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Cell Isolating from Bovine Pericardial Fluid and Culturing for Next Tissue Engineering Applications

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Abstract: Hearth is an important organ that is a responsible for pumping blood whole body, and so cardiac diseases are critically important all over the world. Pericardial fluid (PF) and its component are important for diagnosis and treatment for heart disease. In literature, PF has been analysed especially for pericarditis disease, and there has been any research on PF for tissue engineering application. The aim of this study is isolating the cells from the pericardial fluid and culturing these cells in vitro in order to be used as cell line in tissue engineering studies. PF contains heterogen cell population which are lymphocytes, glanulocytes, macrophages, eosinophils and basophils, These cells were cultured in five different media (Alpha MEM, DMEM-Low, EMEM, MCDB 131, and Med 199) in this study. As a result of cell culture was performed and concluded that, cell proliferation and viability were the best in culture with Alpha MEM media according to the microscope examination.

Keywords: Pericardial fluid, Heart, Cell culturing, Tissue engineering

Introduction

Heart is located in a sac that called as the pericardium. Pericardium is a serious membrane and, enclosed the heart. Pericardium sac has two layer, an outer layer (fibrous pericardium) and inner layer (serous pericardium). Fibrous pericardium consists on connective tissue which is outer layer of pericardium, and serous pericardium is inner layer of pericardium and divided into two layers, visceral and parietal pericardium. Between these two layers a fluid filled pericardial cavity is located and that is known as pericardial fluid (Chinchoy et al., 20015).

The heart system is destroyed by genetic and environmental factors and cardiac diseases are occurred and caused morbidity and mortality all over the world. Cardiac damage such as heart attack must be regenerate quickly. Tissue engineering studies aim to treat damaged tissue and, the damaged tissue can be replaced with in vitro developed tissue engineering organs or tissues.

Inflammation of the pericardium causes of pericarditis which is the most threatening disease for human and bovines. One of the pericarditis forms is effusive that accumulation of a protein rich fluid with in pericardial sac. Fibrin deposition on the epicardium that fibrous pericarditis, and fibrosis on this area lead to constrictive pericarditis (Pekins et al., 2004; Athar et al., 2012). PF is also an indicator of heart that pathological alteration can be observed. In chronic heart failure, expression of several inflammatory cytokines such as interferon and interleukin and T-cells were increased (Iskandar et al., 2017).

PF is a plasma ultra filtrate and difficult to define its cell population. On normal condition without disease, volume of PF is changeable according to body size, such as in rabbits 0.4-1.9 mL and in adult human about 20-60 mL (Vogiatzidis et al., 2015). Cell population of PF in healthy human is heterogeneous. This heterogen cell population are mesothelial cells, lymphocytes, granulocytes, macrophages, eosinophils and basophils (Gibson and Segal, 1978a; Benhaiem-Sigaux et al., 1985). PF also contains some proteins that albumin, globulins, macroglobulins, and fibrinogen (Gibson et al., 1978b; Holt 1970).

PF is a suitable environment for cell accommodation especially mesenchymal stem cells (MSCs) (Blázquez 2015). In this study, pericardial fluid cells were isolated from pericardial fluid and cultured in five different media respectively modified minimum essential medium (Alpha MEM), Dulbecco's modified Eagle's medium low glucose (DMEM-Low), Eagle's Minimum Essential Medium (EMEM), MCDB 131 (reduced serum supplemented medium), Med 199 (Table 1).

Method

Obtaining pericardial fluid from bovine heart

In this study, PF samples were obtained from three slaughtering bovines belonging to institution of meat and dairy slaughterhouse. Firstly, thoracic cavity was opened by being careful not to damage of pericardium, immediately after slaughter from an abattoir. The pericardial fluids, aspirated from the pericardial cavities without any organ removed, by the side of heart apex with 50-mL sterile syringes that were maintained at room temperature and transported to the laboratory as seen in Figure 2. The PF (liquor pericardi) have been obtained from three bovine aged between 2-3 years, and weighed between 350–450 kilograms.

