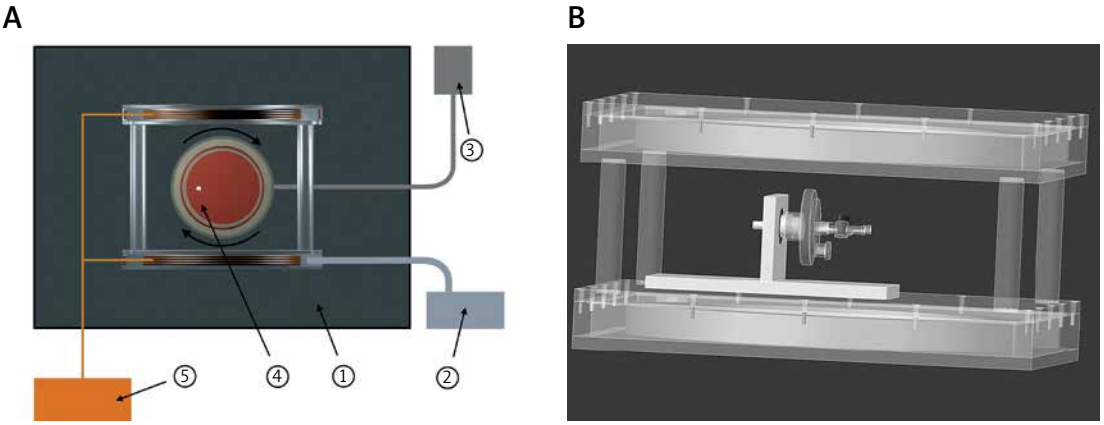

Figure 1. SMG/LF-EMF bioreactor system: **A** – Schematic drawing of setup compounds: incubator (1), LF-EMF solenoid with external cooling system (2), external motor gear of the SMG (3) connected to RCCS vessel by a flexible shaft, floating pellet (white) within the RCCS vessel (4), external frequency generator of the LF-EMF (5). **B** – Drawing of the position of the SMG bioreactor and RCCS vessel within the Helmholtz coils

triple flasks (Nunc, Roskilde, Denmark) in a humidified atmosphere, 5% CO_2, at 37°C, in growth medium (α-medium (Biochrom, Berlin, Germany) containing 10% fetal calf serum (PAA, Pasching, Austria), 50 U/ml penicillin/streptomycin (Biochrom), 2 mM glutamine and 5 ng/ml recombinant human fibroblast growth factor 2 (FGF-basic; Pepro Tech, Rocky Hill, NJ)). At 70–80% confluency, cells were passaged using 0.05% trypsin containing 0.02% EDTA (Biochrom). To generate pellet cultures, 4 × 10^5 hMSCs were centrifuged (150 g, 5 min) at passage 4 in a 15 ml falcon tube (TPP, Switzerland). For differentiation, pellets were cultured over a period of 21 days in differentiation medium (high-glucose Dulbecco's Modified Eagle's Medium (DMEM; Biochrom) supplemented with 10 mg/l insulin, 5.5 mg/l transferrin, 5 µg/l selenium, 0.5 mg/ml bovine serum albumin, 4.7 µg/ml linoleic acid, 0.1 µM dexamethasone, 0.2 mM L-ascorbic acid-2-phosphate, 0.35 mM L-proline (all Sigma-Aldrich, Steinheim, Germany), 30 U/ml penicillin/streptomycin (Biochrom) and 10 ng/ml recombinant human transforming growth factor-β3 (TGF-β_3; R&D Systems, Wiesbaden, Germany)).

Pellet cultures were distributed into four groups: 1) controls cultured under 1g conditions (n = 14); 2) SMG (n = 4); 3) LF-EMF (n = 14); and 4) LF-EMF/SMG (n = 5). The pellet culture system is limited to one culture per tube/RCCS. Pellet cultures were also maintained in the absence of the growth factor TGF-β_3.

Histology and immunohistochemistry

Pellets were embedded in Tissue-Tek (Sakura, Zoeterwoude, the Netherlands) prior to cryo-sectioning. Serial sections were stained with safranin-O (Fluka, Buchs, Switzerland) and fast green (Chroma, Münster, Germany) as previously described [21] to estimate the content and distribution of proteoglycans. For immunohistochemistry, samples were treated with chondroitinase and incubated overnight at 4°C with a primary antibody for collagen type II (Department of Medical and Physiological Chemistry, Uppsala, Sweden) diluted 1 : 6. For negative controls, the first antibody was omitted. Then samples were treated with a goat-derived biotinylated mouse-specific antibody (Vector Laboratories, Burlingame, USA) 1 : 200 in TBS for 1 h at room temperature (RT). Bound antibodies were stained with the VECTASTAIN ABC-Kit (Vector Laboratories, Burlingame, USA) and AEC (Sigma-Aldrich, Steinheim, Germany).

