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Nanotopology potentiates growth hormone signalling and osteogenesis of mesenchymal stem cells

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ABSTRACT

Custom engineered materials can influence the differentiation of human mesenchymal stem cells (MSCs) towards osteoblasts, chondrocytes and adipocytes, through the control of chemistry, stiffness and nanoscale topography. Here we demonstrate that polycaprolactone growth surfaces engineered with disordered (but controlled) 120 nm diameter dots (NSQ50), but not flat surfaces, promote osteogenic conversion of MSCs in the absence of other osteogenic stimuli. Differentiating MSCs on NSQ50 were found to express growth hormone receptors (GH) and stimulation with recombinant human GH (rhGH) further enhanced NSQ50-driven osteogenic conversion of MSCs. This increased osteogenesis coincided with an enhanced ability of GH to activate ERK MAP kinase on NSQ50, but not on flat topology. The importance of ERK for MSC differentiation was demonstrated by using the inhibitor of ERK activation, U0126, which completely suppressed osteogenesis of GH-stimulated MSCs on NSQ50. The ability of GH to activate ERK in MSCs may therefore be a central control mechanism underlying bone development and growth.

1. Introduction

The growth hormone (GH)/insulin-like growth factor 1 (IGF-1) axis is closely linked to chondrogenesis and osteogenesis [17]. This is evidenced by GH deficiency and deletion of GHRs in mice and humans leading to a decrease in bone mass and mineral density [6,16]. Moreover, in rodent models, GH enhances osteoblast precursor pool size and increases osteoblast differentiation [11]. These osteogenic actions of GH appear to occur independently of the classical route of transcriptional control by GH, via activation of the transcription factor STAT5 [16]. GHRs have been detected on murine bone marrow mesenchymal stem cells (MSCs), suggesting a role in osteogenesis [8] and osteogenesis of MSCs appears to be dependent on signalling pathways downstream of GH [3] and involves the activity of the MAP kinase, ERK [5].

MSCs are readily found within the human bone marrow where they undergo self-renewal and differentiate into osteoblasts (bone), chondrocytes (cartilage), adipocytes (fat), fibroblasts (connective tissue) and myoblasts (muscle) and their multi-potency can be demonstrated using defined culture medium, which leads to simultaneous formation of bone, cartilage, fat and reticular tissues through the formation of a range of developmental stages [12,14]. Currently, the major problem for detailed MSC studies is their tendency for spontaneous differentiation, resulting in a heterogeneous population of mainly fibroblastic cells when grown on plastic surfaces in vitro. However, in recent years, advances in high-resolution electron beam lithography (EBL) means that nanoscale topographies can be precisely fabricated and then embossed into biocompatible polymers. These nanometric growth surfaces have been shown to promote osteogenesis from stem and progenitor mesenchymal populations in the absence of osteogenic supplements [4]. Whilst this osteogenic property can be seen as a potential superior implant material in orthopaedics [4], it also provides a better control substrate for MSC growth and bone differentiation without recourse to complex media formulations. In the current study we use a nanometric growth surface to explore the effects of GH on the osteogenic conversion of human MSCs in vitro. This revealed a novel, regulatory interplay between nanoscale topography and GH signalling through the ERK MAP kinase cascade in the control of the osteogenic conversion of human MSCs. For the first time we demonstrate how nanometric topology can influence signalling from hormone receptors to direct the differentiation of MSCs.

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2. Materials and methods

2.1. Materials

Anti-recombinant bovine GH antiserum was a generous gift from Professor David Flint (University of Strathclyde).

2.2. Preparation of cell growth surfaces

The cell culture growth surfaces used throughout this study were nanoscale topography near square 50 (NSQ50) or flat surfaces embossed onto the biodegradable, polyester polycaprolactone (PCL). The nanoscale growth substrate was fabricated by electron beam lithography to form arrays of 120 nm diameter pits of 100 nm depth and average 300 nm pitch in a square arrangement. The EBL tool was programmed to introduce random displacements of up to ±50 nm in X and Y, maintaining an average 300 nm pitch, as previously described [4]. After fabrication, nickel dies were made directly from the patterned resist samples. A thin (50 nm) layer of Ni–V was then sputter-coated on the samples. This layer acted as an electrode in the subsequent electroplating process. The dies were plated to a thickness of about 300 μm. Polymeric replicas were made in PCL by hot embossing. Beads of PCL were melted on a hot plate and the nickel die was pressed by a thumb into it for about 10 s and then transferred to an ice box to allow the sample to cool. All the subsequent replicas possessed a nano-imprinted area of 1 cm^2. As a control surface, flat PCL with similar area was used, which was made by hot-embossing over a glass slide (Ra = 1.174 nm over 10 μm).

