ADHESION, GROWTH AND OSTEOGENIC DIFFERENTIATION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS ON POSITIVELY AND NEGATIVELY CHARGED FERROELECTRIC CRYSTAL SURFACES

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Introduction

Cell-biomaterial interaction strongly depends on the physical and chemical properties of the material surface, such as its polarity, wettability, roughness and topography, rigidity and deformability, pH, electrical charge and conductivity (for a review, see [1]). In this study, we focused on the adhesion, growth and osteogenic differentiation of human bone marrow mesenchymal stem cells (hBM-MSC) on uncharged and electrically-charged surfaces with different polarization – positive or negative.

Materials and Methods

The study was carried out on commercially available $LiNbO_3$ substrates (MTI Corporation), namely single crystalline plates, optical grade, dimensions 10 × 10 × 0.5 mm^3, two-sides polished, surface roughness <0.8 nm, (0001) orientation poled perpendicularly to the surface (one surface with the positive charge and the opposite one with the negative charge) and (0100) orientation poled parallel to the surface with zero charge due to the polarization [2]. The samples were sterilized by 70% ethanol, inserted into 24-well cell culture plates (well diameter 15 mm) and seeded with hBM-MSC. Each well contained approx. 10 000 cells/cm^2 and 1 ml of Mesenchymal Stem Cell Medium (ScienCell Research Laboratories). After 6 days, when the cells reached confluence, one half of samples received α-MEM medium supplemented with dexamethasone (10nM), β-glycerolphosphate (20mM) and ascorbic acid (50 μM), and the second half received pure α-MEM. Both media contained 15% of foetal bovine serum, L-glutamine (2mM) and gentamicin (40 μg/ml). The cells on the samples were evaluated for their number, metabolic activity (estimated by conversion of resazurin), type I collagen production (using a Sircol kit), activity of alkaline phosphatase (ALP), calcium deposition (Calcium Colorimetric Assay) and expression of osteogenic markers collagen I, ALP and osteocalcin (using real-time PCR).

Results and Discussion

The number of initially adhering cells on day 1 after seeding, their spreading, shape, and their metabolic activity, production of type I collagen, activity of ALP and Ca deposition in the following days of cultivation (days 6 and 20) were comparable on all three tested surfaces. However, significant differences were found in the expression of mRNA for type I collagen, ALP and osteocalcin, i.e. an early, medium-term and late markers of osteogenic cell differentiation, respectively (FIG. 1). On day 20, the expression of type I collagen was significantly lower in cells on negatively-charged than on non-charged surfaces. Moreover, the expression of ALP and osteocalcin was higher in cells on positively-charged than on negatively-charged surfaces. These differences were generally more pronounced in standard cell culture medium than in osteogenic medium, which could, at least partly, mask the influence of the material surface properties on the cell behaviour. Thus, positively-charged $LiNbO_3$ surfaces seemed to be more suitable for the osteogenic differentiation of bone marrow mesenchymal stem cells than the negatively-charged surfaces.

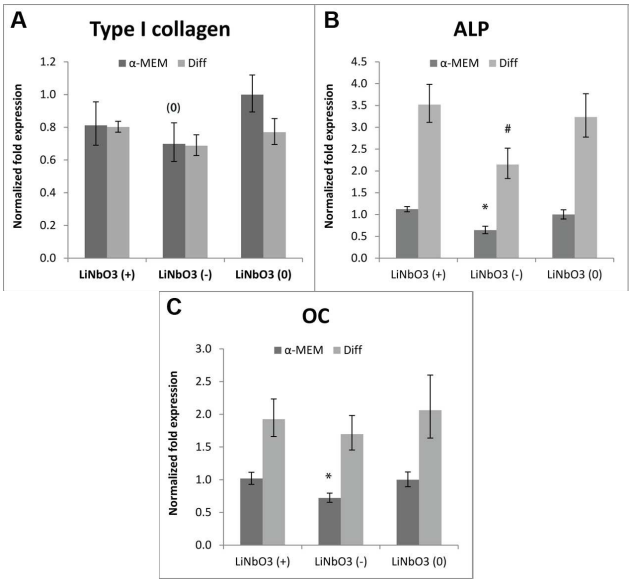

FIG. 1. Gene expression of type I collagen, alkaline phosphatase (ALP) and osteocalcin (OC) in 20 day-old cultures on on positively charged (+), negatively charged (-) and uncharged (0) $LiNbO_3$ surfaces. For the last 14 days, the cells were cultured in standard medium (α-MEM) or osteogenic medium (Diff). Mean ± S.D. from 4 measurements for each experimental group. ANOVA, Student-Newman-Keuls method. Statistical significance: * in comparison with the corresponding samples in α-MEM, # in comparison with the corresponding samples in osteogenic medium, and (0) in comparison with uncharged $LiNbO_3$ sample in α-MEM.

Conclusions

The surface charge of $LiNbO_3$ due to ferroelectric polarization had no significant impact on the adhesion, growth, production of type I collagen and activity of ALP in human bone marrow mesenchymal stem cells. However, the expression of osteogenic markers alkaline phosphatase and osteocalcin was higher in cells on positively-charged than on negatively-charged surfaces.

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References

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