Chondrocytes treated with different shock wave devices

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Summary

**Background:** Shock wave treatment is used for several orthopedic diseases and there are different devices available. Until now, there have been no experimental studies on the effects of these different generators.

**Methods:** We carried out an experimental study to compare the effects of three focused generators (electro-magnetic, piezoelectric and electro-hydraulic) as well as a radial generator on healthy and osteoarthritis chondrocytes.

**Results:** By the analysis of our results, we may exclude significant differences between the different generators, even though there is a greater action specificity for electro-magnetic and piezoelectric generators.

**Conclusions:** The smaller size of the focus of the latter two generators guarantees a greater concentration of energy in the target. The biological effect of the increase of IL-10 and reduction of both N-Cadherin and E-Catenin in chondrocytes in healthy subjects and those affected by osteoarthritis confirms the therapeutic potential of ESWT in cartilage diseases, such as osteoarthritis. In clinical practice it is important to introduce the parameter of total energy. This allows us to standardize the treatment and to manage the variability related to the different types of device and size of the focus.

**Level of evidence:** IIb.

**KEY WORDS:** shock waves, devices, chondrocytes.

Introduction

In rehabilitation, there are four different methods of physical stimulation techniques: inductive (electromagnetic fields), capacitive (electric fields), faradic (electric current) and mechanical vibration (shock wave, radial wave)1. A Shockwave (SW) has an acoustic wave characterized by a quick pressure increase and by a rapid decrease of values below those of the atmosphere, in a few nanoseconds2. This physical characteristic determines the focalization of energy in a small area (focus) with the maximum concentration of energy at some cm of depth of the subcutaneous tissue. The shock wave may be produced by an electro-generator (EI), electromagnetic (EM) and piezoelectric (PE). The SW is responsible for angiogenic effects, modulation of inflammation, as well as proliferative and analgesic effects on the tissue3-5. In literature there emerged that the SW induces at the cellular level different metabolic pathways, such as modulation of membrane permeability, the expression of various cytokines and the synthesis of growth factors and nitric oxide. In 2001, there was introduced another type of acoustic wave, called radial wave (RSW), generated by a ballistic system6. In the case of the radial wave, the increase in pressure value of the acoustic wave needs a longer time. Furthermore, the maximum energy is found at the interface between skin and transducer and is reduced in a quadratic function related to penetration depth. Until now, there has been a lack of studies on the biological effects. Nevertheless some Authors have reported that the attenuation of the focus may be responsible for reducing the effects of the shock wave7. The main indications of shock wave and radial wave in the treatment of musculoskeletal diseases are for tendinopathies, calcific or not, and fracture healing delays8. The application in the cartilage diseases, as osteoarthritis, is still preliminary. Only a few clinical and experimental researches have studied the effects of the shock waves and the radial waves on chondrocytes and articular cartilage in the human model9-16.
The first end-point of this study is to compare the different effects of the shock waves, generated by the three main devices (EI, EM and EP), and the radial waves. The second end-point is to verify the effects of the shock waves and radial waves on the human chondrocyte cell line in normal conditions and in osteo-arthritis.

### Materials and methods

Patients (9 males and 9 females) with primary severe osteoarthritis (OA) (grade 4 Kellgren and Lawrence radiographic staging) who had undergone joint replacement surgery were recruited into the study and the remaining material was acquired. Osteoarthritic cartilage samples were obtained after knee replacement surgeries. Normal cartilage samples were obtained from traumatic knee lesions. Cartilage was taken from the femoral and tibial sides of knee. Due to the limited size of the sample, we did not perform all experiments on each tissue sample. Patient characteristics are reported in Table I. The research was conducted according to international standards and this study was approved by the local medical ethical committee. The study meets the ethical standards of the Journal.

### Results

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### Methods

#### Materials

- **Collagenase (C0130 Sigma)** in DMEM/F12 serum free
- **Penicillin/Streptomycin (P11-010 Gibco Life Tecnology)** and Amphotericin B 1X (P11-001 Gibco Life Tecnology)

#### Methods

- **Culture and stimulation**: Cells were cultured and stimulated with different devices.
- **Ascorbic acid**: 25 µg/ml ascorbic acid was added to cultured medium.
- **Incubation**: Cells were incubated with 0.2% Collagenase overnight at 37°C.

#### Staining

- **Primary antibodies**: Anti-N-Cadherin (Abcam, 76057) and β-Catenin (Abcam, 6302) were used.
- **Secondary antibodies**: Biotinylated polyclonal anti-rabbit antibody was used.
- **Staining Kit**: Vectorstain ABC System kit were used.
- **Counterstaining**: Mayer’s Hematoxylin Solution was used.
- **Analysis**: FAC S analysis was used to study the expression of N-Cadherin and β-Catenin.

