Natural Cardiac Extracellular Matrix Sheet as a Biomaterial for Cardiomyocyte Transplantation

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ABSTRACT

Cardiovascular diseases associated with myocardial infarction are among the major causes of death worldwide due to the limited regenerative capacity of cardiac tissues. Although various approaches, such as biosynthetic biomaterials, have been developed to promote postinfarction cardiac regeneration, a number of limitations, including the immune complications caused by biodegradation of these scaffolds and insufficient cell migration, need to be overcome prior to their clinical application. Hence, the development of natural biomaterials to support myocardial regeneration is crucial. Here, we investigated the effects of a natural biomaterial, cardiac extracellular matrix (ECM) on the proliferation and maintenance of cardiomyocytes in order to assess its suitability for cardiomyocyte expansion. The ECM components not only provide mechanical support, but also induce and preserve the required phenotypic and functional characteristics of the cells. We prepared ECM sheets from decellularized cardiac sections. Cardiomyocytes were then cultured with and without these cardiac ECM sheets. We compared the proliferation rates and phenotypes, and cardiac gene and protein expression, of the cultured cardiomyocytes by automatic cell counting and the MTT assay, microscopy, and RT-PCR and western blotting, respectively. The cardiomyocytes cultured with the natural cardiac ECM sheets exhibited higher proliferation rates and cardiac gene and protein expression than those cultured without the ECM sheets. Our results demonstrate that the ECM sheets are suitable for use in cardiomyocyte transplantation and can provide a novel in vitro model for investigating cell and ECM interactions. We hypothesize that these ECM sheets can be used in the future to improve cardiac transplantation strategies.

DESPITE the advancements in medicine, cardiovascular diseases related to myocardial infarction continue to be among the major health problems worldwide due to the limited regenerative capacity of damaged heart tissues [1]. Therefore, the development of novel cardiac tissue repair and regeneration strategies will be the future challenge in cardiac tissue engineering [2]. Recent studies in cardiovascular tissue engineering have focused on developing biomimetic and tissue-specific biomaterials by exploring the biological and chemical components in the cardiac microenvironment [3,4].

Various types of stem cells, including fetal cardiomyocytes, embryonic stem cells, skeletal myoblasts, crude bone marrow stem cells, hematopoietic stem cells, fibroblasts, smooth muscle cells, and induced pluripotent stem cells, have been studied for their ability to promote myocardial repair, and they have demonstrated varying levels of success in cardiomyocyte transplantation [4,5]. Furthermore, several biomaterials, including cardiac patches, left ventricular restraints, and various injectable products, have been developed over the

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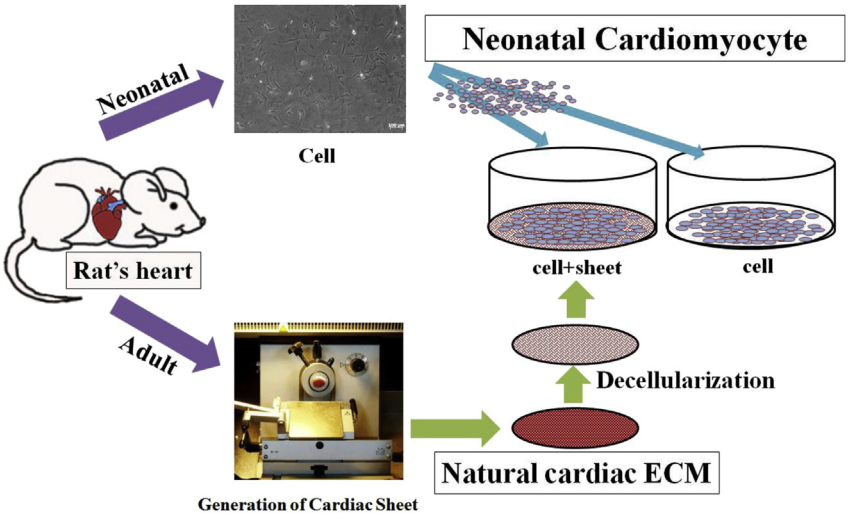

Fig 1. Experimental design. The schematic illustration shows the process of generating natural extracellular matrix (ECM) sheets from adult rat heart. The cardiomyocytes isolated from neonatal rats were cultured with and without cardiac ECM and used for subsequent experiments.

years. The efficiency of injectable biomaterials for the delivery of stem cells, as well as their effects on myocardium regeneration after infarction, have been reviewed previously [3,4,6]. Alginate, fibrin, intestinal submucosa, and chitosan are a few of the injectable biomaterials that have been demonstrated to improve regeneration of the infarcted myocardium [3].

