Rheumatol Int (2013) 33:1567–1575
DOI 10.1007/s00296-012-2600-4

ORIGINAL ARTICLE

Human osteoarthritic chondrocytes exposed to extremely low-frequency electromagnetic fields (ELF) and therapeutic application of musically modulated electromagnetic fields (TAMMEF) systems: a comparative study

Claudio Corallo · Nila Volpi · Daniela Franci · Daniela Vannoni · Roberto Leoncini · Giacomo Landi · Massimo Guarna · Antonio Montella · Antonietta Albanese · Emilio Battisti · Antonella Fioravanti · Ranuccio Nuti · Nicola Giordano

Received: 28 April 2012 / Accepted: 8 December 2012 / Published online: 23 December 2012

Abstract Osteoarthritis (OA) is the most common joint disease, characterized by matrix degradation and changes in chondrocyte morphology and metabolism. Literature reported that electromagnetic fields (EMFs) can produce benefits in OA patients, even if EMFs mechanism of action is debated. Human osteoarthritic chondrocytes isolated from femoral heads were cultured in vitro in bidimensional (2-D) flasks and in three-dimensional (3-D) alginate beads to mimic closely cartilage environment in vivo. Cells were exposed 30 min/day for 2 weeks to extremely low-frequency electromagnetic field (ELF) with fixed frequency (100 Hz) and to therapeutic application of musically modulated electromagnetic field (TAMMEF) with variable frequencies, intensities, and waveforms. Cell viability was measured at days 7 and 14, while healthy-cell density, heavily vacuolized (hv) cell density, and cluster density were measured by light microscopy only for 3-D cultures after treatments. Cell morphology was observed for 2-D and 3-D cultures by transmission electron microscopy (TEM). Chondrocyte exposure to TAMMEF enhances cell viability at days 7 and 14 compared to ELF. Light microscopy analysis showed that TAMMEF enhances healthy-cell density, reduces hv-cell density and clustering, compared to ELF. Furthermore, TEM analysis showed different morphology for 2-D (fibroblast-like) and 3-D (rounded shape) cultures, confirming light microscopy results. In conclusion, EMFs are effective and safe for OA chondrocytes. TAMMEF can positively interfere with OA chondrocytes representing an innovative non-pharmacological approach to treat OA.

C. Corallo (✉) · D. Vannoni · R. Leoncini · G. Landi · A. Montella · R. Nuti · N. Giordano
Department of Internal Medicine, Endocrine and Metabolic Sciences and Biochemistry, University of Siena, Ospedale S. Maria alle Scotte, Viale Bracci, 53100 Siena, Italy
e-mail: corallo.claudio@gmail.com

D. Vannoni
e-mail: vannoni@unisi.it

R. Leoncini
e-mail: leoncini@unisi.it

G. Landi
e-mail: landi31@unisi.it

A. Montella
e-mail: toto.montella@gmail.com

R. Nuti
e-mail: nutir@unisi.it

N. Giordano
e-mail: giordanon@unisi.it

C. Corallo · A. Montella · A. Albanese · E. Battisti · N. Giordano
TAMMEF Centre, University of Siena, Siena, Italy
e-mail: albanesea@unisi.it

E. Battisti
e-mail: battistie@unisi.it

N. Volpi · D. Franci · M. Guarna
Department of Biomedical Sciences, University of Siena, Siena, Italy
e-mail: volpi@unisi.it

D. Franci
e-mail: daniela.franci@unisi.it

M. Guarna
e-mail: massimo.guarna@unisi.it

A. Fioravanti
Department of Clinical Medicine and Immunological Sciences, Rheumatology Unit, University of Siena, Siena, Italy
e-mail: acquapendente8@yahoo.it

Keywords Osteoarthritis · Human primary chondrocytes · Alginate beads cultures · Electromagnetic fields · TAMMEF

Introduction

Osteoarthritis (OA) is one of the most frequently occurring rheumatic diseases. It is a slowly progressive degenerative disorder, characterized by gradual loss of articular cartilage due to disruption of chondrocyte–matrix associations and alterations of metabolic responses in the chondrocyte [1]. The etiology of OA is still not completely understood and represents an open debate in the literature [2]. Pharmacologic interventions have focused primarily on improving symptoms, by the use of non-steroidal antiinflammatory drugs (NSAIDs) and by means of new agents, the so-called "Chondroprotective" or "Structure-modifying" Agents, which seem to counter arthritic degenerative processes and encourage normalization of the synovial fluid and cartilage matrix [3, 4]. Although most of these agents have shown promising effects in vitro, none has been proven to produce clinically meaningful structure-modifying effects in humans with osteoarthritis [5]. Therefore, the treatment of OA is usually unsatisfactory at present and the need to create effective and valid alternatives to standard pharmacological therapies rose up [6]. Some investigators hypothesized that the stimulation of bone and cartilage through the application of specific electric and magnetic fields could interfere in mechanisms such us inflammation, growth and repair [7–9]. Literature reports that bone and cartilage can be stimulated by electromagnetic fields (EMFs) to increase the synthesis of extracellular matrix [10, 11]. Furthermore, extracellular matrix of the hyaline cartilage is piezoelectric, converting electromagnetic oscillations to mechanical vibrations and vice versa [12]. The analgesic efficacy and tolerability of a low-frequency electromagnetic field (ELF), modulated at a frequency of 100 Hz with a sinusoidal waveform and mean induction of a few gauss, has been demonstrated in osteoporosis [13], rheumatoid arthritis [14] and osteoarthritis [15, 16]. The potential effects of electromagnetic fields on human cells are related to many factors, such as the method of cell culture and the properties of EMFs (frequency, intensity, waveform) [17–21]. In the present study, we introduce, in comparison with the most traditional ELF, the new TAMMEF system (Therapeutic Application of Musically Modulated Electromagnetic Field), whose codes or parameters such as frequency, intensity and waveform are modified in time, randomly varying within the respective ranges, so that all the possible codes can occur during a single application (usually 30 min). Furthermore, EMFs used for in vivo treatments are often characterized by higher energies than EMFs used for in vitro studies because EMFs applied in vitro directly interfere with cells without possible interference with other tissues or organs [11–15]. For this reason, the results obtained in vitro are difficult to compare to that obtained in vivo. The aim of the present study is to compare the in vitro effects (in terms of cell viability, healthy-cell density, hv-cell density and clustering density) between standard EMFs treatments and the new TAMMEF system on human osteoarthritic chondrocytes cultured under standard conditions and in three-dimensional alginate beads. We chose to analyze heavily vacuolized cells and clustering formation because little is known in the literature about these two phenomena. For instance, it is reported that during OA, chondrocytes are eliminated following two mechanisms such as apoptosis and autophagy (by cytoplasmic vacuolization) [22, 23]. Furthermore, the term of "Chondroptosis" was used for the first time to design cell death type present in OA cartilage which combines some apoptotic and some autophagic processes [24]. Finally, the concept of "cluster" represents an open debate in the literature because the exact steps leading to formation of these cell clusters in OA cartilage have not been clearly identified [25].