RNA preparation

Pellets from every group were separately disrupted under frozen conditions at 300 rpm using a Micro-Dismembrator S (Sartorius, Goettingen, Germany). RNA was directly isolated from freeze-milled preparations using 1 ml of TRIzol (Invitrogen, Germany). After adding 0.2 ml of chloroform (Sigma-Aldrich, Steinheim, Germany) and vigorously shaking, samples were incubated at RT for 10 min. Then samples were centrifuged at 15,000 × g for 20 min at 4°C, and the aqueous phase was transferred to a fresh tube. RNA was directly isolated using RNeasy Mini Kit (Qiagen).

Microarray analysis

Isolated total RNA was checked for purity (NanoDrop ND-1000, ThermoFisher, Waltham, USA) and integrity (Bioanalyzer 2100, Agilent, Santa Clara, USA). Control (WF) cultures were analyzed versus LF-EMF cultures (WF + EMF). 150 ng of total RNA was used for cDNA synthesis, amplification, fragmentation, and labelling using the Nugen Applause WTA ST and Encore Biotin labelling kits (Nugen, San Antonio, USA). Labelled probes were hy-

bridised to Affymetrix HuGene 2.0 ST GeneChips, washed, stained in an Affymetrix FS450 station, and scanned on an Affymetrix GeneChip Scanner 3000 (Affymetrix, Santa Clara, USA). The array CEL-files were RMA normalised with the appropriate program package from Affymetrix and analysed for differential expression in R using the packages limma and siggenes. The false discovery rate was set to 0.1 and no fold-change cutoff was applied. All arrays passed the quality control checks implemented in the R package arrayQualityMetrics [22].

Quantitative real-time PCR analysis

For cDNA synthesis, 0.3 µg of total RNA was reverse-transcribed using QuantiTect Rev. Transcription Kit (Qiagen, Hilden, Germany) with DNase treatment. Quantitative RT-PCR was performed with a LightCycler (Roche Applied Science, Mannheim, Germany) using 2 µl of SYBR Green Master Mix (Roche Applied Science, Mannheim, Germany) and 2.5 µl of 1 : 4 diluted cDNA in a 10 µl final volume. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward TGC ACC ACC AAC TGC TTA GC, reverse GGC ATG GAC TGT GGT CAT GAG [23]; collagen type II α_1 chain (COL2A1) forward GTT ATC GAG TAC CGG TCA CAG AAG, reverse AGT ACT TGG GTC CTT TGG GTT TG [24]; collagen type 10 α_1 chain (COLXA1) forward CAA GGC ACC ATC TCC AGG AA, reverse AAA GGG TAT TTG TGG CAG CAT ATT [25] and cartilage-specific proteoglycan core protein (aggrecan) forward CAG CAC CAG CAT CCC AGA, reverse CAG CAG TTG ATT CTG ATT CAC G [24] were used as primers. The following RT-PCR conditions were used for COL2A1 and aggrecan: polymerase activation 95°C for 10 min, 40 cycles at 95°C for 10 s, 65°C for 10 s and 72°C for 15 s. Conditions for GAPDH and COLXA1: 95°C for 10 min, 40 cycles at 95°C for 10 s, 60°C for 10 s and 72°C for 15 s. Reactions were performed in triplicate. Relative quantification of the target gene expression was generated normalizing to GAPDH. The LF-EMF, SMG and LF-EMF/SMG values were normalized to controls cultured with TGF-β_3 under 1g conditions.

Statistical analysis

Analysis was conducted using Microsoft Excel 2010 and Prism 5.02 software for Windows. Values were reported as the mean, standard deviation and range. Statistical analysis was performed using the Mann-Whitney *U*-test as data were not normally distributed. The level of significance was set at $p \leq 0.05$.

Results

LF-EMF application

The magnetic flux density in the layer of the pellet cultures within the bioreactor has a homogeneous spatial distribution due to the Helmholtz configuration and is 5 mT ± 5%. The electric field at a certain distance from the coil centre was calculated with good approximation by $E_{max} = 2\ h\ \pi\ f\ B_x$ [25], where B_x is the peak value of the magnetic flux density, f its frequency and h is the height of the medium. With the given values the maximum induced electric field is below 1 mV/m in the region of the pellet. The current density was calculated by $J = \sigma * E_{max}$, where σ is the conductance of the medium. With the given values and a conductance of 1.6 S/m for the medium [26] the current density is below 0.9 mA/m².