2.3. Extraction of bone marrow MSCs

Stromal progenitors containing MSCs were extracted from bone marrow samples obtained from haematologically normal adults

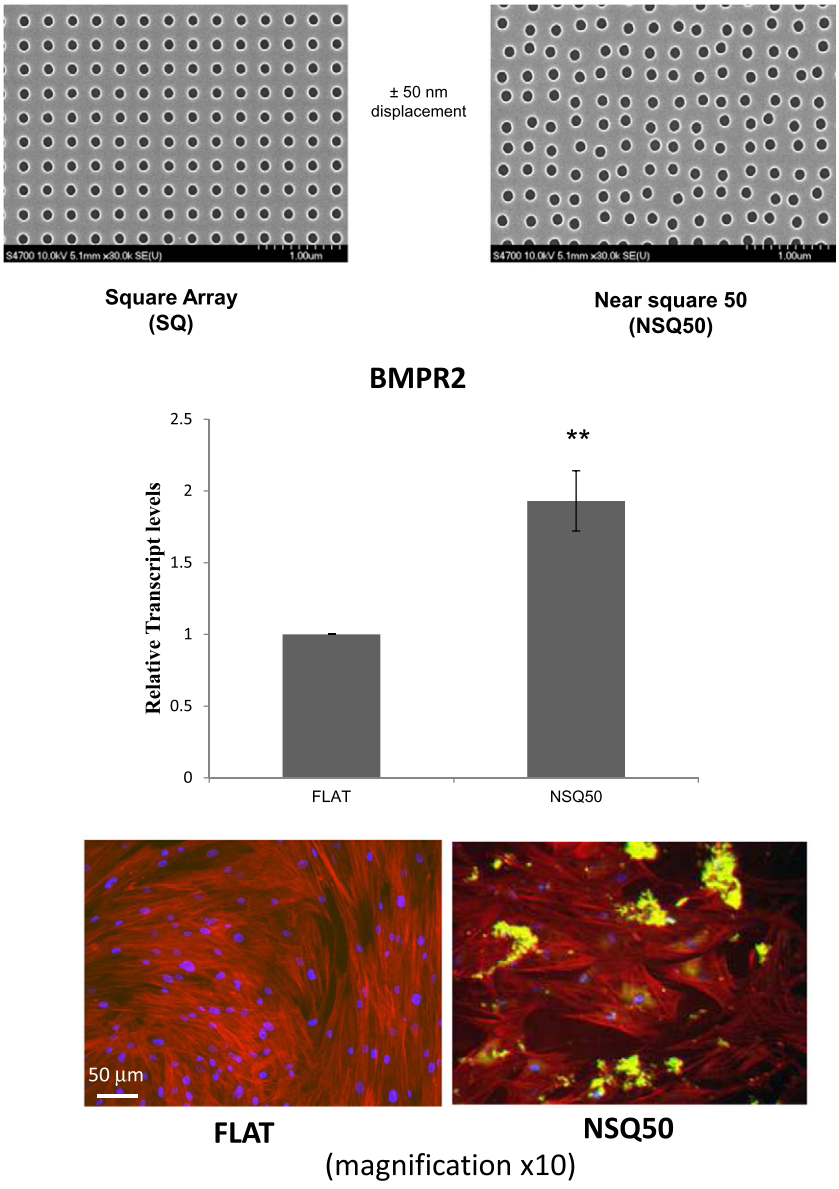

Fig. 1. In the *upper panel* are nanotopographies fabricated by electron beam lithography. Both contain 120 nm diameter pits (100 nm deep). The left image shows a square array with an even average centre–centre spacing of 300 nm. The image on the right is a disordered square (NSQ50) array with dots displaced randomly at ±50 nm on both axes from their position in a true square. NB. In the *lower panel* are qRT-PCR results measuring transcript levels for BMPR2 in MSCs cultured on flat or NSQ surfaces. The relative transcript levels were normalised to cells cultured on the flat substrates and are means ± SD for three separate experiments. Statistical differences are indicated, $^{**}p < 0.01$. Osteopontin (OPN) staining of MSCs after 28 days of culture with the indicated treatments is also shown in the *lower panel*. OPN is stained green, actin is stained red and nuclei are stained blue.