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NOVEL IN VITRO PEMF EXPOSURE SYSTEM FOR A LARGE NUMBER OF CELL DISHES

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Abstract

Pulsed electromagnetic field (PEMF) stimulation has been utilized clinically to enhance the healing process of bone fractures. The underlying mechanisms are still unclear and the clinical results of PEMF treatment is in contrast with reports on in vitro experiments. These osteoblast cells are known to be sensitive to changes in temperature, climate and mechanical stimulation, especially vibrations like ultrasound. Studies are proposed by different groups which shall assess the potential effect of PEMF signal on differentiation and proliferation of these cell lines in five different maturation time points.

Consequently a PEMF setup is required to expose a large number of samples under controlled conditions. In this study a novel double blind PEMF exposure system is presented that allows to investigate potential PEMF effects to inherent parameter of osteoblast cells for 14 standard tissue culture test plates (up to 3 Petri test plates for each time point). The setup provides a uniform magnetic field up to 2 mT over a frequency range from DC to 20 kHz. Measurements regarding temperature and magnetic flux density distribution, as well as vibration have been conducted for controlled exposure conditions. Since the setup impedes the coupling of mechanical vibration into the sample cells it is also most suitable for PEMF experiments involving other "mechano-sensitive" cells like cartilage or muscle.

Introduction

Tissue Engineering is an emerging market of biotechnology that provides replacement tissue for patients suffering from tissue loss due to injury, disease, genetic abnormality, aging, and/or aging-related diseases. Combining the principles of the life sciences, physical science and engineering, this dynamic and interdisciplinary field seeks to develop biological substitutes for damaged tissues and organs in vitro. The Tissue engineering industries are considered by many analysts as one of the fastest expanding markets worldwide classified as one with substantial development potential. The Orthopaedic and plastic reconstructive industry faces a potential market of 300 Mil. € [8]. Nevertheless, the monetary benefits are controversial discussed due to high costs. Therefore, a significant reduction in costs of the tissue engineered bone would be required, i.e. an optimisation of in vitro osteoblast cell growth.

The discovery of the piezoelectric effect in bones provides the possibility to utilise electromagnetic fields to change the behaviour of bone cells [2]. Pulsed electromagnetic field (PEMF) stimulation has been successfully utilized clinically in the last 40 years to enhance the healing process of fractures by improving bone formation [1]. Bone tissue healing is promoted by enhanced bone cell proliferation and differentiation, thus exogenously applied stimuli that specifically support one or the other, have great therapeutic potential. Clinical PEMF devices have been shown to affect proliferation and differentiation of bone cells in vitro [5].

Figure 1: Experimental results of differentiation assay (ALP active) for confluent cultures of MC-3T3-E1 osteoblast like cells [7] are shown together with the applied PEMF signal.
For example, preliminary in vitro experiments [7] investigating the potential effects of pulsed PEMF on MC-3T3-E1 cells indicate a decrease or delay in differentiation as determined by ALP activity (Figure 1). But the underlying mechanisms are still unclear and the clinical success of PEMF treatment is in contrast with reports on in vitro experiments [3]. Consequently, reliable identification and replication of the effects in vitro is necessary. Such studies require the investigation of individual cell lines in different maturation time points up to 28 days under controlled conditions. In this study, a novel PEMF exposure system is presented that allows to investigate potential effects of PEMF on osteoblast cells in a large number of cells for 14 standard tissue culture test plates (up to 3 Petri test plates for each time point).

**Materials & Methods**

In order to uniformly expose a large number of cell dishes simultaneously, the number of coils together with their coil area has to be optimized. A large coil area results in a high inductance limiting the achievable field strength at a given current as well as the spectral content of the signal that can be applied. In addition, high currents in the coils at PEMF frequencies ($f < 20 \text{ kHz}$) are a source of acoustic vibration and heat. Vibration measurements of a cell dish placed in the center of a standard two pair Helmholtz coil setup showed high acceleration at higher frequencies, especially at non-sinusoidal stimulation (Fig. 2). Osteoblast cells are known to be sensitive to changes in temperature, climate, and mechanical stimulation, in particular vibrations like ultrasound [7]. Consequently, the exposure of the cell cultures to external mechanical stress, like vibration, should be reduced. Other requirements include viable environmental conditions like temperature of $T = 310 \text{ K} \pm 0.3 \text{ K}$, a humidity of 89%, and an air/carbon dioxide (95%/5%) atmosphere with constant oxygen level (18%), as well as no contacts to toxic chemical substances like acids and alkaline. Furthermore, the setup should provide long-term stability and easy access to the cell dishes. The parts of the setup should be autoclaveable. All relevant physical parameter have to be monitored.

**Figure 2:** Left: Measurement results of vibrational acceleration of a standard Petri dish excited by different signals; Right: Numerical results of the magnetic field uniformity for various frequencies using CST EM Solver.

**Figure 3:** Left: Schematic CAD sketch of internal structure; Right: Picture of the realized setup.
Results

A novel PEMF exposure setup was designed maximising the exposure volume whilst minimising mechanical vibration of the cells. Both cell groups, exposed and sham control, consists each of 14 standard cell dishes are placed in two separate µ-metal boxes connected by a fan fitting together into one standard incubator (Figure 3 and 4). Hence all cells are exposed to the same climate and temperature. The exposure chamber contains a holding structure. The cell dishes are stacked on a separate holder allowing for fast access to the cell dishes, mounted on a plate attached to the holder by elastic strings. The six coils are all attached on elastic strings connected to the holder. This setting ensures maximal vibration decoupling. The coils are water cooled for temperature control and for additional damping of high frequencies acoustic vibrations. The coil size, position and individual number of windings are optimised for high field uniformity\(^1\) over a wide frequency range taking into account the frequency depending properties of the µ-metal box (Figure 2). A very compact design of an exposure to box volume ratio around 6.5 % is achieved together with a shielding factor of more than 60 dB between both chambers. All parts contained in the box are manufactured of polycarbonate for easy treatment by autoclave. A sensor network monitors online the temperature, vibration and magnetic flux density. A CPU controlled feeding network capable to generate maximum peak magnetic flux density of 2 mT (\(f < 20 \text{ kHz}\)) allows for different study protocols up to 30 days.

Conclusions

A novel in vitro PEMF exposure setup is presented exhibiting a uniform magnetic field for a large number of cell dishes allowing for long term cell growth experiments under controlled conditions. Special attention was given to minimize mechanical vibration leading to biological reactions in mechano-sensitive cell lines. Future work will focus on the combination of the experimental setup with a high throughput microbiological analysis methods in order to optimise and individualise protocols for enhanced bone cell growth for different cell lines.

References


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\(^1\) The field uniformity is defined as \(u = |B - B_0| / B_0\) where \(B_0\) denotes the fields at the centre of exp. volume [4].