The research protocol was approved by our local ethics committee, and each donor provided informed written consent.

Materials and methods

Cell cultures

Human chondrocytes were obtained from 20 OA patients (10 males and 10 females, age 55–80 years) undergoing to total femoral head replacement. Articular cartilage slices isolated from the femoral heads were dissected under aseptic conditions. Chondrocytes were isolated enzymatically from 20 OA cartilage samples and pooled before the experiments. All enzymatic solutions were made in DMEM (Sigma-Aldrich. St. Louis, MO, USA) supplemented with penicillin (100 U/mL) (Sigma-Aldrich. St. Louis, MO, USA), streptomycin (100 μg/mL) (Sigma-Aldrich. St. Louis, MO, USA), 0.25 μg/ml amphotericin B (Sigma-Aldrich. St. Louis, MO, USA), and 10 % FBS (Sigma-Aldrich. St. Louis, MO, USA). Articular cartilage was treated with 0.1 % sheep testes hyaluronidase (Sigma-Aldrich. St. Louis, MO, USA), in DMEM for 30 min at 37 °C. The hyaluronidase solution was then replaced by 0.5 % of Streptomyces griseus Pronase E (Sigma-Aldrich. St. Louis, MO, USA), in DMEM for 60 min at 37 °C. Cartilage was then washed twice with DMEM containing 10 % FBS and digested with type II collagenase (1 mg/mL) (Sigma-Aldrich. St. Louis, MO, USA), in DMEM at 37 °C for

45 min at 37 °C. Cell suspension was filtered with cell strainer 70 μm (BD Biosciences Franklin Lakes, NJ, USA), rinsed in the medium and centrifuged at 100×*g* for 10 min. Viability and number of isolated cells were estimated by vital staining using trypan blue (Sigma-Aldrich. St. Louis, MO, USA). Usually, 1×10^6 chondrocytes could be obtained from 1 g of cartilage of each patient. Chondrocytes (about 1×10^6 cells) were cultured in DMEM supplemented with 100 U/penicillin, 100 μg/mL streptomycin 2 mM glutamine, and 10 % FCS onto 75 cm^2 flasks (BD Costar Cambridge, MA, USA) for bidimensional cultures and incubated at 37 °C in a atmosphere of 5 % CO_2 95 % air, till confluence (2–3 weeks). To obtain three-dimensional cultures, cell suspension (about 1×10^6 cells) was mixed to a 1.2 % solution of sterile alginate in 0.15 M NaCl. Then, alginate/cell suspension was extruded through a 22-gauge needle and dropped into a 102 mM $CaCl_2$ solution. The beads with 3 mm diameter were allowed to polymerize for 10 min and then transferred in six-well multiwall plates (BD Costar Cambridge, MA, USA) (5 beads per well) with DMEM supplemented with 100 U/penicillin, 100 μg/mL streptomycin 2 mM glutamine, 10 % FCS and 25 μg/ml ascorbic acid (Sigma-Aldrich. St. Louis, MO, USA) and incubated at 37 °C in a atmosphere of 5 % CO_2 95 % air.

Treatments with ELF and TAMMEF systems

To perform our experiments, two different types of EMFs were used (Fig. 1a): (a) ELF EMFs (Extremely Low-Frequency Electromagnetic Fields used as gold standard) modulated at a standard frequency of 100 Hz with a sinusoidal waveform and a mean induction of a few gauss; (b) TAMMEF system (Therapeutic Application of Musically Modulated Electromagnetic Fields), whose parameters or "codes" (frequency, intensity and waveform) vary in time, since they are generated by a piece of music (Rachmaninov's Piano Concerto number 2 in C minor, Op.18 for piano and orchestra). We chose a 100 Hz fixed frequency for ELF MF because in the most frequently adopted in the literature and in our previous studies [14, 15]. To conduct the experiments, the flasks and multiwells containing OA chondrocytes were positioned between the magnetic poles in order to be directly within the generated EMF (Fig. 1b). The cells of each patient were divided into two groups: (a) 2-D cell culture; (b) 3-D alginate beads cell culture. For each group, the cells were splitted in three parts in order to receive the following treatments: (1) ELF EMFs (30 min/day for 2 weeks); (2) TAMMEF (30 min/day for 2 weeks); (3) Sham Exposed (S.E.) cells served as the reference control. These cells were removed from the incubator and maintained at a distance far from the EMFs (30 min/day for 2 weeks).

