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Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2014) xxx–xxx

nanomedicine
Nanotechnology, Biology, and Medicine

nanomedjournal.com

# Substrate topography determines the fate of chondrogenesis from human mesenchymal stem cells resulting in specific cartilage phenotype formation

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Received 28 November 2013; accepted 5 April 2014

## Abstract

To reproduce a complex and functional tissue, it is crucial to provide a biomimetic cellular microenvironment that not only incorporates biochemical cues, but also physical features including the nano-topographical patterning, for cell/matrix interaction. We developed spatially-controlled nano-topography in the form of nano-pillar, nano-hole and nano-grill on polycaprolactone surface via thermal nanoimprinting. The effects of chondroitin sulfate-coated nano-topographies on cell characteristics and chondrogenic differentiation of human mesenchymal stem cell (MSC) were investigated. Our results show that various nano-topographical patterns triggered changes in MSC morphology and cytoskeletal structure, affecting cell aggregation and differentiation. Compared to non-patterned surface, nano-pillar and nano-hole topography enhanced MSC chondrogenesis and facilitated hyaline cartilage formation. MSCs experienced delayed chondrogenesis on nano-grill topography and were induced to fibro/superficial zone cartilage formation. This study demonstrates the sensitivity of MSC differentiation to surface nano-topography and highlights the importance of incorporating topographical design in scaffolds for cartilage tissue engineering.


*Key words:* Mesenchymal stem cell; Chondrogenesis; Nanotopography; Zonal cartilage

## Introduction

Treatment of defective adult cartilage tissue poses a significant clinical challenge. The adult cartilage tissue has a poor self-regenerative capacity due to its low cellular mitotic activity, low supply of progenitor cells and its avascularity.[1] Although there were attempts to use culture-expanded autologous articular chondrocytes for the treatment of cartilage defects, the limited source of healthy donor cartilage and the low proliferative capacity of the isolated chondrocytes pose a major

Disclosure of potential conflicts of interest: Authors declare no competing interests.

This study was funded by Mechanobiology Institute (National University of Singapore). The use of human samples was with the approval of IRB-NUS Reference Code: 08-097.

Authors' contribution: YNW and JBKL designed and performed experiments, analyzed data and wrote the manuscript. AYH performed experiments. HYL provided technology and research planning. JHPH provided clinical samples and critical reading of the manuscript. CTL provided technology, equipment and analyzed data. ZY and EHL planned research, obtained funding, and wrote the manuscript.

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http://dx.doi.org/10.1016/j.nano.2014.04.002
1549-9634/

challenge in providing adequate cell numbers for viable cartilage repair.[2] Furthermore, prolonged period of ex-vivo expansion results in extensive de-differentiation of the chondrocytes which yield cartilage of compromised functionality.[3]

Cartilage tissue engineering using stem cells to generate chondrocytes is becoming an expanding research field as it offers a promising approach for cartilage repair that provides an alternative approach to the existing clinical problem. In particular, mesenchymal stem cells (MSCs) offer an attractive cell source due to their ease of extraction from human bone marrow, high proliferation capacity and their ability to be differentiated into chondrocytes.[4,5] However, there remain challenges in using MSCs to repair cartilage as cartilage generated from MSCs is inferior in its biochemical content and mechanical strength when compared to physiological cartilage.[6,7] In addition, MSC-based cartilage tissue regeneration results in homogeneous cartilage tissue structures with little resemblance to the native zonal organization of articular cartilage.[8] Articular cartilage can be subdivided into different zones, namely, the superficial, middle and deep zones. The morphology of the chondrocytes, as well as the composition and structural arrangement of the extracellular matrix (ECM) components, varies greatly between these zones. The quantity of proteoglycans, the complex interactions of collagen with other matrix components, and the zone specific alignment of collagen fibers are paramount to the mechanical properties and thus the overall function of articular cartilage.[8,9]

