Mechanical Stimulation of Cardiomyocytes Seeded on the Carbon Nanotube Forest Scaffold for Producing Mature and Functional Cells

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Introduction

Tissue engineering (TE) is a growing field, which has great potential in modern medicine [1]. It is based on the simple concept that the function of injured tissue or organ can be restored using cells (either from the patient or donor) and providing them with scaffolds, which will help them grow and develop. Currently, the focus is on two types of cells embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) [2]. The choice of the proper material for scaffold design is also crucial, as every property, material type, structure, 3D alignment, stiffness, conductivity, affects cell behavior [2] [1]. Finally, research has shown that certain cues and signals can enhance the differentiation process [1].

Cardiovascular diseases take the lives of 17 million people each year [3]. TE can provide the solution to treat most common of those, like myocardial infarction and congestive heart failure [2]. Cardiomyocytes (CMs) makeup heart tissue and are responsible for its contractions, which enables blood to be pumped throughout the body [4]. Unfortunately, they have a very small recovery ability, so any loss of cells leaves patients with a decrease in the cardiovascular system function. Those losses could be compensated, however sufficient methods of how to mature cells inside of the human body have not been developed yet.

Carbon nanotubes (CNTs) show great potential in serving as a scaffold for maturing CM, as construct made of aligned fibers evince electroconductive properties [2]. Such a material could then "merge" with the patient's own tissue and maintain the transmission of electrical signals between cells, behaving like the native heart. Moreover, CNTs could be adequately grown and tailored in order to obtain desired properties like stiffens, dimensions and alignment.

Recent studies have shown that mechanical stimulation of cell-scaffold construct before implementing it into the organism has a great potential in orienting cells on the desired differentiation path, which would be my primary interest [5] [6] [7].

Research objectives

Our main goal is to develop a proper approach in growing CM. We want to find out how dynamic loading affects CNTs with seeded cells and how exactly should it be applied in order to obtain the best results.

Methods

We would use day 1-3 neonatal rat hearts to obtain cardiomyocytes. Thermo Scientific^{TM} Price^{TM} Primary Cardiomyocyte Isolation Kit protocol would be followed. Briefly, each heart would be minced into 1-3 mm^3 pieces, first CM Isolation Enzyme 1 is added, cells are incubated, and the enzyme is removed. CM Isolation Enzyme 2 follows the same procedure. The tissue is broken up

by pipetting and complete medium (DMEM) is added. Cell suspension volume is then pipetted onto previously prepared CNTs (seeding density $2.5x10^5$ cells/cm^2) and cultured on the 24-well plates with DMEM in the incubator for 24h, before transferring into apparatus. Medium provides cells with necessary nutrition, consisting of fetal bovine serum (FBS, growth supplements mainly) and penicillin-streptomycin component. It must be changed, first after 24h and then every 3 days.

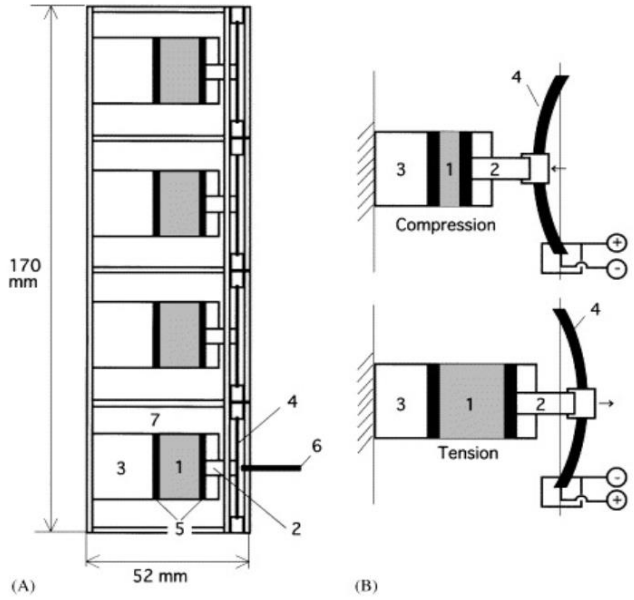

Fig.1 Apparatus used by Tanaka et al. [7]*, dimensions and number of chambers would be adjusted to our project and the sketch would be developed using NX software*

For the apparatus, we would follow the piezoelectric actuator described previously [7]. The device will have five chambers. Each chamber holds one sample (1), which is fully immersed in medium (7) and fixed with stripes of adhesive material (5). Bimorph-type piezoelectric actuator (4) is bent once the voltage is applied, which caused movement of the piston (2) and applies tension to the samples. Apparatus would be first modeled in NX and then 3D printed using the sources provided by the MTU library. The full construct would be placed in the incubator, providing stable conditions for the cells (37^0 C and 5% of CO_2).

We have decided to apply dynamic, non-continuous stretch as it mimics the environment present during native cell differentiation accurately and has been proven to result in the better formation of cardiac tissue [5] [6]. We would apply 2 different types of stress in 2 different times and one sample would be a control. All samples would be cultured for 14 days. First sample: 10% strain applied during days 1-4 for 30min, 2Hz applied in sinusoidal waveform. Second sample: 10% strain applied in days 1-4 for 30min, gradually growing to reach maximum 2Hz at 15min (Gaussian distribution). The third and fourth samples would be exposed to the same strain except during days 7-10.

Results will be measured using CM staining along with confocal microscopy. Expression of genes indicating cells maturation (TNNT2, MYH6, MYH7, and CACNA1c) will be done via a quantitative polymerase chain reaction.

Future Outlook

Combining mechanical and electrical stimulation together would provide a comprehensive method to obtain mature cardiomyocytes. The ultimate goal is to make a hybrid synthetic-biological actuator that could be implemented into the patient's body and serve just like the native heart tissue.

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