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Isolation and Culture of Cardiac Fibroblasts from Adult Xenopus Heart

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Abstract

Recent progress reveals that the damaged adult Xenopus tropicalis heart is able to be regenerated in a nearly scar-free manner in around 30 days after apical resection. It is believed that cardiac fibroblasts will paly an important role in the scar-free regeneration of adult Xenopus heart. However, the methodology for isolation and culture of adult cardiac fibroblasts in adult Xenopus heart is not yet reported. In this study, an in vitro methodology for isolating and culturing cardiac fibroblasts of adult Xenopus heart was successfully established.

Keywords

Xenopus; Heart; Cardiac Fibroblasts; Culture.

1. Introduction

Cardiovascular disease (CVD) is big challenge for human health [1]. According to a report from the World Health Organization in 2019, ischemic heart disease is one of the top ten causes of human death and is at the top of the list [2]. After the occurrence of ischemic heart disease, such as myocardial infarction, which is caused by the obstruction of blood supply to the heart, ischemia and hypoxia can cause the myocardium to work abnormally and even the death of myocardial cells, eventually leading to heart failure, which will affect the quality of life and even life span of the patient [3]. Previous studies generally believed that adult mammalian cardiomyocytes are non-renewable after adulthood [4], while the adult cardiomyocytes of amphibians such as Xenopus and fish, such as zebrafish have a certain proliferation potential [5-6]. After the original cardiomyocytes are damaged, they can repair the heart by generating new cardiomyocytes through cell division, thereby restoring certain heart functions.

Recently, it was reported that the damaged adult Xenopus tropicalis heart is able to be regenerated in a nearly scar-free manner in around 30 days after apical resection [7]. In addition, it was found that after adult heart injury, short-term fibrosis occurred near the injured area. The emergence of this phenomenon suggests that the regeneration after heart injury is likely to be related to cardiac fibroblasts. However, today, the methodology of isolation and culture of cardiac fibroblasts of adult Xenopus heart is not yet reported. This study aims to establish a methodology for isolation and culture of cardiac fibroblasts of adult Xenopus heart, which is able to provide a reliable in vitro model to investigate the functional role of cardiac fibroblasts involved in adult xenopus heart regeneration.

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2. Materials and Methods

2.1 Materials

2.1.1 Experimental Animal

Xenopus laevis albino frogs were applied in this study. Animal care, surgery and handling procedures were performed according to regulations established by The Ministry of Science and Technology of the People's Republic of China ([2006] 398) and approved by Jinan University Animal Care Committee.

2.1.2 Reagents

1× PBS (pH=7.4), 75% alcohol, tyrisin (Gibco), collagenase P (Roche), Mouse anti-Vimentin antibody (accam ab8978), FITC–conjugated Donkey Anti-Mouse IgG(H+L) (Proteintech), Hoechest (Thermo Scientific™), Fetal Bovine Serum (Gibco).

2.2 Methods

2.2.1 Isolation and Culture of Xenopus Laevis Albino Cardiac Fibroblasts

Albino Xenopus laevis frog (> 6 months) was treated with Tricaine methanesulfonate bath (MS-222; 1 mg/mL; TCI, Shanghai, China) which was prepared with sterile double distill water at room temperature for 2-4 min and hibernated on ice for 60 s. Then the frog was transferred into a beaker that filled with 75% alcohol and was soaked for about 3 minutes for disinfection. After that, the frog was were quickly transferred into the ultra-clean workbench, and the ventricle were isolated with scissors. The isolated ventrivle was soon immersed in a glass dish containing pre-cooled 1× PBS (pH=7.4) to wash the remained blood cells. The cleaned ventricle tissue were transfered to another glass dish and were chopped into small pieces by a sterile blade. The minced ventricle tissue was collectd and transferred into a tube containing enzyme solution (1mg tyrisin + 0.5mg collagenase P in 1mL DMEM), and incubated for about 40 minutes in 32℃ water bath. During this time, the tube which is included minced ventricle tissue was inverted every 5 minutes. When the tissue was digested and all the enzyme tissue was dissociated, 1ml 10% FBS-DMEM was added to terminate the digestion. Then, the mixture was firstly filtered by a 100μm filter membrane, then a 40μm filter membrane. The filtered cell suspension was transfer into new tubes and was centrifuged at 1,600 rpm, 4℃ for 5 minutes to collect the isolated cells. After centrifugation, all the supernatant was removed from the tubes, and 1ml of 10% FBS-DMEM was added to resuspend the cells, which were then planted into a 6cm dish. The cells were placed into an incubater at 26℃, and the cell culture medium of them was changed every 2 days.