During tissue development, cells have been known to be influenced not only by the biochemical cues and bioactive growth factors, but also by the topographic cues in their ECM microenvironment.[10,11] Topographic influence of cells to micrometer range features was reported by Curtis in 1964[10] and has since been extensively studied and well-established.[11,12] Besides microtopography, the natural ECM that cells is exposed to typically have nanoscale topographies as well. Recent advances in nanofabrication techniques[13,14] have driven current research interests to explore how the topography of a surface, when engineered with similar nanoscale features mimicking those in ECM, can be used to control cell behavior. Adaptation to nanoscale topography alone has been shown to elicit different responses from fibroblasts, smooth muscle cells, osteoblasts, and MSCs, resulting in increased cell attachment, proliferation, and expression of matrix components.[15-19] Although nano-topographic surface and its effect on MSC chondrogenesis have been previously reported, the results shown have been varying.[20,21] A common limitation among these works is that the topography of interest was random and was not spatially-defined. The ability to specifically and spatially control the patterning of nano-features is a pre-requisite to allow systematic studies on the influence of nanotopography on MSC chondrogenesis, especially to investigate how specific nano-topographies regulate the formation of different zonal cartilage.

In this study, a more systematic and controllable approach was employed to study the effect of spatially controlled nanotopography on MSC chondrogenesis. Specific nanotopographies were fabricated using nano-imprinting. Nanoimprinting has been touted as the next generation nanopatterning technique offering high resolution (down to 10 nm), scalability and cost effectiveness.[22] Three distinct nanopatterns, namely, nano-grill, nano-hole and nano-pillar were chosen to represent the topographical cues presented by the cartilage collagen fibril orientation.[23] Roughly, nano-grill represents the parallel collagen fibers of the superficial and deep zone cartilage; nano-hole represents the pore formed among the randomly oriented collagen fibers; and nano-pillar representing intersecting point of the random collagen fibers. These nano features were directly patterned on the polycaprolactone (PCL) film, creating distinct nano-patterned surfaces (see Supplementary Figure 1). Chondroitin sulfate (CS) was immobilized on these surfaces to provide a chondro-inductive biochemical cue.[24,25] The effect of CS-coated nano-topographies on the seeded MSCs' morphology, cytoskeleton arrangement; proliferation and chondrogenic differentiation were investigated and compared to a CS-coated non-patterned pristine PCL surface.

## Methods

### *Polycaprolactone (PCL) film preparation*

PCL film was prepared by modified thermo-pressure method.[26] Briefly, PCL beads were heat-pressed at 80 °C, 500 bar for 12 hours in argon environment, followed by cooling down to room temperature overnight.

### *Nano-patterning of PCL film via nanoimprinting*

Silicon molds, (nano-grill, nano-hole: Institute of Microelectronics; nano-pillar: NIL Technology) were comprised of three different types of the inverse nano-features: (1) nano-grill: 250 nm line, 250 nm space and 150 nm height; (2) nano-hole: 225 nm diameter hole, 400 nm pitch and 300 nm height; (3) nano-pillar: 250 nm diameter pillar, 500 nm pitch and 250 nm height. The desired nano-topographies were directly patterned onto the PCL film by thermal nano-imprinting (Obducat AB Nanoimprinter) under optimized condition of 80 °C, at a pressure of 60 bar, for 5 min (Supplementary Figure 1).

### *Characterization of the nano-patterned PCL film*

The morphology of both the pristine PCL film and the nano-patterned PCL film was examined by using a scanning electron microscope (SEM) (JEOL 5600).

### *Chondroitin sulfate (CS) immobilization and measurement*

The CS immobilization was modified from the report.[27] Briefly, PCL films were aminolyzed with 20% 1,6-diaminohexane/ethanol solution for 15 min, washed by PBS, and treated with a carbodiimide solution (48 mM EDC and 6 mM NHS in 50 mM MES buffer, pH = 5.5) and 10% CS MES solutions (CS concentration at 1, 5, 10 mg/ml) for 24 hours at 37.5 °C. The amount of CS immobilized on PCL film was measured using Blyscan sulfated glycosaminoglycan assay kit (Biocolor Ltd, Newtownabbey, Ireland) following the manufacturer's protocol.