DEVELOPMENT OF INSULIN DELIVERY DEVICES COMPOSED OF LANGERHANS ISLETS AND CARDIOMYOCYTES

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ABSTRACT

In this study, an implanted self-perfusion insulin delivery device composed of cardiomyocytes and Langerhans islets for diabetes patient is proposed. Firstly, cardiomyocytes were cultured in closed space on polydimethlsiloxane (PDMS) microchip in the proper condition (-incubation time to adhere 2.0 h, medium perfusion rate 1.0 μm/min) and confluent state was achieved. Cardiomyocytes were then cultured and about 11 % of cells adhered in a PDMS hollow sphere. Secondly, co-culture of cardiomyocytes and Langerhans islets were achieved and insulin production was confirmed in a micro titer plate. Thus we established the fundamental technology of implanted insulin delivery device.

KEYWORDS
Cardiomyocyte, Langerhans islets, Insulin pump, Micro device, Bio actuator

INTRODUCTION

Insulin-treated diabetes patients require to inject insulin every day. To achieve point-of-care device for these patient, it is important to develop implanted insulin pumps. However, mechanical pumps which constantly require electric sources or medicine supplies are difficult to use permanently. By contrast, we have previously developed a micropump actuated by a cardiomyocytes sheet [1]. But it also needs medicine supplies and being used long time was very difficult. Here, we propose an implanted self-perfusion insulin delivery device composed of cardiomyocytes cultured inside the pump and Langerhans islets. The device is expected to keep driving permanently by bioenergy from blood because cells are cultured inside the pump. To realize this concept, cardiomyocytes must be cultured inside closed spaces and also cardiomyocytes and Langerhans islets must be co-cultured. The objective of the study is development of such fundamental technologies to create the insulin delivery device.

THEORY

The concept of insulin delivery device we propose is shown in Figure1. A hollow sphere and thin tubes is made of PDMS, which is soft enough to be contracted and biocompatible. Cardiomyocytes cultured inside the sphere drive it as a pump by contraction and take blood inside through connected thin tubes with blood vessels. Pancreas B cells in the Langerhans islets cultured in the tubes produce insulin in response to glucose concentration in the blood. Cardiomyocytes and Langerhans islets are cultured by bioenergy from perfused blood, so they can live and work semipermanently (self-perfusion).

In order to realize this concept, some problems must be cleared. Cardiomyocytes require more oxygen and nutrition than other kinds of cells because of contraction. In addition to it, cardiomyocytes don’t multiply. So, to culture them in closed space, investigation of the culture condition is needed. Also, co-culture condition of cardiomyocytes and Langerhans islets is needed to be investigated. Although there are other problems, for example, optimization of the pump performance, improvement of biocompatibility, and improvement stability or durability of the device, we treated the first two problems in this study.
EXPERIMENTAL

First, the culture method of cardiomyocytes in closed spaces was established. To estimate optimum conditions, we cultured primary cardiomyocytes from neonatal rat in a 0.8 mm diameter circle cross-sectional channel of a PDMS microchip. Proper incubation time for cells adhesion and flow rate of medium perfusion were investigated using the microchip. Cardiomyocytes were then cultured inside a PDMS hollow sphere at the same condition regarding cell concentration, time for attachment and perfusion flow rate per cross-section. Second, co-culture of cardiomyocytes and Langerhans islets was realized. We co-cultured Langerhans islets with cardiomyocytes in a 24 well microtiter plate. We prepared three conditions (A: Cardiomyocytes and Langerhans islets (in medium), B: Langerhans islets (in medium), C: Langerhans islets (in Buffer, no glucose)) and were fluorescently-stained by Calcein-AM, which stains only alive cells and intensities of the fluorescence of the cells is proportional to concentration of insulin. Insulin is produced in response to concentration of glucose in medium by Langerhans islets.

RESULTS AND DISCUSSION

In the first experiment, confluent state and spontaneous beating was accomplished at the condition of incubate time for cell attachment (after introduction of cell suspension: $2.0 \times 10^6$ cells/ml): 2.0 h and velocity of medium perfusion: 1.0 μl/min (Figure. 2,3). In the PDMS hollow sphere, a part of cardiomyocytes attached (about 11 %) (Figure.4). The result may be caused by roughness at the interior surface of hollow sphere. Although the rate of cell adhesion was low, we established culture method of cardiomyocytes in closed spaces for a self perfusion micro pump actuated by cardiomyocytes. In the second experiment, co-culture was successful as show in Figure.5 (Condition A). Moreover, as shown in Figure.6, intensities of fluorescence of Langerhans islets in condition A and B was stronger than in condition C, which indicate that in condition A and B, Langerhans islets produced insulin.
CONCLUSION
In conclusion, we established fundamental technologies to create an insulin delivery devices composed of cardiomyocytes and Langerhans islets. We will improve the fabrication of PDMS hollow sphere to reduce the roughness at inside surface and in the future, implant the device into body as insulin delivery device.

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Figure 3 Fluorescently-stained confluent cardiomyocytes in PDMS microchip (0.80mm diameter)

Figure 4 Fluorescently-stained cardiomyocytes’ nuclei in PDMS hollow sphere

Figure 5 Cardiomyocytes and Langerhans islet fluorescently-stained with Calcein-AM

Figure 6 Intensities of fluorescence of Langerhans islets at each condition (n=4)