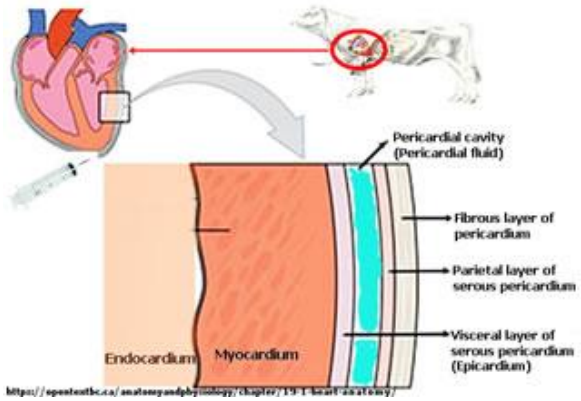


Figure 1. Pericardium structure and obtaining pericardial fluid from bovine heart

Figure 2. Three pericardial fluid samples.

Cell isolation from PF and Cell Culturing

Amount of three pericardial fluid samples were obtained respectively 103-105 mL (sample one), 90-100mL (sample two) and 100mL (sample three), and centrifuged at two times. First centrifuge of pericardial fluid was made at 400x g for 5 minutes to remove particulate in the pericardial fluid. Pellet, contained particulate was discarded and supernatant was transfered to 50 mL fresh falcon tubes. Second centrifuge was made at 4500x g for 5 minutes and supernatant was transfered to 50 mL fresh falcon tubes and filtrated with through 0.22 µm syringe filter. This sterilated supernatant acellular fluid was coded as PRS and then removed to -20^{o}C storage for later used in media supplemented. Pellet was contained pericardial fluids' cell populations was coded as PF cells were suspended in 200 µL PRS and then cultured in five different (DMEM-Low, MEM alpha, EMEM, MCDB 131, and MED 199) media supplemented with 10% fetal bovine serum (FBS), 250 U/mL penicilin, 250 µg/mL streptomicin, 25 mM glutamin, 20 µg/mL bovine insulin and maintained in 5% CO2, at 37^{0}C and humudified atmosphere. For the 2D monolayer culture (well plate), 1x10^{5} cells/cm^{2} were seeded in six well plate (Fig.3). The 2D monolayer cells were grown to 80% confluense then rinsed twice DPBS buffer. Adhered cells were passaged when cell population was confluenced at 80%-90% by 0.25 % trypsin solution and seeded a new culture.

Table 1. Five different media

Media
Alpha MEM
DMEM-Low
EMEM
MCDB 131
Med 199

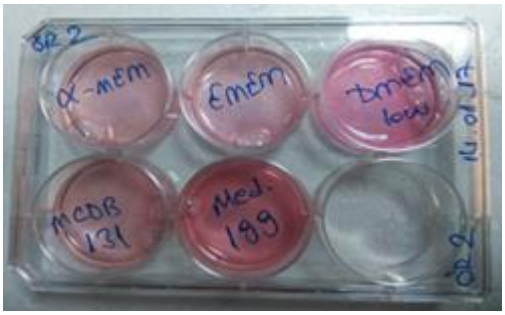


Figure 3. The image of PF cells culturing in six well plates.

Results and Discussion

Approximately after 24 hour of cell culture incubation cell images were taken by inverted microscopes. Numerous non-attached cells were seen so that these cells were moved a new six well plate, and media added on all adherent cells in six well plates. Thus, both a cell density was reduced in the medium and fresh medium was added in six well plates. After the 48 hour cell culturing that PF cells were adhered on six well plate and, images were taken by inverted microscope.

When the five different media were evaluated according to their psychic efficacy, it is thought that the alpha MEM media provides the most effective support for cell survival and proliferation.