The magnetic flux density of the Helmholtz-coils was simulated (Finite Element Method Magnetics (FEMM); Meeker, D. (2010), version 4.0.1) (Figure 2 A). The simulation was validated by measuring the B-field along the axis of the Helmholtz coils with a Gaussmeter (Bell 640, F.W. Bell, Orlando, FL) (Figure 2 B). The earth's static magnetic field perpendicular to the axis of the Helmholtz coils was measured at 45 mT.

SMG bioreactor

Bioreactors simulating microgravity are used to avoid the enormous costs of space flight experiments. The SMG condition often results in a certain amount of shear stress. Within this study a low resulting shear stress of 0.16 dyn/cm² ($(T_{max} = 3\mu * V_{pellet}/2\ r_{pellet}$) viscosity of the medium (µ): 1.05 kg/ms; sedimentation rate (V_{pellet}): 0.04 m/s; mean pellet radius (r_{pellet}): 0.4 mm) was obtained. Values were obtained by measuring caviar globes as dummy pellets (data not shown).

A conventional commercially available Rotatory Cell Culture System (RCCS) developed by NASA to simulate microgravity generates low EMF in the layer of the pellets in the range of 0.1–0.5 mT with a sinusoidal modulation depending on the rotation speed of the motor. The controlled bioreactor used in this study excluded low inadvertent EMF by using an external motor gear.

Histology and immunohistochemistry

At the end of the differentiation period, positive staining for safranin-O and collagen type II was found in the centre of all pellet cultures treated with growth factors. Without growth factors, staining for safranin-O and collagen type II was negative. Control pellet cultures treated with growth factors showed more uniform staining for safranin-O and collagen type II. Cultures treated with LF-EMF alone revealed intense staining of safranin-O in the middle of the pellet and similar localized staining for collagen type II. In pellets cultured under single SMG there was weaker safranin-O and collagen type II staining. Pellets exposed to SMG and LF-EMF showed strong safr-

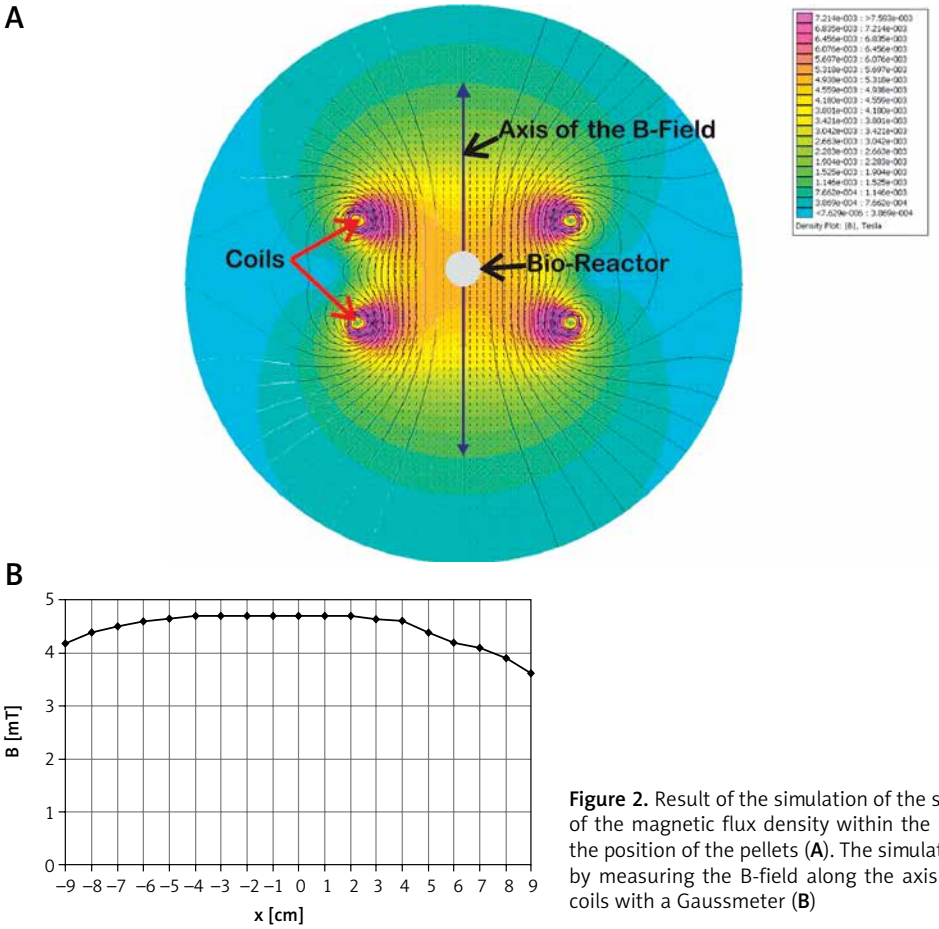

Figure 2. Result of the simulation of the spatial distribution of the magnetic flux density within the Helmholtz coils in the position of the pellets (**A**). The simulation was validated by measuring the B-field along the axis of the Helmholtz coils with a Gaussmeter (**B**)

anin-O staining in the centre and uniform staining for collagen type II (Figure 3).