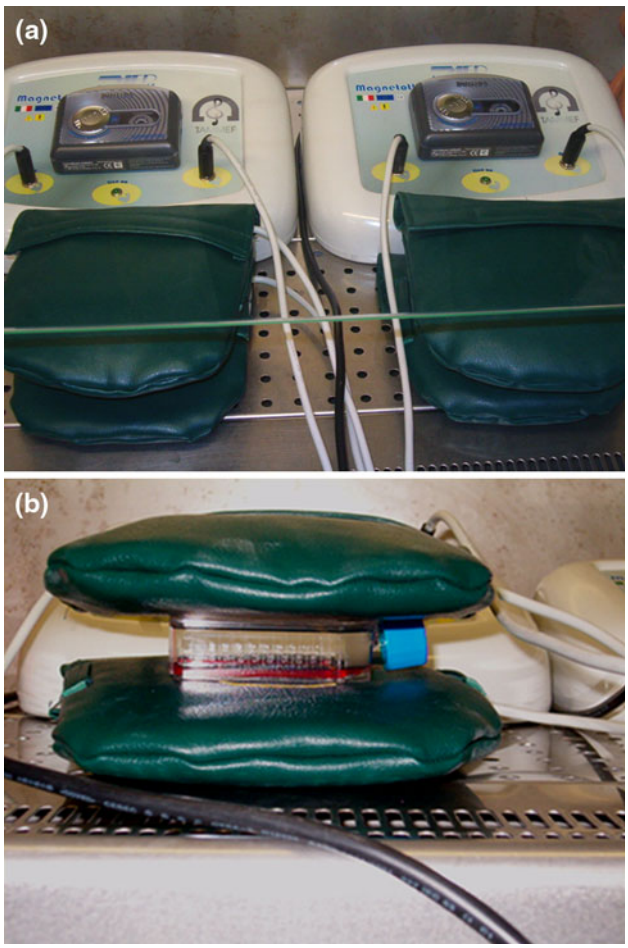

Fig. 1 The apparatus used to generate the ELF EMF and TAMMEF. **a** To conduct an experiment, cell samples were positioned between the polar expansions in order to be directly in the generated EMF. **b** Sham Exposed cells were maintained at a distance far from the EMF; thus, it served as a control. The experiments were carried out at room temperature at time (30 min) for 2 weeks

Cell viability assay

Cell viability was measured during the treatment with EMFs at days 7 and 14 using the alamarBlue® assay (Invitrogen S.R.L, MI, ITALY). The culture medium was removed and replaced with 10 % (v/v) alamarBlue® in DMEM without FBS and incubated for 4 h (37 °C, 5 % CO_2). The system incorporates the fluorometric/colorimetric REDOX indicator which changes from oxidized form (non-fluorescent, blue) to reduced form (fluorescent, red). The changing of the color, from initial blue to final red, is directly proportional to the amount of the reagent reduced and provides an estimation of the number of viable cells submitted to the test. Fluorescence intensity was read using a Multiskan EX microplate spectrofluorometer 200–240 V (Thermo Fisher Scientific, 81 Waltham MA 02454 USA) at excitation 530 nm and emission 590 nm.

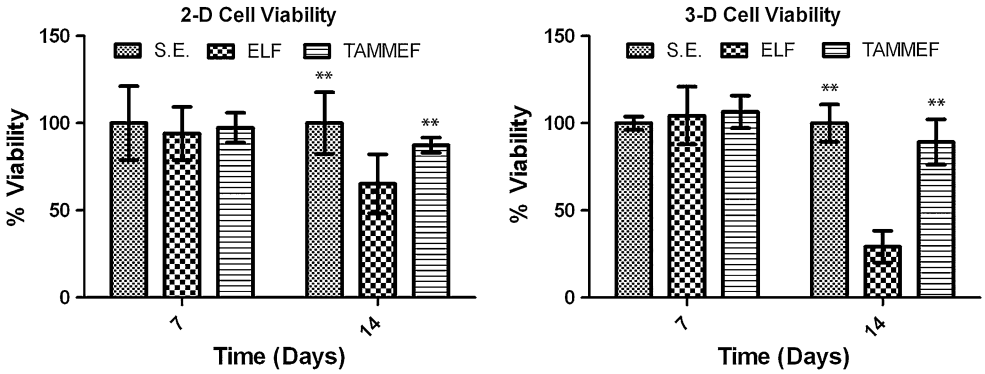

Fig. 2 2D and 3D cell viability recorded after 7 and 14 days of treatments

Light and electron microscopy

After trypsinization (only for 2-D cell culture), 2-D and 3-D alginate beads cells were fixed at 4 °C in Karnovsky 0.1 M solution, 2.5 % glutaraldehyde and 4 % formaldehyde, at pH 7.4, then washed twice in cacodylate buffer 0.1 M, pH 7.4 and post-fixed for 2 hours in 1 % osmium tetroxide at 4 °C. After four washes in cacodylate buffer, cells were dehydrated in ethanol gradient, transferred into propylene oxide, included into araldite resin and polymerized at 60 °C for 24 h. Semithin sections were colored with toluidine blue and observed at Axioscope (Zeiss Jena GmbH, Germany) microscope. Ultrathin sections were contrasted with uranyl acetate and lead citrate and were observed at Philips TEM CM-10 (Huntsville, Alabama, USA) transmission electron microscopy.