### Table I. Patients characteristics.

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<tr>
<th>Number</th>
<th>FACS analysis</th>
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<tr>
<td>HD</td>
<td>8</td>
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<td>OA</td>
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data did not show any difference in the expression of IL-10 in healthy (HD) compared to osteoarthritis (OA) chondrocytes after different shock waves stimulation. We studied the expression of N-Cadherin in osteoarthritic (OA) cultured chondrocytes after different stimulations (EM, PE) by FACS staining. The value of non-stimulated cells (OA: 41.3 +/- 18.6) was statistically higher than treated cells (OA: PE 37.4 +/- 19.6; EM 37.5 +/- 16.9) (p =0.04). As regards the PE and EM stimulated cells, there was a tendency towards a decrease in values (p>0.05).

Then we evaluated the expression of β-Catenin in HD and OA cultured chondrocytes after the same stimulations by ICC staining. The cells not stimulated with SW (HD: 2.2 +/- 0.3; OA: 2.5 +/- 0.1) showed higher values than those stimulated (HD: PE 1.6 +/- 0.7; EM 1.3 +/- 0.2; OA: PE 1.5 +/- 0.6; EM 1.4 +/- 0.3) (p=0.02). We found a trend towards a decrease in values in the cells stimulated with EM generator (p>0.05). Furthermore, we evaluated the expression of N-Cadherin in HD and OA cultured chondrocytes after the same stimulations by ICC staining. As regards the non-stimulated cells (HD: 2.1 +/- 0.4; OA: 2.4 +/- 0.2), we revealed statistically greater values (HD: PE 1.3 +/- 0.6; EM 1.2 +/- 0.2; OA: PE 1.7 +/- 0.5; EM 1.3 +/- 1.5) (p=0.01). We found a trend towards a decrease in values in the cells stimulated using EM generator (p>0.05).

Discussion

We conducted this study in order to respond to the controversy in the scientific community as regards whether or not there are differences between the different acoustic wave generators. The results, despite the limited sample and the difficulty to increase the number of cases, thereby hindering reproducibility, allows us to make interesting hypotheses regarding previous knowledge reported in the literature. Until now, there has not been conducted an experimental or clinical study which compares directly all of the different generators. In 2006, Martini et al., in an experimental study compared the effects of electromagnetic and electrohydraulic devices. They found that the electromagnetic device induces fewer cytodestructive effects and more proliferation stimulation on osteoclast cells18. Our results do not support this first hypothesis, because we verified that different types of generators caused similar effects in cell biological response. Furthermore, the tendency to increased action with EM and PE devices, may be interpreted in relation to the smaller size of the focus of these generators, with consequent major focalization of the treatment and major concentration of the dissipated energy.

The second aim of the work was to analyze the effects of the SW on the cartilage. In the animal model it was verified that the application of SW does not induce pathological changes in the articular cartilage9,11,13,19,20. Furthermore, SW improves joint function, reduces inflammatory cytokines which are responsible for osteoarthritic degeneration and enhances the recovery effects13. Recently, three clinical studies support the potential therapeutic utility of SW in the treatment of OA21-23. IL-10 inhibits the synthesis of different pro-inflammatory cytokines (IFN-γ, IL-2, IL-3, TNF-α and GM-CSF) which are hyper-expressed in arthritic conditions. The overexpression of N-Cadherin and β-Catenin is associated with degeneration diseases24. Our results show a trend towards an increased expression of IL-10 and a decreased...
expression of N-Cadherin and β-Catenin after stimulation with different devices. Our data do not allow to confirm that the extracorporeal shockwave therapy is effective in the treatment of osteoarthritis. Further experimental and clinical studies are needed to validate the clinical application of the SW in the treatment of OA and to assign levels of evidence of effectiveness. In conclusion, by observation of the results of our study, we may support an overlap of effects of different shock waves generators. The introduction of the total energy parameter with respect to the EDF (Energy Flux Density) parameter only, currently used to quantify the energy delivered during ESW treatment, may allow us to overcome the possible differences determined by the focal range of different sizes of each generator. This will be useful in order to conform the treatment protocols and the results of the therapies provided with each generator. Also the stimulation with radial waves would appear to be able to determine the biological and therapeutic effects, notwithstanding the physical differences between the radial wave and the shock wave. This is consistent with the new recent experiences on the biological effects and the therapeutic potential of different mechanical stimulations, from mechnano-transduction to vibration\cite{25,26}. The applications of shock waves in the treatment of cartilage and osteoarthritis will represent the next frontier, after wide spread application of this therapy in tendon pathologies\cite{27,28}. Further clinical and in vivo studies are needed to test the effects on humans and define specific treatment protocols.

**Conflict of interest**

The Author has no financial or personal relationships with other people or organizations that could inappropriately influence their work.

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