2.2.2 Sub-Culture of Isolated Cells and Preparation of Cells for Staining

The cells were passaged when the cell density reached about 80%. All the cell culture medium was removed, and 1ml pre-warmed trypsin was added into the cell culture dish. After digesting for about 2 minutes at 26℃, the digestion was terminated by adding 1ml of 10% FBS-DMEM into the cell suspension. Then, the cell suspension was transferred into a 15ml centrifuge tube, and was centrifuged at 1,600 rpm at room temperture for 5 minutes. After that, all the supernatant was removed, and 1 ml of 10% FBS-DMEM was added into the centrifuge tube to resuspend the cells. 300μL of the cell suspension was respectively planted into two 6-cm cell cultre dishes, and the rest of it was seperately planted into 4 wells of 24-well plated that had been placed with the glass slide.

2.2.3 Immunofluorescence Staining for Fibroblasts Marker

When the cell density in the 24-well plate reached about 50%, 1mL of 4% paraformaldehyde which had been pre-warmed was added to each well, the cells were fixed overnight at 4℃. After three washes with 1× PBS (pH=7.4), the glass slide which is included cultured cells was drawed a circle by a histochemical staining pen. 100μL of 0.1% Triton™ X-100 (dissolved in 1× PBS) was added to each slide at room temperature for 15 minutes to disorganize the cell membrane. Then 100μL of 1× PBS was added to for 5 minutes washes for 3 times. After that, 100μL of 1% BSA (dissolved in 1× PBS) was added to each slide at room temperature (26℃) for one hour, and then 50μL of mouse anti-

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vimentin (1:100 dissovled in 1%BSA) primary antibody was added to each cell slide and incubated at room temperature (26℃) for 2 hours. After three washes with 1× PBS (pH=7.4), 50μL of FITC–conjugated Donkey Anti-Mouse IgG(H+L) (1:1000 dissolved in 1× PBS) secondary antibody was added and incubated for one hour at room temperature (26℃). After staining, the glass slide was sealed with antifade mounting medium and photographed.

3. Results

3.1 Cell Culture and Identification of Primary Isolated Xenopus Laevis Heart Fibroblasts in Vitro

The primmary isolated cells were revealed as circular polygon and spindle shage morphology. It was grown to around confluence after 6-day in vitro culutre in a 6 cm dish. The cultured cells are able to be passaged (Figure 1). The passage-2 isolated cells were applied to confirm the expression of fibroblasts marker, vimentin. Our anti-Vimentin immunofluorescence staining demonstrated that the isolated cells were vimentin positive (Figure 2). Thus, the results of morphology, growth characteristics and marker staining supports that the isolated cells are cardiac fibroblast. The established methodology of present study is able to isolate and in vitro culture the cardiac fibroblasts of adult Xenopus heart.