Microarray analysis

Low-dose LF-EMF was applied to exert non-thermal cell stress on hMSCs during chondrogenic differentiation. Microarray analysis of hMSC cultures exposed to LF-EMF was used to analyse the gene expression pattern and to provide a better understanding of LF-EMF mechanisms. At passage 5 under optimal conditions, the microarray analysis provided no significant changes in gene expression caused by LF-EMF treatment. Rigorous control of input RNA and microarray array data showed optimal quality. The microarray findings were consistent with the findings obtained by RT-PCR and our results obtained from former studies using optimal chondrogenic differentiation conditions [15]. The following link was created to allow review of records: GSE57298: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=srqhyuourzahzgp&acc=GSE57298.

Quantitative real-time PCR analysis

Chondrogenic differentiation was confirmed in cultures supplemented with TGF-β_3 by RT-PCR for COL2A1 and aggrecan. All results were normalized to control cultures treated with TGF-β_3. Untreated pellet cultures without growth factors did not express any of the chondrogenic markers (data not shown) and were therefore not used as normalizing controls.

The SMG significantly reduced COLXA1 expression compared to control cultures treated with TGF-β_3 (Figure 4). The application of SMG also significantly decreased the expression of COL2A1 and aggrecan. Pure LF-EMF showed no significant effect on the expression of COL2A1, aggrecan and COLXA1 compared to the control as described by gene expression analysis. LF-EMF/SMG showed significantly higher COL2A1 expression compared to SMG, but did not reach control levels. LF-EMF/SMG showed an insignificant increase of COLXA1 expression compared to SMG. The COL2A1/COLXA1 ratio reached comparable values for control conditions and LF-EMF application, followed by LF-EMF/SMG and then SMG (Figure 4).

Discussion

A bioreactor system was applied, which makes it possible to examine the effect of LF-EMF and SMG on *in vitro* chondrogenesis of hMSCs in 3D