Alginate beads cell cultures light microscopy analysis

Three-dimensional cell-alginate beads treated with ELF, TAMMEF and S.E. were fixed as previously described after 14 days of treatments and cut into semithin sections. For each alginate bead, 9 semithin sections, distanced 20 μm, were obtained. In each section, healthy-cell (no autophagic vesicles, no apoptotic blebbing) density, heavily vacuolized (hv) cell density and cluster density were evaluated with AxioVision 4.6 software (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). For this study, cells were classified as hv-cells when vacuoles occupied more than 50 % of their cytoplasmic space. Data statistical analysis was performed with GraphPad Prism 5.0 software (1992–2007 GraphPad Software, Inc).

Statistical analysis

Statistical analysis was performed using GraphPad Prism® 5 for Windows. To analyze different protein expressions, one-way ANOVA and Newman–Keuls Multiple Comparison Test were performed. For cell viability statistics, data

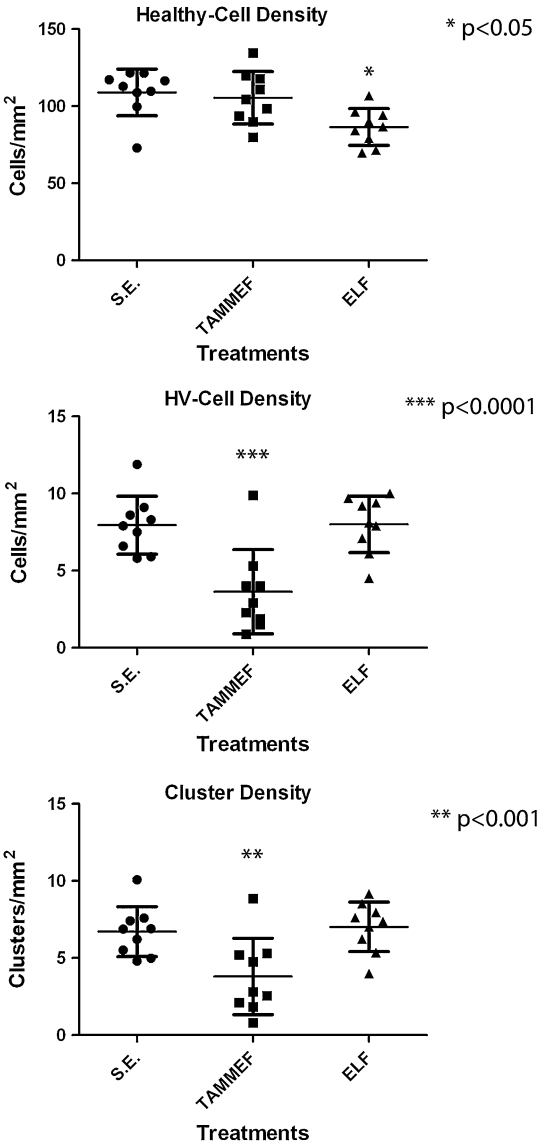

Fig. 3 Healthy-cell density, heavily vacuolized (hv) cell density and clusters density calculated as the mean of 9 semithin sections with axial distance of 20 μm for each alginate bead for S.E., ELF and TAMMEF cells after 14 days of treatments

are reported as the percentage increment compared to control (100 %). For light microscopy statistics, densities were calculated as the mean of 9 semithin sections with axial distance of 20 μm for each alginate bead. Data are reported as the means ± standard deviations.

Results

Cell viability

Figure 2 shows cell viability at days 7 and 14 for both 2-D and 3-D cultures. Six independent samples for each culture condition and for each treatment were analyzed to perform statistical calculations. For 2-D cell culture, there is no statistically relevant change in cell viability between ELF, TAMMEF and S.E. after 7 days of treatments. But, after 14 days of treatments, there is a statistically relevant decrease in cell viability of ELF-treated cells compared to TAMMEF (Anova, $p = 0.04$) and S.E. (Anova, $p = 0.03$), while there are no statistically relevant changes in cell viability between TAMMEF and S.E. (Anova, $p = 0.20$). For 3-D cell culture, there is no statistically relevant change in cell viability after 7 days of treatments between ELF, TAMMEF and S.E., while after 14 days of treatment, there is a statistically relevant decrease in cell viability of ELF-treated cells compared to TAMMEF (Anova, $p = 0.003$) and S.E. (Anova, $p = 0.003$). No relevant differences were found between TAMMEF and S.E. (Anova, $p = 0.16$).

Alginate beads cell cultures light microscopy analysis

Figure 3 shows healthy-cell density, hv-cell density and clustering density. Healthy-cell mean density was lower in ELF-treated cells respect to S.E. ($p < 0.05$), and TAMMEF ($p < 0.05$). Moreover, the hv-cell mean density was lower in TAMMEF-treated cells compared to S.E. ($p < 0.0001$) and ELF ($p < 0.0001$). Finally, the clustering mean density was lower in TAMMEF-treated cells compared to S.E. ($p < 0.001$) and ELF ($p < 0.001$). Clusters were also observed (Fig. 4).

Electron microscopy

Figure 5 shows transmission electron microscopic analysis of cells cultured in 2-D and 3-D substrates. Chondrocytes cultured in 2-D flasks showed a fibroblast-like elongated phenotype. On the contrary, chondrocytes cultured in 3-D alginate beads showed the typical chondrocyte phenotype consisting in rounded shape chondrocytes. Cells showed features of anabolic activity, such as euchromatic nuclei, abundant RER and Golgi profiles, many transport vesicles, as well as secreted extracellular matrix. Furthermore, both 2-D and 3-D cells treated with ELF system showed more vacuoles and apoptotic bodies in the cytoplasm compared to 2-D and 3-D cells treated with TAMMEF and 2-D (Fig. 3a) and 3-D (Fig. 3b) S.E. cells. Clusters were also observed (Fig. 6).

