DESIGN OF A 3D MICROFLUIDIC SYSTEM FOR LOADING FLUID FLOW ON OSTEOCYTE PROCESS IN VITRO

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1. INTRUDUCTION

Mechanical loading is an important regulator of bone metabolism. However, the cellular level regulation of how bone cells respond to mechanical loading is incompletely understood. Osteocytes are believed to be the critical mechanical sensor cells [1, 2], although the mechanism by which osteocytes perceive mechanical load is not known. Loading induced fluid flow shear stress has been proposed to be the signal that osteocytes sense [3]. Many in vitro studies have shown that bone cells response to shear stress [5-7]. However, most in vitro studies have the cells grown on slides and exposed them to fluid flow, which is very different from in vivo situation, where cells are licated in a 3-D channel system, lacunar-canalicular system. Moreover, as discussed in a recent paper by You et al. [8], the mechanical signal that osteocytes detect might be the fluid flow induced large cell deformation on osteocytic cell process membrane instead of shear stress. Therefore, in order to further understand the mechanism of mechanotransduction on osteocytes, we design a microfluidic system which mimic the canalicular system so that we can load the fluid flow on osteocytes process and then observe the intracellular response.

2. MATERIAL AND METHOD

Microfluidic system: Two layers of transwell polycarbonate membranes (Corning, Life Science) are contained in a flow chamber (Fig.1, Fig.2). The first layer membrane pore size is 3 micro in diameter. The second layer membrane has pore size of 0.4 micro in diameter. Both membranes are 10 micro thick. Cells are cultured on the first layer, processes will grow through the pores of the membranes. The flow inlet and outlet are located on the wall of the middle part and lower part of the chamber. When fluid flow is introduced, the fluid will flow between the middle part and lower part of the chamber through the pores of the second layer transwell membrane. Osteocytes processes in the second layer membrane will then be exposed to fluid flow in the channels surrounding them.

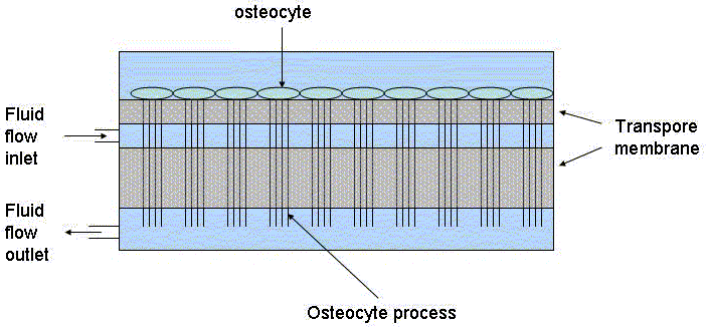


Fig. 1 Schematics of micro-culture system.

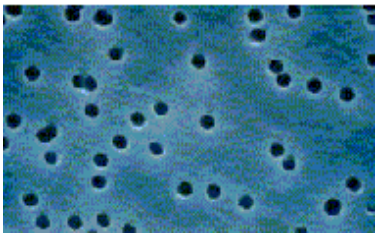
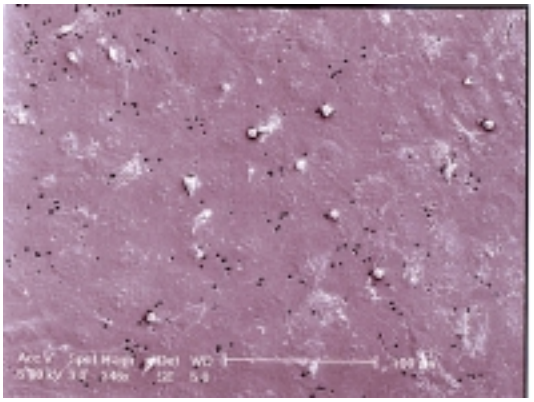


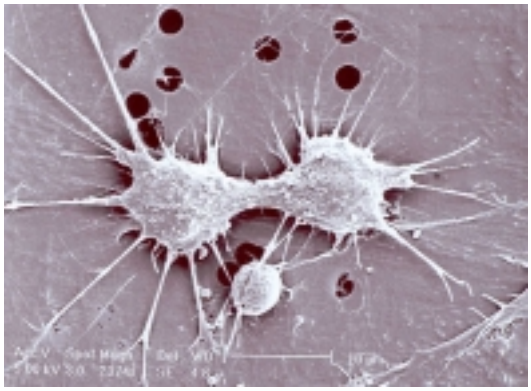
Fig. 2 SEM of the surface of a pore membrane

3. RESULT

Fig. 3 shows that MC3T3-E1, osteoblasts-like cells, can grown on transwell polycarbonate membrane. Some processes can grow into the pores of the membrane.



A.



B.

Fig. 4 SEM of bone cells grow on the transwell membrane. A. at lower magnification, x346, B. at higher magnification, x2374

4. DISCUSSION

Our preliminary study suggest that bone cells can grow on the polycarbonate transwell membrane and the processes can grow into the micro channels of the membrane. Currently we are using MC3T3-E1 cells to test the system. In the future, osteocytes (MLO-Y4) (gift of Dr. Linda Bonewald of the University of Kansas) will be seeded on top of the membrane. After loading fluid flow, we will collect the osteocytes and look at the cell response in terms of osteopontin gene expression. This research will provide insight in mechanotransduction on osteocytes process.

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