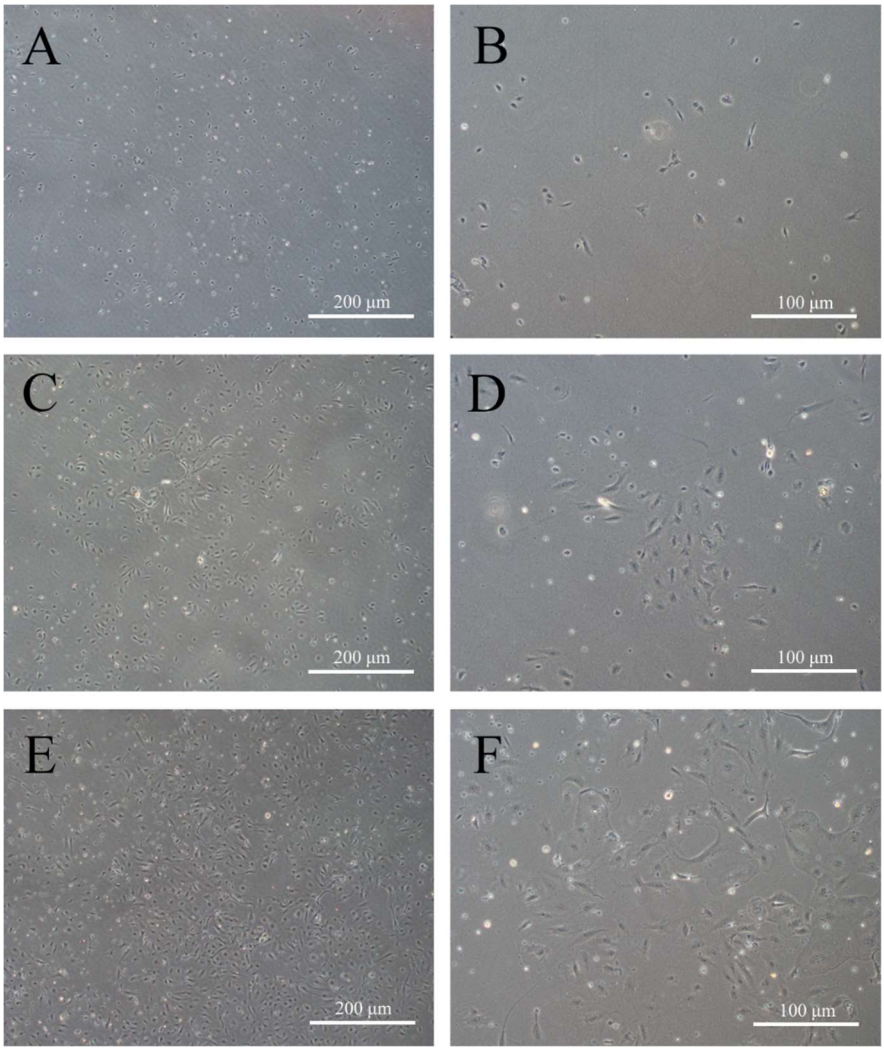

Figure 1. Primary culture of isolated cardiac fibroblasts of adult f Xenopus laevis heart. A-B: 1-day culture of isolated cells. C-D: 4-day culture of isolated cells. E-F: 6-day culture of isolated cells. The primmary isolated cells were revealed as circular polygon and spindle shage morphology. The cultured cells are able to be passaged.