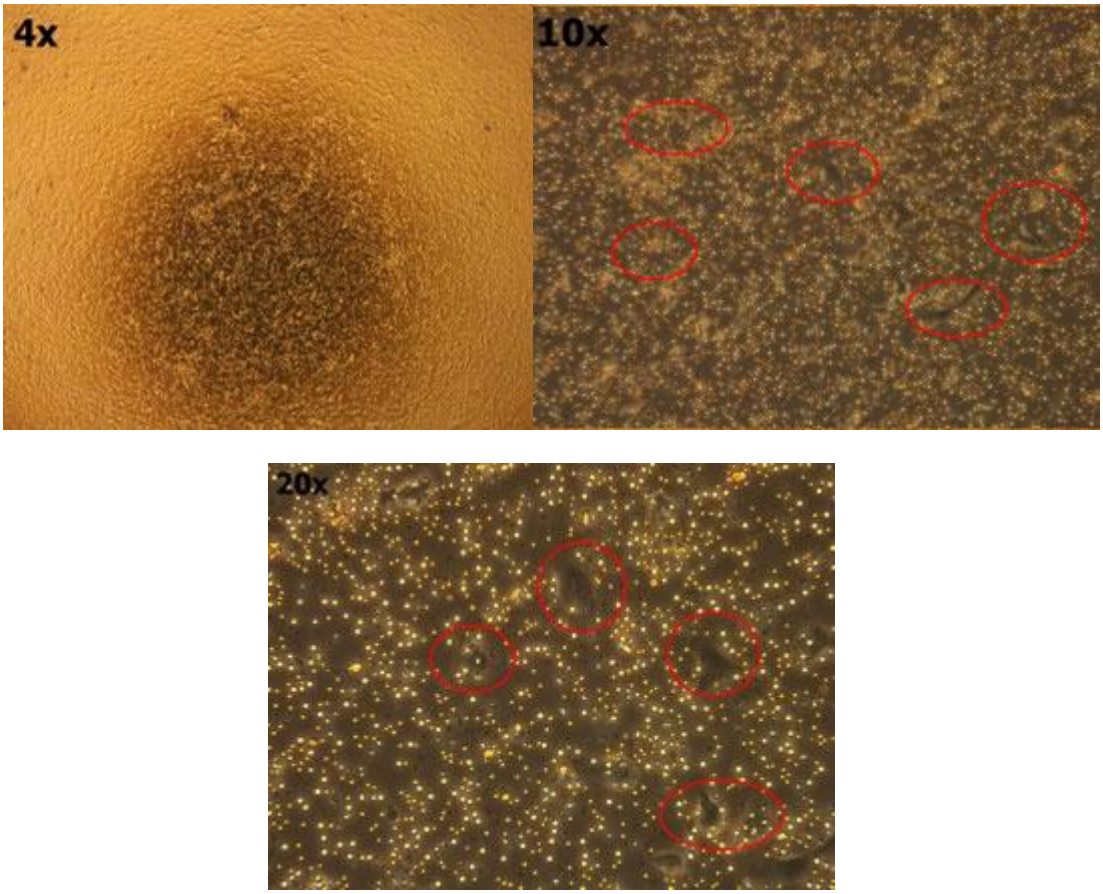
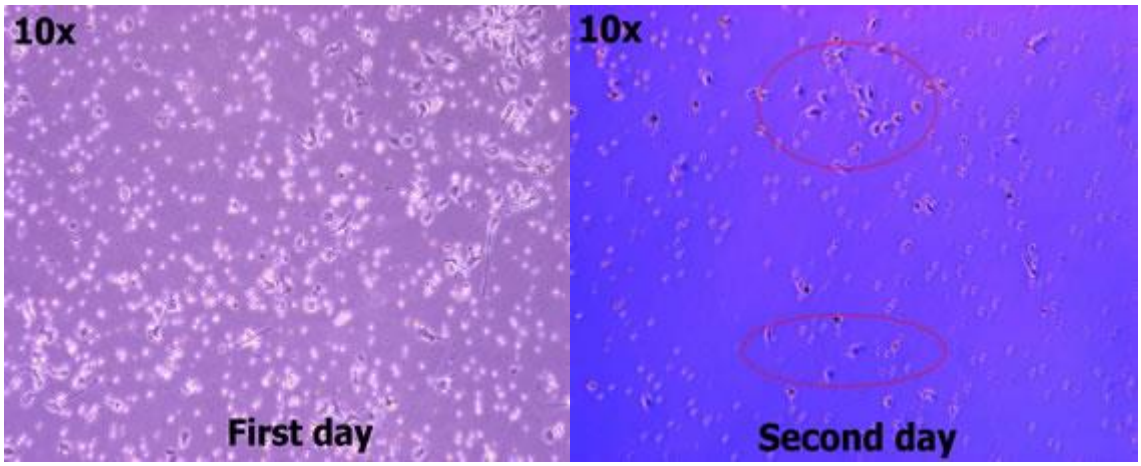


Figure 4. Microscope images of PF cells with high cell dencity at magnification 4x, 10x, and 20x with culturing Alpha MEM media