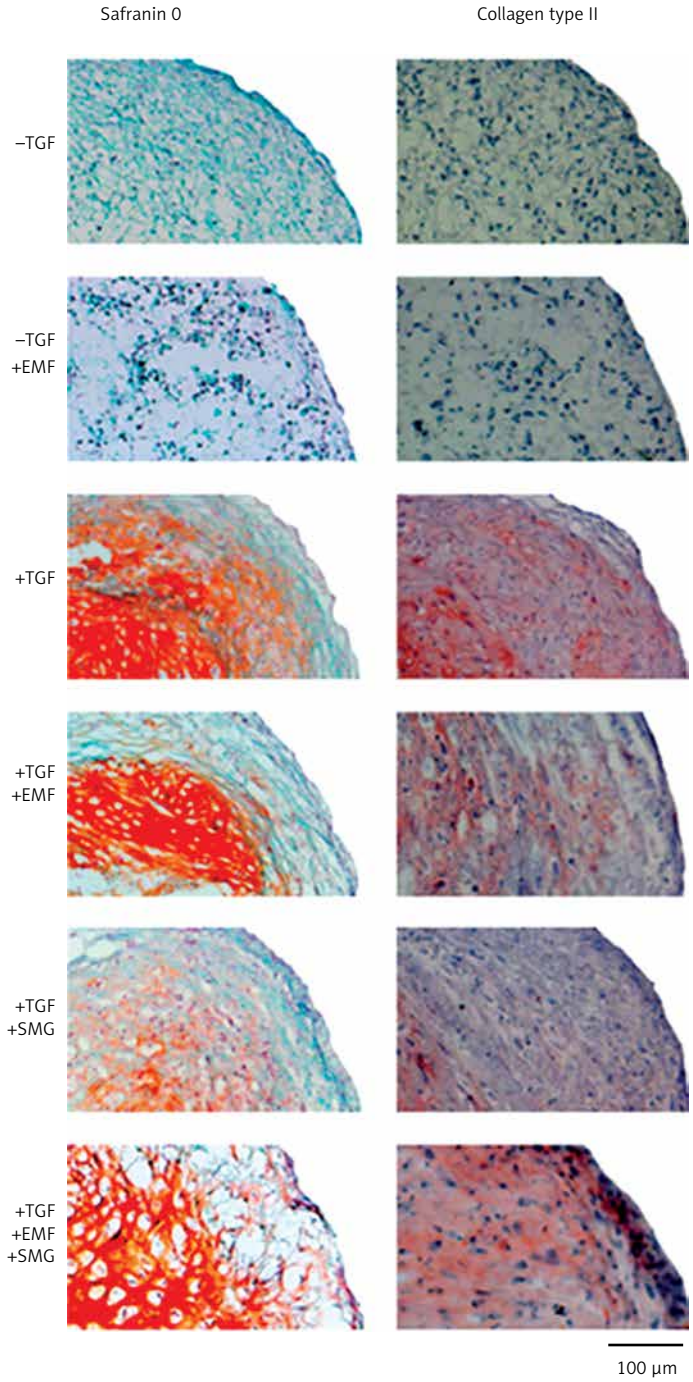

Figure 3. Safranin-0 staining and immunohistochemistry for collagen type II of pellet cultures treated with no additional growth factor (–TGF); no additional growth factor in the presence of electromagnetic fields (–TGF +LF-EMF), TGF-β_3 alone (+TGF); TGF-β_3 in the presence of electromagnetic fields (+TGF +LF-EMF); TGF-β_3 in combination with electromagnetic fields under simulated microgravity conditions (+LF-EMF +TGF +SMG) and TGF-β_3 under simulated microgravity conditions (+LF-EMF +SMG)

culture separately or combined under controlled conditions.

Single LF-EMF showed no effect on gene expression. Single SMG decreased the expression of COLXA1 and COL2A1. LF-EMF/SMG resulted in a significantly higher expression of COL2A1 when compared to SMG. LF-EMF/SMG insignificantly increased COLXA1 when compared to SMG. Altogether, the combination therapy LF-EMF/SMG was not significantly superior to control culture levels treated with growth factors. However, LF-EMF/SMG rescued the COL2A1 expression, which had been lowered by SMG.

Regarding the literature, space flights alter bone metabolism, resulting in osteoporosis [27]. Osteoblasts have been shown to change expres-