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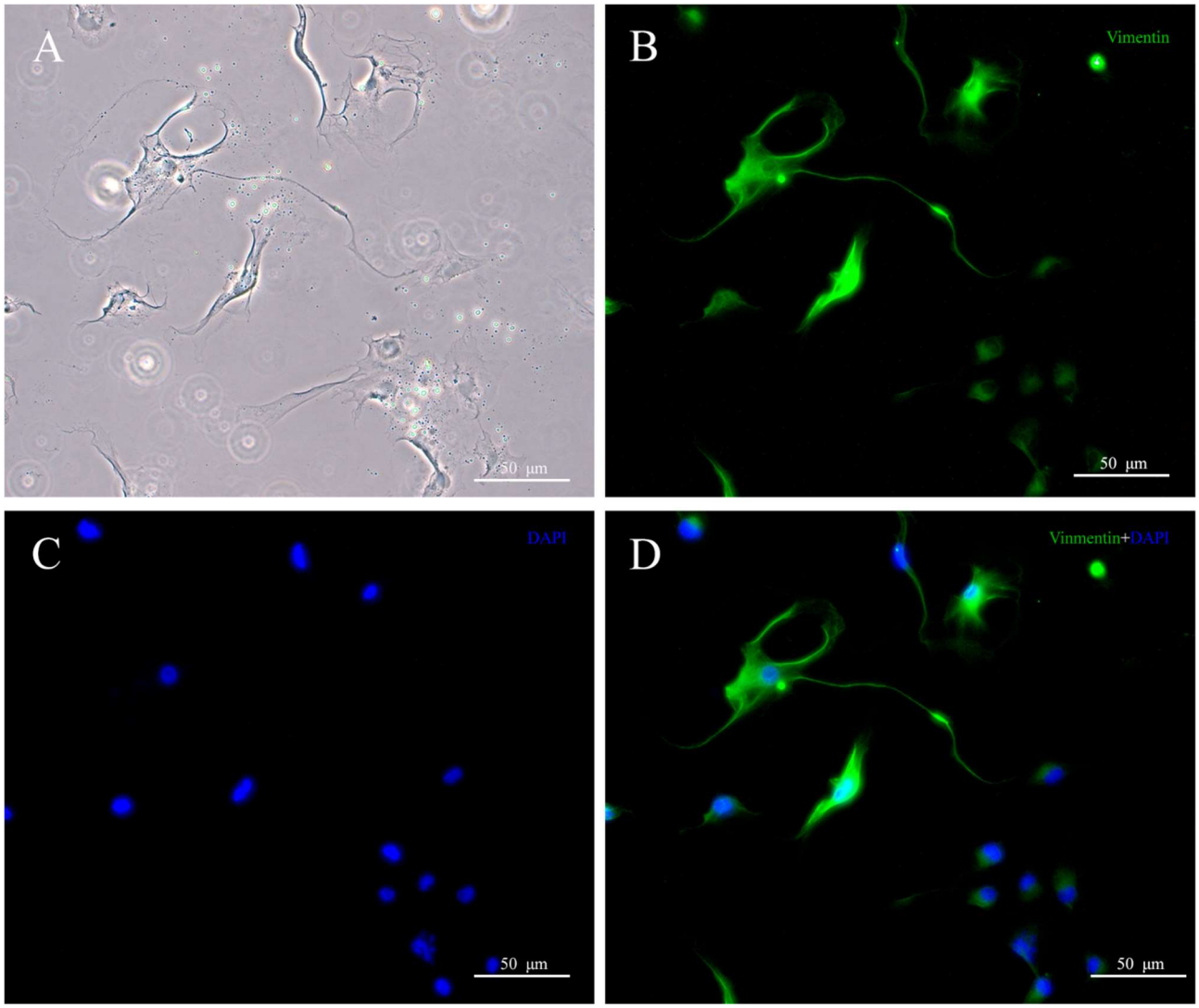

Figure 2. Immunofluorescence staining of anti-vimentin for culture isolated cells in passage-2. A: Bright field. B: anti-Vimentin (Green). C: DAPI (Blue). D: Merged. The isolated cells were vimentin positive.

4. Conclusion

In this study, we have successfully established a new, stable and efficient methodology for isolating and culturing the cardiac fibroblasts of adult xenopus heart. Xenopus are cold-blooded animals that prefer to living at the water with temperature of about 23°C to 26°C. In the beginning we tried culturing the isolated adult cardiac fibroblasts in 37°C same as mouse. However, we found that culture cells experience cell death as the temperature higher than that of suitable survival temperature, 26°C. Therefore, 26°C was identified as suitable temperature for Xenopus cardiac fibroblasts culture. The results of morphology, growth characteristics and marker staining in present study supports that the isolated cells are cardiac fibroblast. The established methodology of present study is able to isolate and in vitro culture the cardiac fibroblasts of adult Xenopus heart. It is believed that the established methodology will be a powerful tool for studying of functional role of cardiac fibroblasts in adult Xenopus heart regeneration.

Acknowledgments

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