Biomimetic Culture Of Mammalian Cells In A Perfusion Bioreactor

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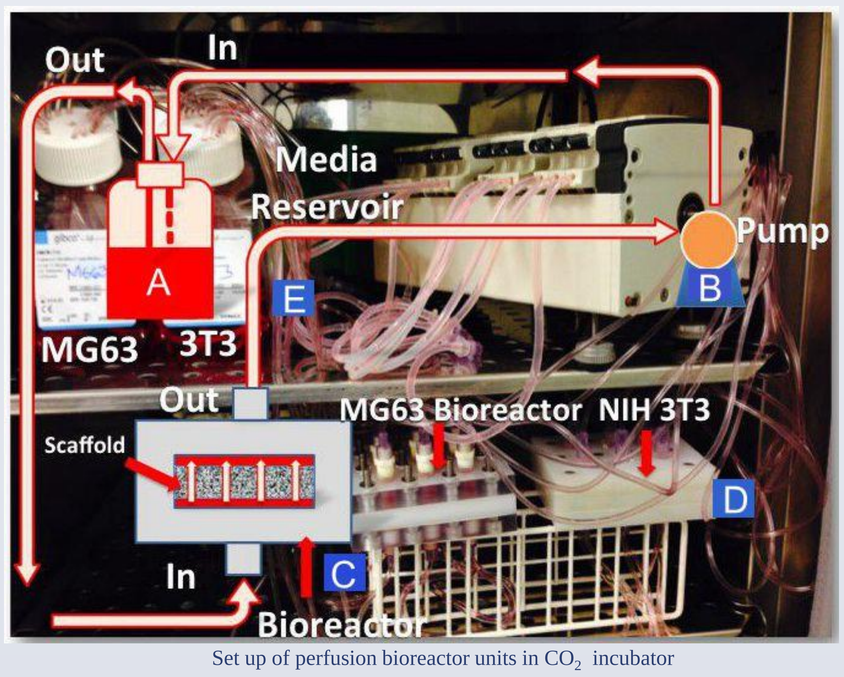
Introduction

In traditional static culture of tissue engineering, the nutrients and oxygen are delivered into scaffolds by simple diffusion [1]. When culturing cells in 3-D scaffolds, limitations on nutrients and oxygen availability cause the cells grow and proliferate at the medium-scaffold interface in preference to the center of the scaffold where cells are low in number with poor viability[2]. Perfusion bioreactors have been developed to create dynamic culturing conditions that can mimic the native environment which different from static culturing. Studies have shown that the perfusion culture has the ability to increase the cell viability and proliferation by providing a constant oxygen supply to the center of the cultured scaffolds [3]. It has also been suggested that, the mechanical forces generated by the fluid flow may also have the ability to stimulate cell activity and differentiation, which leads to the enhancement of tissue regeneration[4]. In this study, a custom made perfusion bioreactor was used to culture mammalian cells (NIH-3T3 & MG63) and the results were compared with static method.

Design of perfusion bioreactor

The perfusion culturing system used in this study contains a bioreactor major body which consists of 6 culture chambers (D, 10 mm in length, 10 mm in diameter), a peristaltic pump and a medium reservoir which filled with culture medium(A). The perfusion bioreactor was made of ultra-high molecular weight polyethylene (UHMWPE), and each bioreactor contains sandwich-like structure which is sealed with 2 silicone gaskets (C). A multi-channel peristaltic pump (B) was used to pump the medium from bottom to the top of each chamber and cycle the medium within the whole perfusion system uninterruptedly. Commercially available luer (E) and silicone tubes were used to completed the fluid circuit.



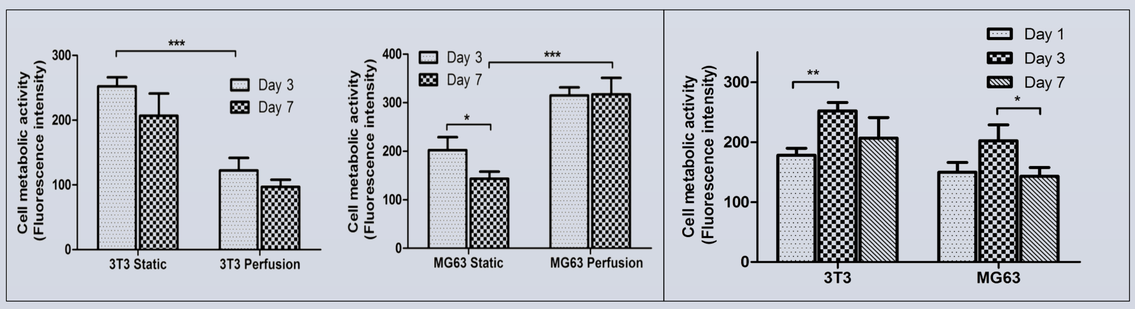
Set up of perfusion bioreactor units in CO_2 incubator