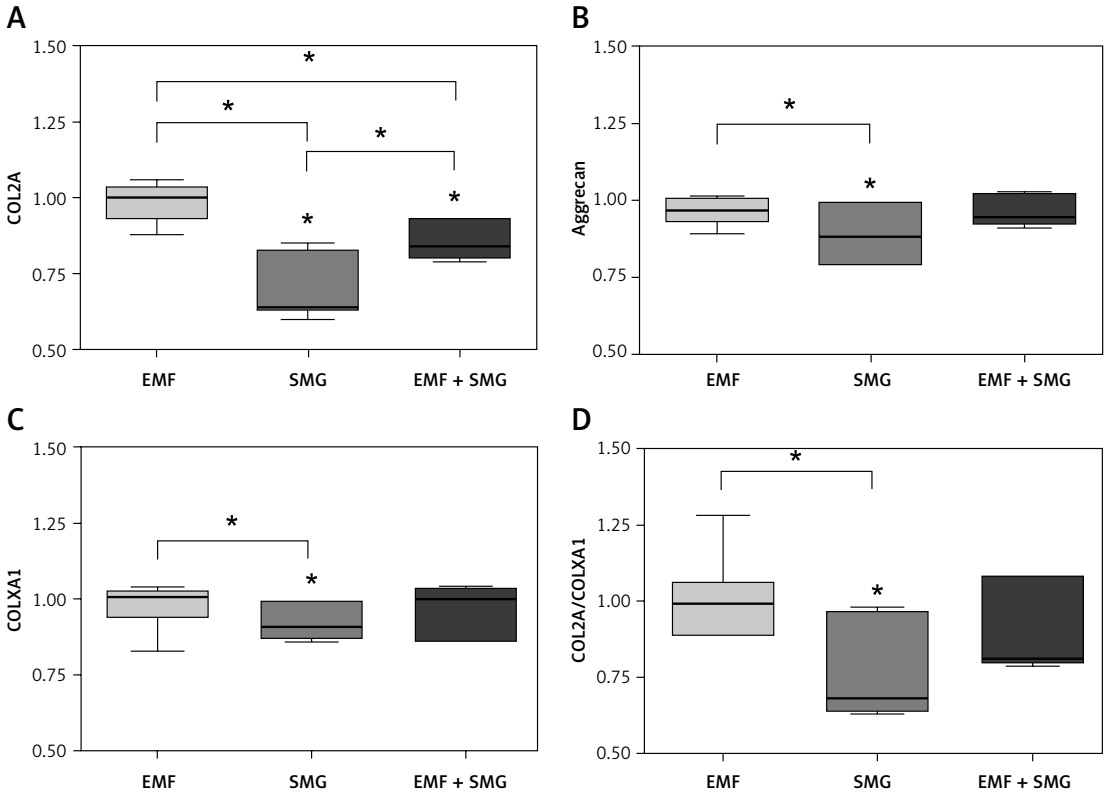

Figure 4. Expression of mRNA for COL2A1, COLXA1, aggrecan and the COL2A1 to COLXA1 ratio in pellet cultures (hMSCs from passage five) treated with TGF-β_3 and cultured under microgravity conditions (+SMG), under microgravity conditions treated with LF-EMF (+SMG +LF-EMF) or stimulated with pure LF-EMF (+LF-EMF). All levels were normalized to control cultures treated with TGF-β_3. Message levels of RNA preparations were analyzed by qRT-PCR and normalized to mRNA levels for GAPDH

sion levels under SMG [28]. Microarray experiments showed lower osteogenic gene expression of hMSCs seeded onto gelatin microcarriers under SMG compared to static culture after 1 week [12]. In our controlled 3D *in vitro* study, 1 week of SMG was shown to be effective in reducing hypertrophy, which is a first step during terminal ossification of hMSCs [9].

Studies investigating the effects of SMG on chondrogenesis do not provide consistent results. Results range from increased collagen type II expression in rabbit MSCs after 4 weeks of SMG in a conventional direct motor gear RCCS [29] to reduced collagen type II expression of porcine chondrocytes when using a random positioning machine [11]. Comparing SMG to real microgravity (RM), lower collagen type II expression in chondrocytes was observed under RM conditions [11, 30]. It therefore cannot be excluded that EMF effects reported in the literature were induced by direct power units, which might have influenced the SMG results. To obtain controlled study conditions, it is necessary to exclude inadvertent EMF effects by a direct motor gear in SMG studies. In this study, under controlled conditions, 1 week of SMG induced a significant reduction of COL2A1 expression.

Electromagnetic fields applications result in divergent data regarding cartilaginous cells [13, 16]. Electromagnetic fields were shown to limit progression of OA in animal models [31–33]. It was reported that EMF counteracted IL-1β activity during chondrogenesis [34]. Electromagnetic fields have also been reported to stimulate growth factor synthesis of TGF-β_1 [35]. In this study LF-EMF had no effect on chondrogenesis under optimal differentiation conditions, which has been reported by our group previously [15]. Furthermore, we could show that LF-EMF application did not result in any gene expression changes of hMSCs during chondrogenic differentiation as examined by microarray analysis. This is of major importance as previous reports have linked EMF with an increased risk of carcinogenesis [16–18] and there is a growing concern in public health regarding EMF [36]. In this study, three weeks of LF-EMF did not cause any inadvertent gene expression changes. However, we cannot exclude that gene expression

changes might occur during EMF treatment under modified conditions.

The combined LF-EMF/SMG application resulted in a COL2A1/COLXA1 ratio that was not significantly different from control cultures treated with TGF-β_3. This implies that the combination therapy was not more effective than plain growth factor application. All parameters (time, shear stress, electromagnetic field) were chosen according to optimal results obtained for hypertrophy (SMG) and chondrogenesis (LF-EMF) regarding single applications [9, 15]. We therefore cannot recommend LF-EMF/SMG treatment in regenerative medicine for improvement of cartilaginous cells. Nevertheless, further studies are necessary to optimize the LF-EMF/SMG parameters and find a combination therapy which might render LF-EMF/SMG useful for tissue engineering.

However, LF-EMF/SMG treatment significantly increased the chondrogenic potential of hMSCs compared to SMG conditions. There was a rescue-LF-EMF effect observed, which was dependent on the effects resulting from SMG treatment. We had demonstrated similar findings of LF-EMF effects under suboptimal cellular conditions where LF-EMF effects were only observed in hMSCs with a lowered differentiation potential [15]. As LF-EMF alone did not show any effect on chondrogenesis, the LF-EMF/SMG effect is probably linked to the altered cellular situation induced by SMG. Increased growth factor synthesis [35] or anti-inflammatory effects of A_{2A} and A_3 adenosine receptors [37], as described for EMF, might be a reason for a re-improvement of the chondrogenic potential. EMF have also been proven to reduce the expression of pro-apoptotic and increase the expression of anti-apoptotic proteins [38]. Moreover, it has been reported that EMF are able to delay cellular senescence mediated by heat shock proteins [39]. Recent data show that EMF might even be able to mediate cell reprogramming into a pluripotent state [40]. Therefore, there are various possible explanations for the influence of EMF on hMSCs during chondrogenic differentiation. Altogether, EMF seems to act on hMSCs regarding differentiation in the form of a rescue effect, which is only observed under suboptimal cellular conditions.