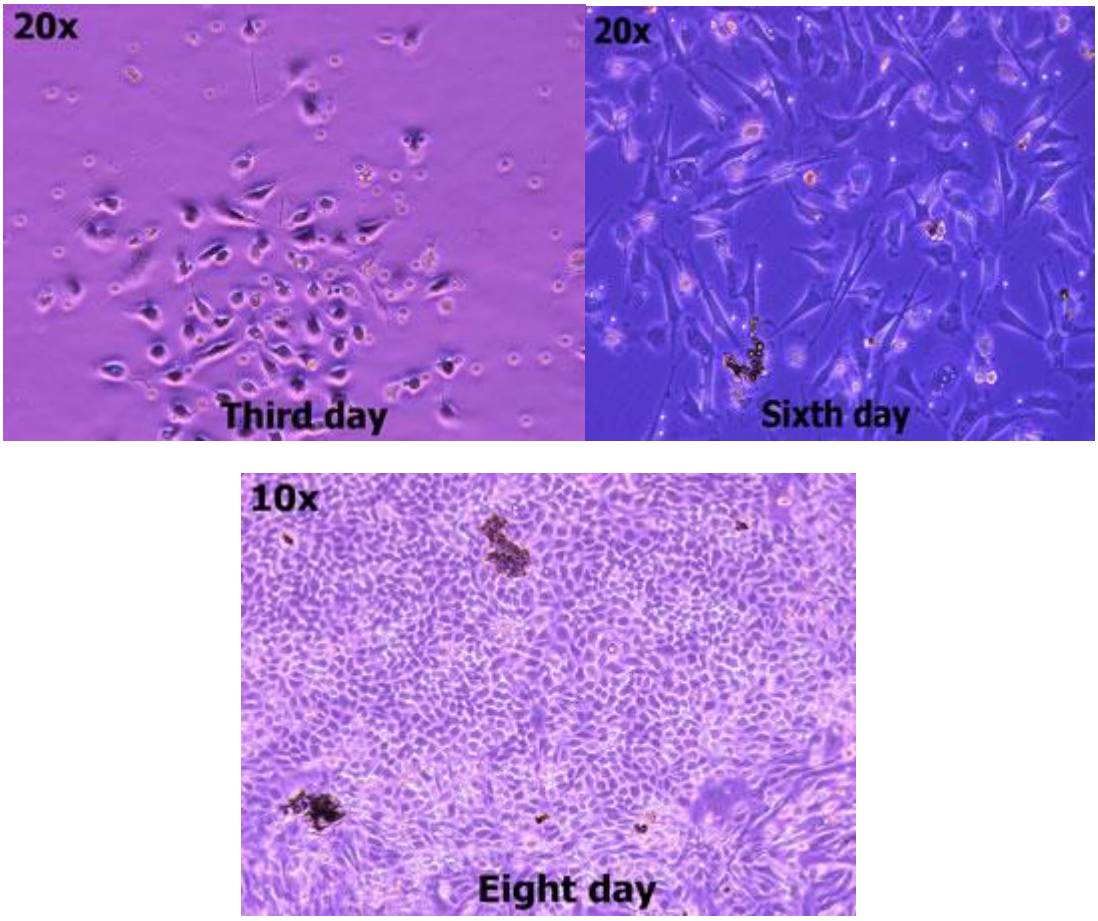


Figure 5. Microscope images of PF cells with culturing in Alpha MEM media at magnification 4x, 10x, and 20x

Conclusion

This study has been a preliminary study for other tissue engineering studies, especially vascular tissue engineering. Cells in PF taken from bovine heart were isolated and cultured in five different media in vitro and it was concluded that cell viability and proliferation were the best in cell culture with MEM alpha media.
After obtaining the PF from bovine heart, PF cells were cultured in five different media for determining the best media. Cell viability and proliferation were concluded that the best in cell culturing with MEM alpha media.

In the future studies, PF cells will be cultured on scaffold (three dimensional environment) and will be used for tissue production studies.
The use of PF in both isolation and in vitro culturing of PF cells as well as in tissue engineering studies was performed as a first with this study. Additionally, this study also important for the use of PF cells as an alternative to the known cell lines used in tissue engineering studies.

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