Materials and methods

Gelatin/chitosan porous scaffolds with 90% porosity and average pore size of 45μm were fabricated and used through this study. Cells were seeded on top of each scaffold. The seeded scaffolds were transferred into bioreactor or continuing culture in well plates. The constant perfusion culturing was set to 0.047 ml/min. The culturing periods were set as 1, 3 and 7 days for static culturing and 3, 7 days for perfusion. Cell viability was determined using Alamar Blue and cell distribution was analysed by fluorescent microscopy. Cell morphology was studied using a scanning electron microscope.

Results

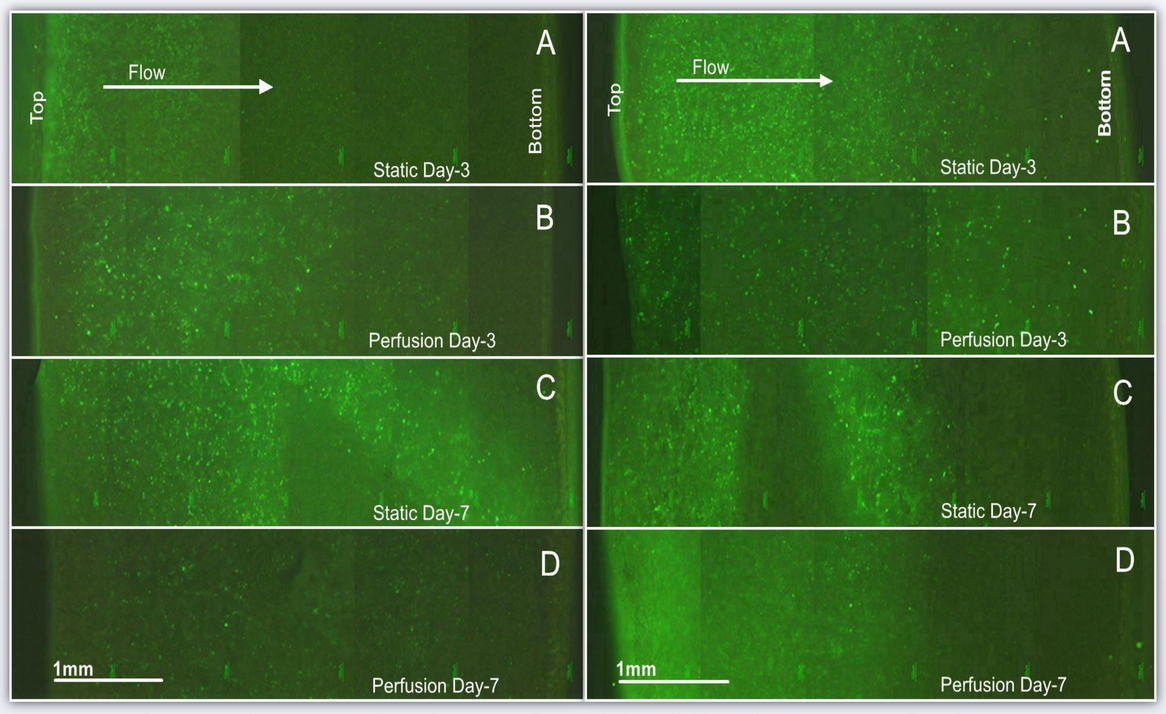
Cell Viability (Alamar Blue assay)



Comparison of 3T3 and MG63 viabilities at static and perfusion culturing conditions at on day 3 and day7(left), Viabilities of 3T3 and MG63 in static culturing at day 1, 3 and 7(right).

The viability level of 3T3 cultured in static condition was 2-fold compared to perfusion condition at defined time points. On the contrary, MG63 seeded scaffolds show higher cell proliferation and metabolism activities in perfusion and lower in static condition at the selected time points respectively. At static culturing, the viabilities of both cell lines increased from day 1 to day 3 but had lower viabilities at longer culturing time(day 7), and the viability of MG63 cells at culturing day 7 was lower compare to day 1.

