Piezoelectric bone stimulation: should we move closer to nature?

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Introduction

Bone is a natural composite with piezoelectric properties. Bone mass and structure are dependent on mechanical stress and adaptive response at cellular and tissue levels, but the role piezoelectricity plays in bone physiology is yet to be completely understood. Bone piezoelectrical properties have rouse interest, in the context of bone physiology and electro-mechanics. It has been related to bone remodeling mechanisms, and to streaming potential mechanisms [1,2]. Physical activity enhances bone density, through mechanical stimulation [3,4]. Osteocytes and osteoblasts are essential for mechanosensing and mechanotransduction, and cell response depends on strain and loading frequency [5,6]. The aim of this work was to experimentally validate the use of piezoelectric materials as a mean of directly straining bone cells by converse piezoelectric effect.

Materials and Methods

Polymeric piezoelectric films (Polyvinylidene Fluoride (PVDF)) were used as substrate for cell growth. These thin films consist of a 12x13 mm^2 active

area, printed with silver ink electrodes on both surfaces in a 15x40 mm^2 die-cut piezoelectric polymer substrate, polarized along the thickness.

MCT3T3-E1 cells were cultured under standard conditions and on the surface of β-PVDF films, subjected to static and dynamic conditions. In dynamic conditions the substrates were deformed by applying a 5 V current, at 1Hz and 3Hz for 15 minutes. To estimate the magnitude of stress/strain, finite numerical models were applied and theoretical data was complemented by optic experimental data. Cell viability and proliferation were addressed through resarzurin method; nitric oxide was measured in medium after stimulation and normalized to protein content, 24 and 48 hours after seeding.

Normal distribution of the results was verified using the Shapiro-Wilk test, homogeneity of variance accessed through the Levene test and differences between groups tested using one-way ANOVA (at a level of 0.05). The statistical analysis was done using software OriginPro 7.5 (OriginLab Corporation, USA).

Immunofluorescence studies were conducted on the cytoskeleton.

Results and conclusions

The Finite Numerical Method estimated displacement varying from 6.44 to 77.32 nm in the y direction in uncoated films, with strain levels around 2,2 μstrain along the surface. The Electronic Speckle Pattern Interferometry (ESPI) method showed the displacement was higher where the cells were seeded, in the central area of the coated devices (Figure 1).

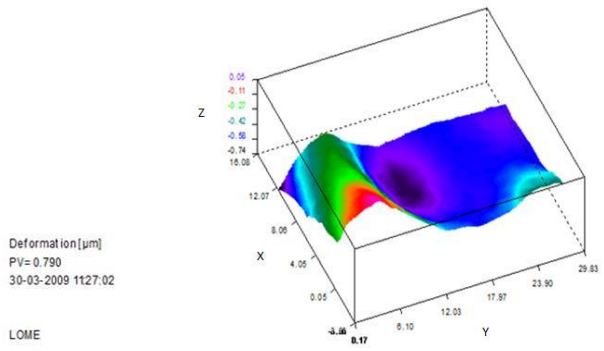

Figure 1. Tridimensional displacement variation occurring along the coated PVDF actuator surface (axis-zz), using ESPI.

Cell viability was affected by the substrate (PVDF vs. customized cell culture dish). Viability was significantly decreased in the groups grown on the device surface but higher in the group subjected to stimulation (Figure 2).

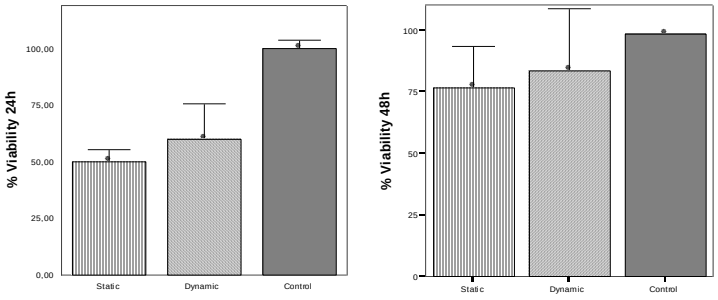

Figure 2. Cell viability 24 and 48 hours after seeding and daily stimulation of the dynamic group, results are expressed in percent related to controls (standard cell culture dish). Bars show Means± Standard Error of the Mean.

Nitric oxide in culture medium after stimulation was significantly higher in dynamic conditions vs. static (Figure 3).

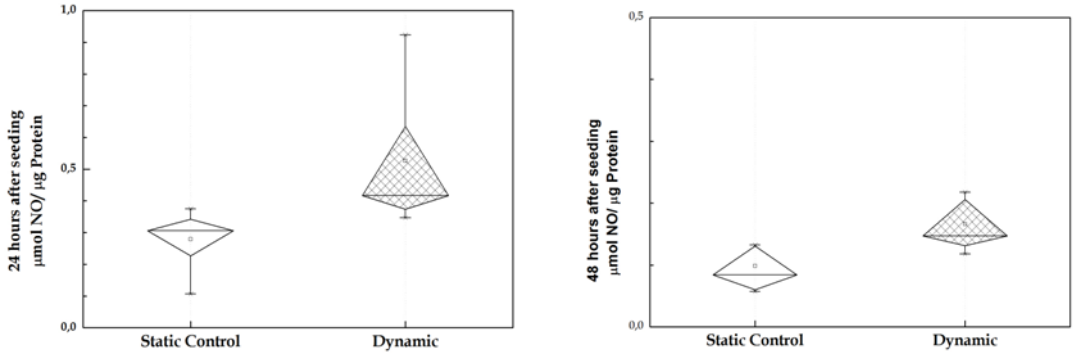

Figure 3. NO measurement (µmol/ml) in culture medium normalized to total protein content (µg/ml) in static vs. dynamic conditions, 24 and 48 hours after seeding, and immediately after stimulation at 1 and 3 Hz. Values are significantly higher in the dynamic group.

Indirect immunofluorescence conducted on cytoskeleton show differences between cells cultured under static vs. dynamic conditions.

The results suggest that the design and development of biomaterials aiming osteoconductivity and osteoinductivity should consider providing them with piezoelectric properties. The results also show osteoblasts are able to sense and respond to minimal displacements of the substrate in a reproducible manner. Piezoelectric based devices may provide effective bone mechanical stimulation.

Acknowlegments

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