Discussion

In our opinion, this study documents first of all that EMFs could positively interfere with OA chondrocytes in cultures. In particular, the results obtained with TAMMEF system showed that EMfs do not affect cell viability, do not induce uncontrolled proliferation and are not dangerous for in vitro studies [26–29]. Differences in the growth rate between treated and untreated chondrocyte cultures can be explained as a possible interference that electromagnetic fields may have on the state of cells' electric charge, in terms of the ability of chondrocytes to restore the correct intracellular energetic equilibrium [30]. Moreover, the

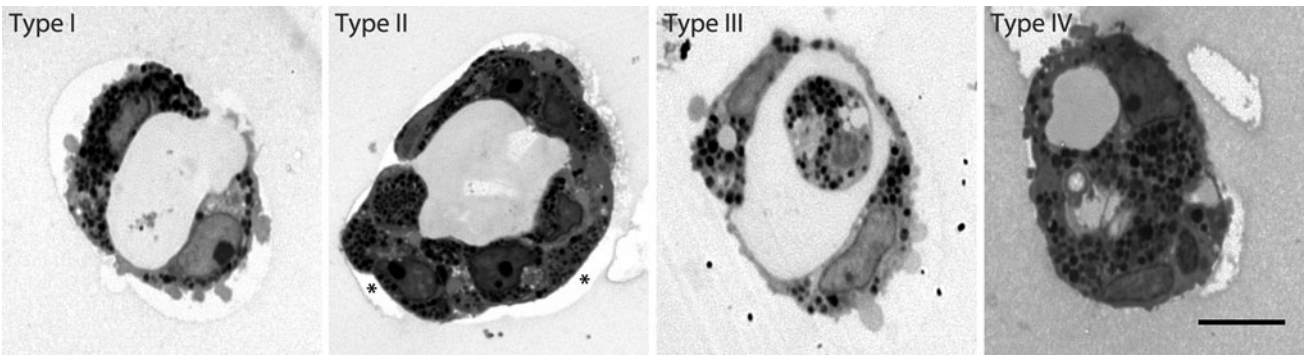

Fig. 4 Clusters of varying morphology (light microscopy, *scale bar* 10 μm) following Ross' classification. In type 1 clusters, one layered chondrocytes in an oval conformation surrounds a central area filled with amorphous material. Type 2 clusters suggest a more advanced process, with concentric cell layers (*asterisk*) surrounding the cavity in a spiral fashion. In type 3 clusters, flattened cells surround an area containing a viable cell. Type 4 cluster composed of tightly packed cells, with indistinct cell boundaries