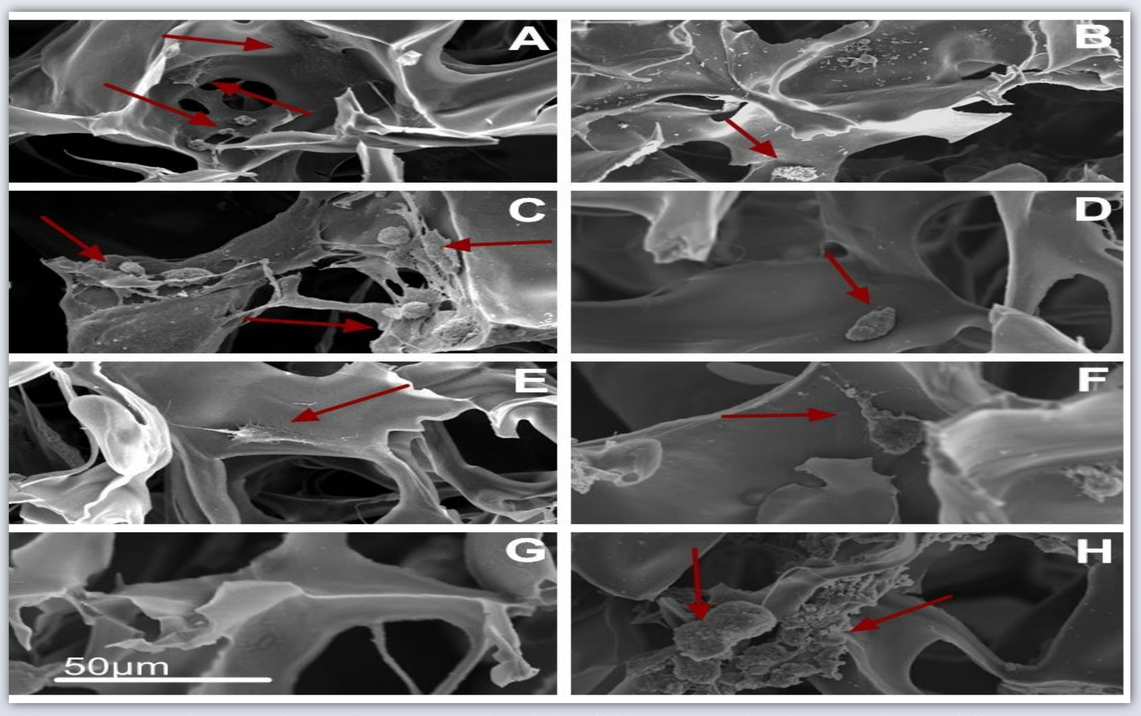
Cell distribution



Cross-sectional images of NIH-3T3(Left) and MG63(right) seeded scaffolds with static(A,C) and perfusion(B,D) culturing condition on day 3 and 7.

Necrotic centres were observed on both 3T3 and MG63 seeded scaffolds at culturing day 7 under static culturing while cells distributed homogenously in perfusion culturing.

Cell morphology



SEM cross-sectional images of NIH-3T3 & MG63 cell-seeded scaffolds in static and perfusion condition at different time points A,B) day 3 of 3T3 static and perfusion; C,D) day 7 of 3T3 static and perfusion; E,F) day 3 of Mg63 static and perfusion; G,H) day 7 of Mg63 static and perfusion

Both cell lines had attachment to the surface of the porous wall on day 3(A,E). On day 7, MG-63 cells were difficult to be observed but 3T3 with flatten shapes were found on the porous structure (C,G). In perfusion culturing, cells with spherical shapes were found within both cell lines at day 3(B,F). On day 7, number of 3T3 became very limited to be detected at selected field where close to the center of scaffold while MG63 accumulated with attachments to the scaffold (6D,H)

Conclusion

Our custom made perfusion bioreactor unit has been used in this study to provide a reliable dynamic culturing condition. By using the perfusion and static culturing method, the viability, morphology and distribution of two different cell lines (MG63 and NIH-3T3) seeded scaffolds were investigated. Comparison between the two culture methods, perfusion culture might benefits the cells' migration and promote cells distribute homogenously. The results indicate MG63 has affinity to the perfusion culturing where NIH-3T3 shows higher viability under static condition. This finding suggests that in *in vitro* tissue development, different cell lines might have different affinities to varied culturing conditions.

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