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ORIGINAL ARTICLE

# Regenerating Heart Using a Novel Compound and Human Wharton Jelly Mesenchymal Stem Cells

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*Background.* Myocardial infarction is a major problem in health system and most conventional therapy is not led to restoration of the health. Stem cell therapy is a method to regenerate the heart but today appropriate cell source and scaffold selection as extracellular matrix to achieve the best effect is disputing.

*Aim of the Study.* In this study a combination of human Wharton jelly mesenchymal stem cells (HWJMSCs) with a novel compound consisting polyethylene glycol (PEG), hyaluronic acid and chitosan is presented to heart regeneration.

*Methods.* After proliferation and expansion of HWJMSCs, these cells were mixed with scaffold and injected into the infarcted rabbit myocardium. After two months cardiac function and infarcted area were evaluated. Immunohistochemistry performed for vessel count and demonstrating of differentiation ability into cardiomyocytes. To confirm this ability PCR was done. Scanning electron microscope was used to evaluate angiogenesis.

*Results.* Improving cardiac function was higher in cell/scaffold group than the others and it was confirmed by SPECT results which showed least defect size in the myocardium. There were a lot of neoangiogenesis in the target group and also cardiomyogenesis observed in cell/scaffold group. PCR results confirmed the presence of differentiated cardiomyocytes and SEM showed well developed vessel in this group.

*Conclusions.* Comparing macroscopic and microscopic results between all groups revealed that HWJMSC in combination with this scaffold led to brilliant results regarding cardiac function, angiogenesis and cardiogenesis. It is recommended using these cells and materials for cardiac tissue engineering and regeneration therapy. © 2017 IMSS. Published by Elsevier Inc.

*Key Words:* Myocardial infarction, Cell therapy, Scaffold, Rabbit, Angiogenesis.

## Introduction

Today, myocardial infarction represents the most cause of mortality in human population (1). To lessen the major adverse effect, preventing of ventricular remodeling, myocardial fibrosis and reduced cardiac function are key parameters to be noticed (2). Nowadays regenerating the heart is one the most interesting challenge in the field of regenerative medicine. The great part of this strategy is cell selection for cardiac regeneration that suffers from deficit of accessible potent cells (3). No reliable results achieved following cell transplantation because of cell loss. To compensate this deficiency, scaffolds have been introduced to cover these limitations. Scaffolds present an environment like extracellular matrix (ECM) for cell attachment and

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differentiation (4). The best material for cardiac regeneration is the one that mimic heart tissue. Many scaffolds have been used to date for cardiac regeneration. Collagen was used by many researchers to prevent remodeling, creating angiogenesis and increase heart function (5–8). Fibrin, chitosan and so on are some scaffolds in this field (9,10).

Hyaluronic acid is a glycosaminoglycan of the ECM that has an important role in cell attachment, healing and connective tissue formation (11). It can be replaced in bare space of damaged myocardium (12). Yoon SJ et al. showed that the 50 kDa hyaluronic acid presents best effects for cardiac regeneration and can differentiate cells into endothelial and cardiomyocytes (13).

Polyethylene glycol (PEG) is a water soluble polymer produced from polymerization of ethylene oxide. This biocompatible and FDA approved polymer can be used as a matrix in tissue engineering to lessen the inflammation (14). PEG increases the viability of cells (15) and promotes differentiation of cells into cardiomyocyte (16).

In attention with these explanations, we were going to select a combination of PEG/hyaluronic acid that expresses all advantages of these materials.

Just as explained, the other part of regeneration therapy is cell selection. Many cells have been used from embryonic (17–20) to adult (21–24) achieved from many sources but there is a vacancy regarding best cell with easy extraction. Human Wharton's jelly mesenchymal stem cells (HWJMSCs) are multipotent cells in the matrix of human umbilical cord (25). These cell populations possess many advantages than the others in myocardial regeneration (26), as follows: HWJMSCs are comfortably accessible from waste material during child delivery without any ethical issues than the others like embryonic stem cells (27). Also, HWJMSCs release angiogenic factors like vascular endothelial growth factor (VEGF), angiopoietin-1, HGF and transforming growth factor β1 (TGF-β1) (28) that leads to angiogenesis, in situ (29). One the important issue regarding these cells is the immunogenicity subject. MARK L. WEISS et al, showed that HWJMSCs do not stimulate T-cell proliferation, secretes interleukin-6, modulate immune response, and finally it can be tolerated in allogeneic injection (30–32). These results indicate that these cells are appropriate for xenotransplantation. These cells can be stored in a cell bank and are ready to use immediately after request. The aim of the present study is the application of a new and attractive component (PEG/Hyaluronic acid) as scaffold with one of the best cell sources in cardiac regeneration therapy (HWJMSCs) relying on clinical results.

## Materials and Methods

### *Cell Preparations*

Human umbilical cords collected from women with caesarian section and no complications during gestation. Informed consent has taken from all women. The cords stored in a sterile flask and immediately transferred to the University for processing. First of all, vessels removed from the cord and its matrix, parceled into 2–3 mm pieces, washed with phosphate buffered saline (PBS), cultured DMEM-F12 with 15% FBS (Gibco), 100 U/ml penicillin, 60 g/mL streptomycin in 37°C incubator and 5% CO2. Cells at 2 passages were collected with 190% confluency. A number of 1 × 106 HWJMSCs for each case have been prepared (33).

### *Scaffold Production*

As previously mentioned, 70% of hyaluronic acid 50 kDa and 28% of PEG 6000 kDa have been selected to mix and prepare a compound as a scaffold for cells. Because of low viscosity of this compound, 2% chitosan has been added to solution to obtain a jell viscosity for injection. These percentages experimentally obtained in our lab to achieve a component with jell consistency.

### *Fourier Transform Infrared Spectroscopy (FTIR)*

The presence of hyaluronic acid, chitosan, and PEG in the hydrogel scaffold was investigated using FTIR.

### *MTT Experiment*

This experiment is able to detect the surviving cells in the compound. About 3 × 103 cells has been mixed with 1 ml of scaffold and prepared for the test. In the MTT assay, tetrazolium salt is dehydrogenated and changed into purple colored crystals during incubation. The final product shows cellular viability. 50 µl of the MTT solution added to well following 3 h incubation at 37°C. The test continued for 5 d to get the optimum results. For reading the results, optical density (OD) detected by ELISA reader (Expert 96, Asys Hitch, Ec Austria) (34).

### *Animal Study*

MI model and cell transplantation: All animal experiments were approved by ethical committee of Tehran university of medical sciences in line with NIH guideline (Guide for the Care and Use of Laboratory Animals, NIH publication 8523, revised 1996). 24 white male Newzealand rabbit weighing 2000–2500 grams divided into four groups, each one six animals, as, control (myocardial infarction + culture medium injection), scaffold (myocardial infarction + scaffold injection), cells (myocardial infarction + cells injection), scaffold/cells (myocardial infarction + cells and scaffold injection). Animals were anaesthetized with Ketamine (50 mg/kg i.p.) plus Xylazine (5 mg/kg i.p) intubated, and maintained by mechanical ventilation plus inhalation anesthesia using Isoflurane 2%. Left-lateral thoracotomy performed after prep and draping and Left anterior descending coronary artery was ligated and infarction proved by ST segment