In conclusion, LF-EMF did not cause any adverse effects during chondrogenic differentiation. The applied combination therapy of LF-EMF/SMG was not significantly superior to control conditions and therefore does not appear to be suitable for cartilage tissue engineering of hMSCs. However, under LF-EMF/SMG there was a rescue effect of LF-EMF observed regarding the chondrogenic potential of hMSCs. Further studies are necessary to improve LF-EMF/SMG combination therapies for hMSCs and evaluate the exact mechanism of LF-EMF rescue.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Hunziker EB. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. Osteoarthritis Cartilage 2002; 10: 432-63.
2. Czyżewska A, Glinkowski WM, Walesiak K, Krawczak K, Cabaj D, Górecki A. Effects of preoperative physiotherapy in hip osteoarthritis patients awaiting total hip replacement. Arch Med Sci 2014; 10: 985-91.
3. Madeira C, Santhagunam A, Salgueiro JB, Cabral JM. Advanced cell therapies for articular cartilage regeneration. Trends Biotechnol 2015; 33: 35-42.
4. Chen S, Fu P, Cong R, Wu H, Pei M. Strategies to minimize hypertrophy in cartilage engineering and regeneration. Genes Dis 2015; 2 276-95.
5. Blaber E, Marcal H, Burns BP. Bioastronautics: the influence of microgravity on astronaut health. Astrobiology 2010; 10: 463-73.
6. Carmeliet G, Nys G, Stockmans I, Bouillon R. Gene expression related to the differentiation of osteoblastic cells is altered by microgravity. Bone 1998; 22: 139S-43S.
7. Landis WJ, Hodgens KJ, Block D, Toma CD, Gerstenfeld LC. Spaceflight effects on cultured embryonic chick bone cells. J Bone Mineral Res 2000; 15: 1099-112.
8. Rucci N, Migliaccio S, Zani BM, Taranta A, Teti A. Characterization of the osteoblast-like cell phenotype under microgravity conditions in the NASA-approved rotating wall vessel bioreactor (RWV). J Cell Biochem 2002; 85: 167-79.
9. Mayer-Wagner S, Hammerschmid F, Redeker JI, et al. Simulated microgravity affects chondrogenesis and hypertrophy of human mesenchymal stem cells. Int Orthop 2014.
10. Duke J, Daane E, Arizpe J, Montufar-Solis D. Chondrogenesis in aggregates of embryonic limb cells grown in a rotating wall vessel. Adv Space Res 1996; 17: 289-93.
11. Stamenkovic V, Keller G, Nesic D, Cogoli A, Grogan SP. Neocartilage formation in 1 g, simulated, and microgravity environments: implications for tissue engineering. Tissue Eng A 2010; 16: 1729-36.
12. Sheyn D, Pelled G, Netanely D, Domany E, Gazit D. The effect of simulated microgravity on human mesenchymal stem cells cultured in an osteogenic differentiation system: a bioinformatics study. Tissue Eng A 2010; 16: 3403-12.

