THREE DIMENSIONAL CULTURE OF OSTEOBLASTS-LIKE CELLS ON POROUS CERAMIC SCAFFOLDS UNDER OSCILLATORY FLOW

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INTRODUCTION

By nutrient transport and mechanical stimulation, perfusion culture systems with unidirectional flow have been proven effective bioreactors for construction of tissue engineering bone in vitro. But bone interstitial flow in vivo is oscillatory in nature. To simulate this flow environment is necessary of applying dexamethasone in flow condition need more investigation in animal experiments. The oscillatory perfusion culture could construct 3D engineering bone with uniform living cells distributed, as well as their matrix probably, whereas the static culture only has living cells on the surface as a thick layer with dead cells in the center and bottom. Thus oscillatory perfusion culture may be a useful method for bone tissue engineering.

METHODS

A perfusion bioreactor, which could provide porous scaffolds with oscillatory flow, was designed (Fig.1). Scaffold was set in each culturing well by silicon cassette for preventing the media from flowing around them. Medium in 6 culturing wells was perfused oscillatory by up-down movement of their silicon membrane bottom, which was driven by a continuous cycle syringe pump.

Mouse MC 3T3-E1 osteoblast-like cells were subcultured by culture medium consisting of α-minimum essential medium (α-MEM) 1% penicillin-streptomycin and 10% fetal bovine serum. Cells were seeded in 100µl medium containing 1.5×10^5 MC 3T3 cells by perfusing through cylindrical β-TCP scaffolds (10mm diameter×8mm height and a porosity of 75%) in the perfusion system. After perfusing the cell suspension in 3ml/min for 3min, scaffolds for static culture were released from the cassettes and set into 24-well plate while another 1.4ml media was added. After static period of 24 hours, culture media were changed with fresh media with or without 10^{-8}M dexamethasone supplement accordingly. Scaffolds for perfusion culture were cultured under oscillatory flow (1ml/min per scaffold, 1/60Hz, total culture media=1.5ml); scaffolds for static culture were cultured statically continuously. Media in 6 culturing wells was renewed every day. After another 6 days of culture, the cellularity of each scaffold was evaluated using the PicoGreen assay kit (Molecular Probes, Eugene, OR) to measure dsDNA content.

RESULTS

As Fig.2, there is no significant difference between the groups. But there is a trend that the groups of same condition with dex have a lower cell viability under oscillatory fluid flow. So the dexamethasone may enhance the effect. But the dexamethasone could also decrease the cell viability under oscillatory fluid flow. So the necessary of applying dexamethasone in flow condition need more investigation in animal experiments.

As shown in Fig.3, perfusion culture with dex has significantly higher average ALP activity than static culture (P< 0.05, n=3). There is also a trend that Perfusion culture with out dex has higher ALP activity than static culture without dex (P=0.06, n=3).

As Fig.4, in static culture (A), living cell (green) only accumulated on the surface of scaffold as a thick layer, except bottom. The cells in center and bottom are mainly dead cells (red). In perfusion culture (B), living cells distributed throughout the scaffold uniformly. With further observation under 10×magnification, we found that the cells in the center of static culture (C) had contracted plasma shape with red stained nuclei, the cells of perfusion culture (D) has a spread shape with only few red stained dead cells. The percentage of living cells in axial part of section view is oscillatory perfusion system images, scale bar (A, B) =5mm, scale bar (C, D) =100µm; E): percentage of living cells with flow seems fewer than those without flow.

DISCUSSION

Although there is a strong tendency that oscillatory flow only could have better osteogenic effect than static culture (P=0.06, n=3), dexamethasone may enhance the effect. But the dexamethasone could also decrease the cell viability under oscillatory fluid flow. So the necessary of applying dexamethasone in flow condition need more investigation in animal experiments. The oscillatory perfusion culture could construct 3D engineering bone with uniform living cells distributed, as well as their matrix probably, whereas the static culture only has living cells on the surface as a thick layer with dead cells in the center and bottom. Thus oscillatory perfusion culture may be a useful method for bone tissue engineering.