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CONTROLLING THE FORMATION OF OSTEOBLAST-OSTEOCYTE INTERACTIONS BY MICROPATTERNING TO STUDY BONE CELL MECHANOBIOLOGY

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ABSTRACT

Over the past 30 years, it has been extensively reported that bone cells are sensitive and responsive to mechanical stimulation. Mechanical cues promote bone strength whereas the lack of physical stimuli results in significant bone loss. However, despite many in vivo and in vitro studies, the molecular mechanisms through which bone cells communicate together to maintain bone tissue homeostasis in response to mechanostimulation are hardly understood. Therefore, we developed 2-dimensional (2D) bone cell arrays by robotic printing and cell micropatterning to study the mechanobiology of bone cells in communication with each other.

KEYWORDS: Bone cells, Mechanobiology, Bone-on-a-chip, Cell micropatterning, Robotic printing

INTRODUCTION

Bone tissue homeostasis relies on the balanced activities of two cell types: bone-forming osteoblasts and bone-degrading osteoclasts. This equilibrium is ensured by the third bone cell type, namely the osteocytes [1]. Upon extreme physical loads, bone tissue may endure cracks, which jeopardizes bone strength [2]. In response, osteocytes first direct the osteoclasts to degrade the damaged bone and subsequently promote the osteoblasts to produce new bone. This process, called bone remodeling, relies on the intrinsic ability of bone cells to recognize and adapt to physical cues [3]. Each bone cell type was demonstrated to be mechanosensitive and responsive, independently of each other [1,4,5]. Nonetheless, their mechanobiology is still poorly understood. Moreover, bone remodeling is a cooperative process. In this regard, critical information with respect to osteoblast-osteocyte-osteoclast communication is still missing. The development of 3-dimensions (3D) printing opened new paths enabling the study of bone cell communication in a physiological environment [6]. However, analyzing 3D printed structures often requires specific imaging methods with lower resolution. On the contrary, 2D cell micropatterning and robotic printing enable creating physiological-like cell arrays with controlled cell-cell interactions, readily observable by conventional high-resolution microscopy techniques.

EXPERIMENTAL

We created bone cell arrays of osteoblasts and osteocytes in communication with each other by means of two different technologies, namely robotic printing and cell micropatterning. Robotic printing of bone cells was achieved with a non-contact printer. Osteoblast and osteocyte suspensions were printed onto glass coverslips previously coated with fibronectin. The substrate was then covered with a microfluidic channel to enable medium perfusion and cell growth. Figure 1A-B shows examples of cell arrays printed to promote specific homo- and heterotypic interactions between osteoblasts and osteocytes. Cell-cell communication was further confirmed by immunostaining for connexin 43, which is the main component of gap junctions within the bone tissue (Figure 1C).

Figure 1: (A, B) Schematic illustration of the printed cell arrays and their corresponding fluorescence images. Osteoblasts and osteocytes were labelled in green and red respectively. Arrows indicate the clear-cut interfaces between the 2 different cell populations. (C) Zoom showing the interaction between an osteoblast (green) and an osteocyte (red). The actin fibers are labelled in magenta and the connexin 43 protein in blue (obj.: 150x).
Complementary to robotic patterning, we used surface micropatterning to design networks of osteoblasts and osteocytes interacting with each other. First, we optimized micropatterns whose geometries and dimensions produced the best in vivo-like phenotype of osteoblasts (square-like morphology) and osteocytes (dendritic-like morphology) (Figure 2A-C). Then, we assembled these micropatterns together into a network controlling the formation of homo- and heterotypic interactions between osteoblasts and osteocytes (Figure 2D).

![Image](image-url)

Figure 2: (A, B, C) Fluorescence images of the micropatterns optimized to generate the in vivo-like morphology of osteoblasts (A) and osteocytes (B,C). The fibronectin micropatterns were labelled in green, the cell actin fibers in red and the nuclei in blue. Scale bar = 10 µm; obj. = 60x. (D) Fluorescence picture of the micropatterned network designed to simultaneously grow osteoblasts (on the square area) and osteocytes (on the star area) in communication with each other. Fibronectin micropatterns were labelled in green (obj.: 10x).

RESULTS AND DISCUSSION

By means of robotic printing, we were able to deposit nanoliter droplets of bone cell suspensions at specific locations with micrometer accuracy. As a result, we could print arrays of osteoblasts and osteocytes in close proximity to each other. By adapting the pitch distance between the droplets, we were able to create clear-cut interfaces of communicating cells (Figure 1).

Surface micropatterning was used to promote bone cells to adopt their in vivo-like cell morphology. The cubic morphology of the osteoblasts could readily be obtained with the “I”-shaped micropattern. On the contrary, the two star-shaped micropatterns (4- and 6-branches) for dendritic osteocytes produced mixed results. Whereas the 6-branches star micropattern resulted in a broad spreading cell, the 4-branches star micropattern generated a more dendritic-like phenotype. The later was thus selected to construct the network of osteocytes interacting with osteoblasts. A mixed cell suspension was seeded onto the network and primary results showed that osteocytes and osteoblasts interacted at the square-star interface. Further experiments will be performed to confirm and extract valuable information on the osteoblast-osteocyte communication under fluid flow shear stress.

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REFERENCES


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