13. Fini M, Pagani S, Giavaresi G, et al. Functional tissue engineering in articular cartilage repair: is there a role for electromagnetic biophysical stimulation? Tissue Eng B Rev 2013; 19: 353-67.
14. Ross CL, Siriwardane M, Almeida-Porada G, et al. The effect of low-frequency electromagnetic field on human bone marrow stem/progenitor cell differentiation. Stem Cell Res 2015; 15: 96-108.
15. Mayer-Wagner S, Passberger A, Sievers B, et al. Effects of low frequency electromagnetic fields on the chondrogenic differentiation of human mesenchymal stem cells. Bioelectromagnetics 2011; 32: 283-90.
16. Li S, Yu B, Zhou D, He C, Zhuo Q, Hulme JM. Electromagnetic fields for treating osteoarthritis. Cochrane Database Syst Rev 2013; 12: CD003523.
17. Balamuralikrishnan B, Balachandar V, Kumar SS, et al. Evaluation of chromosomal alteration in electrical workers occupationally exposed to low frequency of electromagnetic field (EMFs) in Coimbatore population, India. APJCP 2012; 13: 2961-6.
18. Kirschenlohr H, Ellis P, Hesketh R, Metcalfe J. Gene expression profiles in white blood cells of volunteers exposed to a 50 Hz electromagnetic field. Radiation Res 2012; 178: 138-49.
19. Berg-Beckhoff G, Breckenkamp J, Larsen PV, Kowall B. General practitioners' knowledge and concern about electromagnetic fields. Int J Environ Res Public Health 2014; 11: 12969-82.
20. Mueller MB, Tuan RS. Functional characterization of hypertrophy in chondrogenesis of human mesenchymal stem cells. Arthritis Rheumat 2008; 58: 1377-88.
21. Wagner S, Hofstetter W, Chiquet M, et al. Early osteoarthritic changes of human femoral head cartilage subsequent to femoro-acetabular impingement. Osteoarthritis Cartilage 2003; 11: 508-18.
22. Kauffmann A, Gentleman R, Huber W. arrayQualityMetrics: a bioconductor package for quality assessment of microarray data. Bioinformatics 2009; 25: 415-6.
23. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002; 3: RESEARCH0034.
24. Varas L, Ohlsson LB, Honeth G, et al. alpha10 integrin expression is up-regulated on fibroblast growth factor-2-treated mesenchymal stem cells with improved chondrogenic differentiation potential. Stem Cells Develop 2007; 16: 965-78.
25. Zhang LM, Su PQ, Xu CX, Yang JL, Yu WH, Huang DS. Chondrogenic differentiation of human mesenchymal stem cells: a comparison between micromass and pellet culture systems. Biotechnol Lett 2010; 32: 1339-46.
26. Bassen H, Litovitz T, Penafiel M, Meister R. ELF in vitro exposure systems for inducing uniform electric and magnetic fields in cell culture media. Bioelectromagnetics 1992; 13: 183-98.
27. Rambaut PC, Johnston RS. Prolonged weightlessness and calcium loss in man. Acta Astronautica 1979; 6: 1113-22.
28. Kumei Y, Shimokawa H, Katano H, et al. Microgravity induces prostaglandin E2 and interleukin-6 production in normal rat osteoblasts: role in bone demineralization. J Biotechnol 1996; 47: 313-24.
29. Ohyabu Y, Kida N, Kojima H, Taguchi T, Tanaka J, Uemura T. Cartilaginous tissue formation from bone marrow cells using rotating wall vessel (RWV) bioreactor. Biotechnol Bioeng 2006; 95: 1003-8.
30. Freed LE, Langer R, Martin I, Pellis NR, Vunjak-Novakovic G. Tissue engineering of cartilage in space. Proc Natl Acad Sci USA 1997; 94: 13885-90.
31. Veronesi F, Torricelli P, Giavaresi G, et al. In vivo effect of two different pulsed electromagnetic field frequencies on osteoarthritis. J Orthop Res 2014; 32: 677-85.
32. Fini M, Torricelli P, Giavaresi G, et al. Effect of pulsed electromagnetic field stimulation on knee cartilage, subchondral and epyphiseal trabecular bone of aged Dunkin Hartley guinea pigs. Biomed Pharmacother 2008; 62: 709-15.
33. Ciombor DM, Aaron RK, Wang S, Simon B. Modification of osteoarthritis by pulsed electromagnetic field: a morphological study. Osteoarthritis Cartilage 2003; 11: 455-62.
34. Ongaro A, Pellati A, Setti S, et al. Electromagnetic fields counteract IL-1beta activity during chondrogenesis of bovine mesenchymal stem cells. J Tissue Eng Regen Med 2015; 9: E229-38.
35. Aaron RK, Boyan BD, Ciombor DM, Schwartz Z, Simon BJ. Stimulation of growth factor synthesis by electric and electromagnetic fields. Clin Orthop Relat Res 2004; 419: 30-7.
36. Doucet IL. Biological effects of low frequency electromagnetic fields. Med War 1992; 8: 205-12.
37. Vincenzi F, Targa M, Corciulo C, et al. Pulsed electromagnetic fields increased the anti-inflammatory effect of A(2)A and A(3) adenosine receptors in human T/C-28a2 chondrocytes and hFOB 1.19 osteoblasts. PloS One 2013; 8: e65561.
38. Podda MV, Leone L, Barbati SA, et al. Extremely low-frequency electromagnetic fields enhance the survival of newborn neurons in the mouse hippocampus. Eur J Neurosci 2014; 39: 893-903.
39. Perez FP, Zhou X, Morisaki J, Jurivich D. Electromagnetic field therapy delays cellular senescence and death by enhancement of the heat shock response. Exp Gerontol 2008; 43: 307-16.
40. Baek S, Quan X, Kim S, Lengner C, Park JK, Kim J. Electromagnetic fields mediate efficient cell reprogramming into a Pluripotent State. ACS Nano 2014; 8: 10125-38.