Although promising results have been achieved in preclinical and early phase clinical applications of biosynthetic polymers as biomaterials, further investigations are required to establish their clinical effectiveness. We speculated that cotransplantation of cardiomyocytes and "intelligent" natural biomaterials can be a novel strategy to enhance the efficiency of cardiomyocyte transplantation. One such natural biomaterial is the three-dimensional (3D) cardiac extracellular matrix (ECM), which is composed of a network of interstitial collagens that houses the rest of the matrix components. ECM components not only provide structural and mechanical support, but also induce and preserve appropriate phenotypic and functional characteristics of the cardiac cells [7]. Recently, decellularization has been used extensively for developing biological ECM scaffolds. The process involves the application of a combination of detergents and other chemicals to remove the cellular components completely, while maintaining the ECM components and the integrity of the biological scaffold [6,8,9]. In this study, we developed decellularized sheets of natural cardiac ECM to support cellular myocardial regeneration by using thin cardiac tissue sections and investigated their effects on the proliferation and phenotype maintenance of cardiomyocytes.

MATERIALS AND METHODS

Neonatal Rat Cardiomyocyte Isolation

Neonatal ventricles from 1- to 2-day-old Sprague-Dawley (SD) rats (Samtako, Osan, Korea) were dissected, minced on ice, and digested for 10 minutes with an enzyme solution in a water bath at 37°C with gentle shaking by hand. The enzyme solution consisted of 0.1% collagenase type II (Worthington Biochemical Co., N.J., United States), 0.1% DTT (Bio-Rad, Hercules, Calif., United States), 1% glucose (Sigma-Aldrich, St. Louis, Mo., United States), and 0.5% trypsin (Gibco, Carlsbad, Calif., United States) in phosphate-buffered saline (PBS), Supernatants were discarded and the cells were resuspended in fresh collagenase solution for 4–6 subsequent digestions. Cells were harvested by centrifugation at 700 × *g* for 10 minutes and resuspended in Dulbecco's modified Eagle medium/F-12 (DMEM/F-12; Gibco) containing 5% heat-inactivated horse serum (Gibco), 10% heat-inactivated fetal bovine serum (FBS; Gibco), and 1% antibiotic-antimycotic solution (AA; Gibco). The isolated cardiac cells were plated at a seeding density of 2×10^4 cells/cm^2 and incubated at 37°C with 5% CO_2 for culturing.

Preparation of Natural Cardiac ECM Sheets

Hearts were harvested from adult SD rats (Samtako) and were washed with PBS. Subsequently, the hearts were cut into 10-μm sections using a cryomicrotome (Thermo Fisher Scientific, Waltham, Mass., United States) to generate the cardiac ECM sheets following a previously described protocol [10,11]. For sterilization, cardiomyocyte ECM sheets were spread on a Petri dish and air-dried under ultraviolet radiation at room temperature on a clean bench for 12 hours (Fig 1).

Decellularization of the Natural Cardiac ECM Sheets

The ECM sheets were rinsed with PBS and incubated in prewarmed decellularization solution consisting of 0.25% Triton X-100 (Sigma-Aldrich) and 10 mmol/L NH_4OH (Daejung Chemicals, Gyeonggi-Do, Korea) in PBS, for 1.5 hours at 37°C. Subsequently, the supernatant from the decellularization solution was discarded. The decellularized

Table 1. List of the Primers Used in This Study and Their Sequences

Primer		Sequence
Cardiac troponin-T	Forward	AGACTGGAGCGAAGAAGGAAG
	Reverse	TGTTCTGCAAGTGAGCCTCGATC
Cardiac troponin-I	Forward	TGCCTCCACAACACGAGAGAGATC
	Reverse	AAGCACCTCTACTGCAAGGTTGGG
GATA-4	Forward	GTGCCAACTGCCAGACTACC
	Reverse	AGCCTTGTGGGGACAGCTTC
GAPDH	Forward	GGACCAGGTTGTCTCCTGTG
	Reverse	ATTCGAGAGAAGGGAGGGCT