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# OSTEOGENIC DIFFERENTIATION OF HUMAN BONE MARROW STROMAL CELLS ON A POLYCAPROLACTONE MICRO- AND NANO-ROUGHNESS GRADIENT

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**KEYWORDS**

Human bone marrow stromal cells, Micro, Nano, Osteogenesis, Polycaprolactone, Polymer surface, Roughness mediated differentiation, Surface roughness gradient.

**ABSTRACT**

Osteoblastic cells have been reported to respond to roughness features. This topographical cue has been shown to influence osteoblastic proliferation, differentiation and matrix production *in vitro*. We used polycaprolactone gradient surfaces, ranging from the micro to the nanoscale, to access if osteoprogenitors human bone marrow stromal cells (hBMSC) are also influenced towards this phenotype when cultured in the absence of strong soluble osteoinducers such as dexamethasone. Gradients allow for systematic investigation of a large spectrum of average roughness on the same cell population, reducing technical errors of manipulation and culture of several different homogeneous roughness samples. Human BMSCs showed homogeneous quantitative distribution along the 10 mm gradient, but different morphological features on the micro and nano-scale roughness, while alkaline phosphatase and collagen type I differentiation markers were present in the absence of dexamethasone in the medium, more markedly in the microscale roughness. The results indicate that average roughness features in the studied range can provide a successful and cost-effective substitution to soluble osteoinducers, such as

dexamethasone, in the context of human BMSCs osteogenesis.

## INTRODUCTION

The field of tissue engineering uses living cells in a variety of ways to restore, maintain or enhance tissues and organs (Brett et al. 2004). Such regeneration is ideally achieved with the help of a biodegradable polymer onto which cells may be seeded and subsequently implanted into the patient, where the material gradually resorbs, leaving behind a matrix of connective tissue and cells with the appropriate structural and mechanical properties.

Cells are inherently sensitive to their surroundings(Brett et al. 2004) and therefore, for the success of any strategy, it is essential to correctly choose the chemical, physical and topographical cues and signals on the support material that will enable the engineered construct to develop into a fully functional tissue(Tang et al. 2004). Patterned surfaces influence cell adhesion, outgrowth, migration, organization and tissue development. Therefore, an important area of research aims at developing the surface properties that positively influence the cellular behavior.

Among the topographical features that influence bone cells' profile of genes (Brett et al. 2004),(Schneider et al. 2003), therefore affecting osteoblast proliferation, differentiation and matrix production *in vitro* (Martin et

al. 1995), (Ball et al. 2008) is roughness. However, during the bone formation after adult fracture repair and remodeling not only osteoblasts are involved, but also osteo-progenitor cells, namely mesenchymal stem cells (MSCs)(Bruder et al. 1994). MSCs which reside in the bone cavity are known as bone marrow stromal cells (BMSCs) and can differentiate into several stromal cells besides the osteoblasts, such as chondrocytes, adipocytes or endothelial cells (Yin 2006). For this reason, the control of the implant surface features that can influence the BMSC differentiation is of extreme relevance in the context of both bone tissue engineering and bone substitution by prosthetic implants.

Our approach uses a polycaprolactone roughness gradient sample varying from 4.9 µm down to 21.8 nm of average roughness (*Ra)* to study the role of this topographical cue on the human BMSC differentiation into the osteogenic lineage, under culture conditions lacking dexamethasone – a strong soluble osteogenic inducer usually required to guide MSC osteo-differentiation.

## METHODS

### Gradient surface fabrication

The gradient polycaprolactone replicas were produced by hot embossing lithography, where the micro- and nano-features from epoxy masters, produced as described by Wieland(Wieland et al. 2002) and Schuler et al.(Schuler et al. 2009), were imprinted onto PCL membranes. An 120g weight load was applied on the polymer, through the master, at 80ºC for 15 minutes. The PCL was then allowed to cool down and sterilized by air-plasma treatment.

### Characterization of the gradient surface

Gradients were characterized with an optical profilometer. Profiles were measured perpendicular to the long gradient axis (1 cm) and the data evaluated with SensoMap 6.1.0.6001. The roughness parameters were calculated according to DIN EN ISO 4288-98.

### Cell culture

Human bone marrow stromal cells at passage 1 were seeded in a concentrated drop of 5000 cells per $cm^2$ sample in α-MEM supplemented with 10%FBS, 1% PSN, 50mM ascorbic acid phosphate and 2mM β-glycerophosphate, lacking dexamethasone. The positive control samples were cultured in the same medium in the presence of dexamethasone; while the negative control samples were cultured in expansion medium.

Cells were fixed and permeabilised with 4% parafolmaldehyde/0.2% triton x-100 for 8 minutes and stored in PBS without glucose until stained for specific alkaline phosphatase, collagen type 1, actin and DAPI.

## RESULTS

Evaluation from profilometry showed a gradient roughness average of 4.9 µm down to 21.8nm, along the 10 mm size sample.

Immunolabeling results indicated that the earlier osteogenic marker ALP is expressed in osteogenic medium lacking dexamethasone at day 7, contrasting with their absence from the negative control samples. Also, this expression is more clear in the microroughness scale. Also in the absence of dexamethasone, expression of collagen type 1 at day 14 is ubiquitous, but shown to be more intense on the microroughness side. The cellular distribution seems to be homogeneous along the gradient, as accessed by nuclei counting in function of the spatial positioning, but cell morphology is clearly different. Cellular elongation is characteristic of the nanoscale roughness, while cuboidal geometry is more evident in the microscale roughness areas.

## CONCLUSIONS

In the present work, we found evidence that confirm that roughness features in the range of 4.9 µm down to 21.8nm can substitute strong soluble osteoinducers, such as dexamethasone, in the context of osteogenic differentiation of human BMSCs. This approach shows to be an elegant and cost-effective cue in the context of bone tissue engineering and bone prosthetic device surface modification.

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