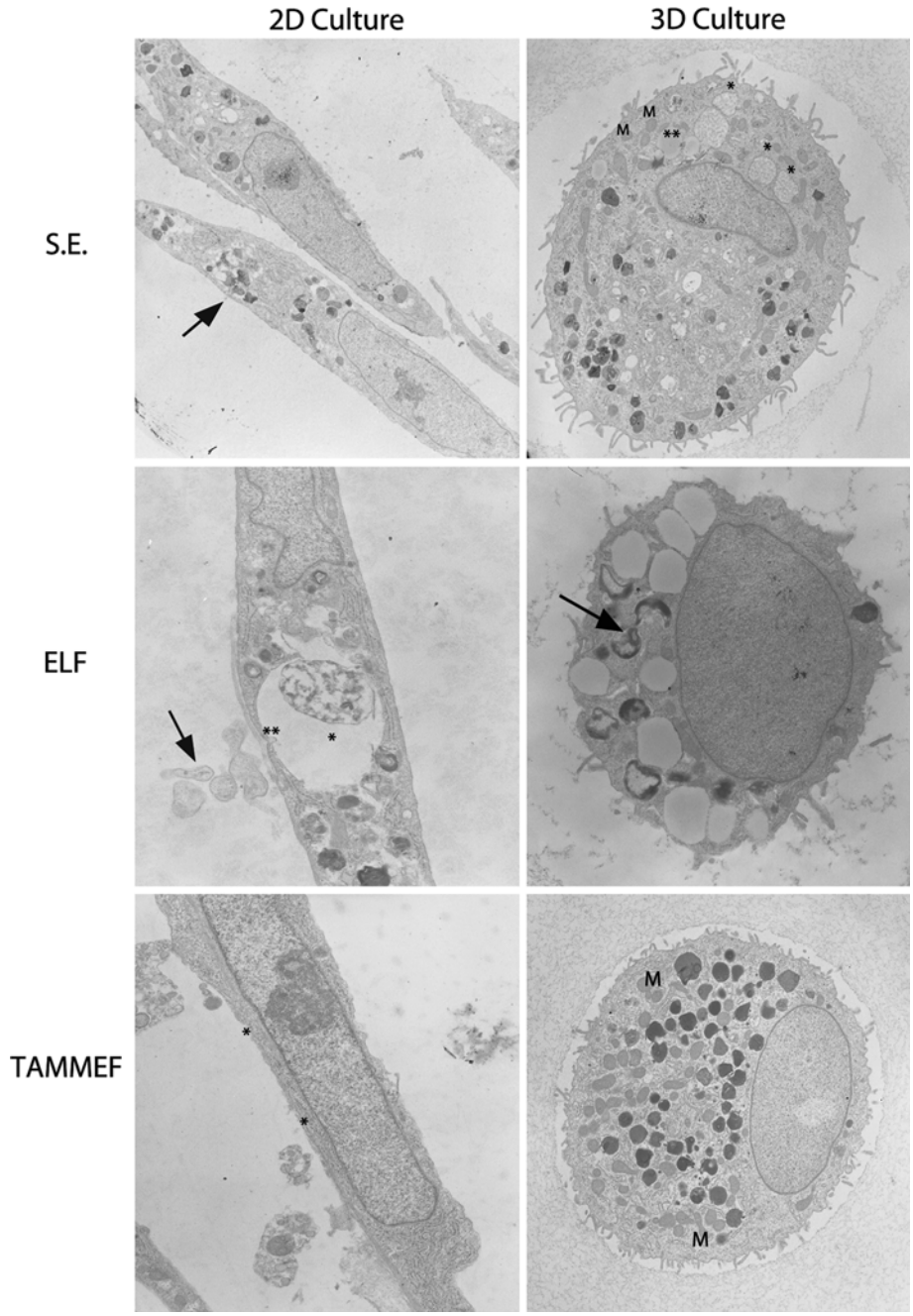

Fig. 5 2D and 3D cultures of Sham Exposed, ELF and TAMMEF 14-day-treated chondrocytes. 2D S.E. cells show a typical, elongated fibroblast-like morphology, some vacuoles with autophagic features (*arrow*) are observed (original magnification ×3,900). 3D S.E. chondrocytes in alginate beads showed typical rounded chondrocyte morphology, mitochondria (*M*) and dilated transport vesicles containing floccular material, likely directed to extracellular matrix (*single asterisk*). Occasional lipid vacuoles (*double asterisk*) and autophagic vacuoles are also observed. 2D ELF. Fibroblast-like elongated morphology of chondrocytes showed features of chondroptosis, with indented nucleus and autophagic vesicles. A large autophagic vacuole (*single asterisk*), containing cell debris and partially membrane limited (*double asterisk*), is observed, as well as extrusion of vesicles in the extracellular environment (*arrow*). 3D ELF cells exhibited early chondroptosis. Cytoplasm is almost filled with large vacuoles and apoptotic bodies showing myelin-like membranes (*arrow*), indicative of autophagy, near apoptotic bodies (o.m. ×8900). 2D TAMMEF cells showed cytoskeletal assembly with fibroblast-like F-actin thick fibers, or stress fiber (*single asterisk*). Abundant RER and Golgi profiles are observed in cytoplasm (*arrow*) (o.m. ×8,900). 3D TAMMEF cells exhibited typical chondrocyte morphology, with abundant mitochondria (*M*) and transport vesicles (o.m. ×8,900)

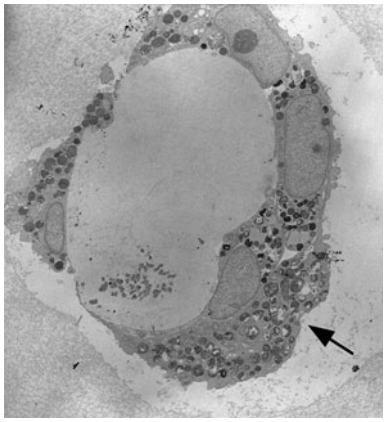

Fig. 6 3D cultured chondrocytes forming a cluster of type 2, following Ross' classification. Cells in more than one layer (*arrow*) surround a cavity containing amorphous secreted material, nuclei are euchromatic and cytoplasm is rich of multivesicular bodies (o.m. ×2,200)

molecular mechanisms through which low-frequency electromagnetic fields could interfere with chondrocyte proliferation and differentiation have been partially clarified [31]. To evaluate the best efficacy of EMFs on cell culture conditions must mimic closely the in vivo environment of the cell type [32]. Hence, the need to cultivate chondrocytes in three-dimensional cultures represents the basis to "maintain" in vitro the original differentiated active phenotype and its metabolic functions. This study suggests that the combination of multiple "codes" (intensities, wave forms and frequencies) generated by TAMMEF system in 30 min enhances cell viability compared to ELF with fixed frequency of 100 Hz, so we hypothesize that the combination of codes may contain precise subsets of frequencies and intensities and waveforms that positively "activate" chondrocytes. However, we do not know yet what is the real positive subset because multiple codes occur in a single application and it also depends on the piece of music chosen [15–18]. Moreover, we hypothesize that ELF system with fixed frequency of 100 Hz is unable to positively activate chondrocytes such as TAMMEF system but, as suggested by our results, ELF codes may negatively interfere with chondrocytes. This negative influence could explain the lowest viability recorded after 14 days of ELF treatments and also the highest percentage of highly vacuolized cells in 3-D ELF alginate beads cultures. Literature reported that during OA, chondrocytes are eliminated such as by active caspase 3 (apoptosis) and by cytoplasmic vacuolization (autophagy) [22, 23]. Furthermore, Roach et al. [24] introduced the term of "Chondroptosis" to design cell death type present in OA cartilage which combines some apoptotic and some autophagic processes. Together, these processes lead to complete self-destruction of chondrocytes, as demonstrated by the presence of empty lacunae. ELF treatments seem to accelerate the second process involved in "Chondroptosis", that is the autophagy caused by cytoplasmic vacuolization, as observed in both 2-D and 3-D ELF-treated cultures. In addition, OA cartilage shows a multiplicity of articular chondrocyte morphologies consisting in apparently normal chondrocytes at the periphery and, through degenerative matrix processes, in cellular loss and erosion in the middle [33]. In the present study, the use of 3-D alginate beads cultures mimics processes associated with the initiation and progression of chondron remodeling, one of the early events in the osteoarthritic process [34]. These events of chondron remodeling consist in swelling and remodeling of the pericellular microenvironment, initiation of cell division and migration associated with the swollen microenvironment, and continued proliferation and modified synthesis to form clonal (toward forming) chondrocyte clusters [25, 35, 36]. Literature reports the identification of four different types of clusters based on their morphology and extracellular matrix composition [33, 37–39]. The first type is composed of rounded cells in oval or round conformation. The second type is formed by concentric layers of cells densely packed together. The third type is composed by a layer of flattened interleaved cells around the outside of the cluster with few cells in the middle. The final cluster type consists of tightly packed cells, with membrane boundaries difficult to discern. In the present study, we recognize the four types of clusters with the lowest density recorded for TAMMEF-treated cells (Fig. 4). However, clustering formation represents an open debate in the literature since the exact steps leading to the formation of these cell clusters have not been clearly identified [25]. Some authors reported that clustering phenomenon is the final stage after cartilage softening, fibrillation and fissures of human OA [34]. In the three-dimensional model exposed in the present study, we cannot discern if the clustering phenomenon is due exclusively to osteoarthritis or it represents the normal evolution of articular chondrocytes because of the unavailability of healthy non-osteoarthritic cartilage and we acknowledge this limitation. However, we could hypothesize that cluster formation is a combination of the final stage of chondroptosis during OA and cell proliferation in alginate bead lacunae.

In conclusion, the results shown in this work encourage the use of electromagnetic fields in rheumatic diseases and in particular the use of the innovative TAMMEF system in OA treatments. Moreover, TAMMEF system could represent a valid non-pharmacological approach in the treatment of this particular disease, especially because NSAIDs are symptomatic drugs and they cannot be used for a long time. The goal of every treatment for OA is not only to reduce pain and stiffness, and to allow for greater movement, but also to slow the progression of the disease. At present, it is

well known that NSAIDs are not able to restore cartilage and that the therapeutic use of the so-called "Chondroprotective" or "Structure-modifying" Agents is extremely controversial. On the contrary, the analgesic activity of electromagnetic fields is well documented in the literature, and it is free of side effects and seems to be able to interfere positively with cartilage metabolism. Furthermore, three-dimensional cultures represent a valid method to study the possible consequences that electromagnetic fields might have on OA chondrocytes because they mimic closely the in vivo articular cartilage environment.

Acknowledgments Thanks to TAMMEF Centre, University of Siena, Siena, Italy.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Mankin HJ, Dorfman H, Lippiello L, Zarins A (1971) Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. J Bone Joint Surg 53A:523–537
2. Fioravanti A, Benetti D, Coppola G, Collodel G (2005) Effect of continuous high hydrostatic pressure on the morphology and cytoskeleton of normal and osteoarthritic human chondrocytes cultivated in alginate gels. Clin Exp Rheumatol 23:847–853
3. Verbruggen G (2006) Chondroprotective drugs in degenerative joint diseases. Rheumatol 45(2):129–138
4. Richette P, Bardin T (2004) Structure-modifying agents for osteoarthritis: an update. Joint Bone Spine 71(1):18–23
5. Bay-Jensen AC, Hoegh-Madsen S, Dam E, Henriksen K, Sondergaard BC, Pastoureau P et al (2010) Which elements are involved in reversible and irreversible cartilage degradation in osteoarthritis? Rheumatol Int 30(4):435–442
6. Colen S, Haverkamp D, Mulier M, van den Bekerom MP (2012) Hyaluronic acid for the treatment of osteoarthritis in all joints except the knee: what is the current evidence? BioDrugs 26(2):101–112
7. Bassett C (1968) Biological significance of piezoelectricity. Calcif Tissue Res 1:252–272
8. Bassett C (1971) Biophysical principles affecting bone structure. In: Bourne GH (ed) Biochemistry and physiology of bone, vol 3. Academic Press, New York, pp 1–68
9. Bassett C, Pawluk RJ, Pilla AA (1974) Acceleration of fracture repair by electromagnetic fields: a surgically noninvasive method. Ann NY Acad Sci 238:242–262
10. Aaron RK, Ciombor DM (1992) Electrical stimulation of bone induction and grafting. In: Habal M, Reddi AH (eds) Bone grafts and bone substitutes. W.B. Saunders, Philadelphia, pp 192–205
11. Rosen AD (2003) Mechanism of action of moderate-intensity static magnetic fields on biological systems. Cell Biochem Biophys 39:163–173
12. Jacobson JI, Gorman R, Yamanashi WS, Saxena BJ, Clayton L (2001) Lowamplitude extremely low frequency magnetic fields for the treatment of osteoarthritic knees: a double-blind clinical study. Altern Ther Health Med 7:54–67
13. Giordano N, Battisti E, Santacroce C, Geraci S, Tanganelli V, Matii G, Gennari C, Gennari L, Rigato M (2001) Evaluation of early biomarkers of cartilage degeneration in the diagnosis and clinic-therapeutic monitoring of primary osteoarthrosis. Clin Ther 152(3):179–182
14. Giordano N, Battisti E, Geraci S, Santacroce C, Lucani B, Fortunato M, Mattii G, Gennari C (2000) Analgesic-antiinflammatory effect of 100 Hz variable magnetic field in R.A. Clin Exp Rheumatol 18:263–267
15. Rigato M, Battisti E, Fortunato M, Giordano N (2002) Comparison between the analgesic and therapeutic effects of a musically modulated electromagnetic field (TAMMEF) and those of a 100 Hz electromagnetic field: blind experiment on patients suffering from cervical spondylosis or shoulder periarthritis. J Med Eng Technol 26:253–258
16. Battisti E, Piazza E, Rigato M, Nuti R, Bianciardi L, Scribano A, Giordano N (2004) Efficacy and safety of a musically modulated electromagnetic field (TAMMEF) in patients affected by knee osteoarthritis. Clinic Exp Rheumatol 22:568–572
17. Ciombor DM, Lester G, Aaron RK, Neame P, Caterson B (2002) Low frequency EMF regulates chondrocyte differentiation and expression of matrix proteins. J Orthop Res 20:40–50
18. Wilmot JJ, Chiego JR, Carlson DS, Hanks CT, Moskwa JJ (1993) Autoradiographic study of the effects of pulsed electromagnetic fields on bone and cartilage growth in juvenile rats. Arch Oral Biol 38:67–74
19. Dini L, Abbro L (2005) Bioeffects of moderate-intensity static fields on cell cultures. Micron 36:195–217
20. Yoshizawa H, Tsuchiya T, Mizoe H, Ozeki H, Kanao S, Yomori H, Sakne C, Hasebe S, Motomura T, Yamakawa T, Mizuno F, Hirose H, Otaka Y (2002) No effect of extremely low-frequency magnetic field observed on cell growth or initial response of cell proliferation in human cancer cell lines. Bioelectromagnetics 23:355–368
21. Gál P, Kilík R, Špaková T, Pataki Š, Sabo J, Pomfy M, Longauer F, Hudká R (2005) He-Ne laser irradiation accelerates inflammatory phase and epithelization of skin wound healing in rats. Biologia 60:691–696
22. Kourí JB, Rojas L, Pérez E, Abbud-Lozoya KA (2002) Modifications of golgi complex in chondrocytes from osteoarthrotic (OA) rat cartilage. J Histochem Cytochem 50:1333
23. Almonte-Becerril M, Navarro-Garcia F, Gonzalez-Robles A, Vega-Lopez MA, Lavalle C, Kouri JB (2010) Cell death of chondrocytes is a combination between apoptosis and autophagy during the pathogenesis of osteoarthritis within an experimental model. Apoptosis 15:631–638
24. Roach I, Aigner T, Kouri JB (2004) Chondroptosis: a variant of apoptotic cell death in chondrocytes? Apoptosis 9:265–277
25. Le Graverand MP, Sciore P, Eggerer J, Rattner JP, Vignon E, Barclay L, Hart DA, Rattner JB (2001) Formation and phenotype of cell clusters in osteoarthritic meniscus. Arthritis Rheumatism 44:1808–1818
26. Fioravanti A, Nerucci F, Collodel G (2002) Biochemical and morphological study of human articular chondrocytes cultivated in the presence of pulsed signal therapy. Ann Rheum Dis 61(11): 1032–1033
27. Pezzetti F, De Mattei M, Caruso A (1999) Effects of pulsed electromagnetic fields on human chondrocytes: an in vitro study. Calcif Tissue Int 65(5):396–401
28. Ongaro A, Pellati A, Masieri FF, Caruso A, Setti S, Cadossi R et al (2011) Chondroprotective effects of pulsed electromagnetic fields on human cartilage explants. Bioelectromagnetics 32(7):543–551
29. Schmidt-Rohlfing B, Silny J, Woodruff S, Gavenis K (2008) Effects of pulsed and sinusoid electromagnetic fields on human chondrocytes cultivated in a collagen matrix. Rheumatol Int 28(10): 971–977
30. Hofbauer LC (2000) The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. J Bone Miner Res 15:2–12

31. Fabbri E, Barbin L, Capuzzo A, Biondi C (1998) Adenyl cyclase activity and glucose release from the liver of the European eel, Anguilla anguilla. Am J Physiol 275:1563–1570
32. Carrossino AM, Recenti R, Carrossino R, Piscitelli E, Gozzini A, Martineti V et al (2007) Methodological models for in vitro amplification and maintenance of human articular chondrocytes from elderly patients. Biogerontology 8:483–498
33. Ross JM, Sherwin AF, Poole CA (2006) In vitro culture of enzymatically isolated chondrons: a possible model for the initiation of osteoarthritis. J Anat 209:793–806
34. McGlashan SR, Cluett EC, Jensen CG, Poole CA (2008) Primary cilia in osteoarthritic chondrocytes: from chondrons to clusters. Dev Dyn 237:2013–2020
35. Durrant LA, Archer CW, Benjamin M, Ralphs JR (1999) Organisation of the chondrocyte cytoskeleton and its response to changing mechanical conditions in organ culture. J Anat 194: 343–353
36. Rothwell AG, Bentley G (1973) Chondrocyte multiplication in osteoarthritic articular cartilage. J Bone Joint Surg Br 55:588–594
37. Girkontaite I, Frischholz S, Lammi P, Wagner K, Swoboda B, Aigner T et al (1996) Immunolocalization of type X collagen in normal fetal and adult osteoarthritic cartilage with monoclonal antibodies. Matrix Biol 15:231–238
38. Poole CA, Matsuoka A, Schofield JR (1991) Chondrons from articular cartilage. III. Morphologic changes in the cellular microenvironment of chondrons isolated from osteoarthritic cartilage. Arthritis Rheum 34:22–35
39. Poole CA, Flint MH, Beaumont BW (1988) Chondrons extracted from canine tibial cartilage: preliminary report on their isolation and structure. J Orthop Res 6:408–419