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An Implantable Electrical Bioreactor for Enhancement of Cell Viability

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***Abstract*—Low survival of injected cells which are prepared by ex-vivo culture is main obstacle in cell-based tissue regeneration. To elevate cell adaptation, we designed an implantable electrical bioreactor where human mesenchymal stromal cells (hMSCs) can be cultured and stimulated electrically. Bioreactor was composed of biocompatible cylindrical Teflon body containing a flexible polyimide electrode and implantable stimulator. The Teflon body has about 300 holes with a diameter of 300um for effective nutrients supply inside the bioreactor and has a length of 17mm and a diameter of 8mm for implantation. After hMSCs seeded on the collagen sponge that serves as scaffold to form a bone tissue graft, they are cultured in the bioreactor with biphasic electric current (BEC) stimulation. BEC stimulation with amplitude of 20/40uA, duration of 100us and a frequency of 100Hz was applied for one week in the early stage of cultivation. Subsequently, after hMSCS were cultured for another week without electrical stimulation, cell response such as cell proliferation, cell attachment and gene expression are evaluated. In vitro and In vivo culture of hMSCs showed 19% and 22% increase in cell proliferation at stimulated groups, compared to unstimulated control. The expression of type I collagen increased significantly at stimulated group. These results suggest that the usage of implantable electrical bioreactor can be a good strategy to enhance the efficiency of stem cell-based tissue engineering.**

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I. INTRODUCTION

STEM cells become remarkable element in the tissue engineering for cytotherapy because of its capability of differentiation into several linages including bone, muscle, vessel and cartilage [1]. But the cell therapy using stem cells is restricted by the low cell survival in the host organism after cell injection [2]. To harvest much of stem cells, it has been suggested to make tissue graft using bioreactor [3]. 3D cultivation using in-vivo bioreactor allows more simple transplantation and is more similar to cellular structure of organism compared with 2D cultivation. Also, auto-graft of the tissue graft cultured in the body of a host can improve the survival of stem cells after transplantation [4].

While there are some researches about induction of osteogenesis and release of cytokine related osteoblast differentiation using electrical stimulation [5-6]. Even though the mechanism of osteoinduction by electrical stimulation is not declared, electrical stimulation has been known as an effective osteoinductive tool excites proliferation of osteoblast and cytokine induction. There are several type of electrical stimulation such as direct charge injection, capacitive coupling and inductive coupling [7]. As a method of electrical stimulation by applying electric field with inductive coupling, pulsed-electromagnetic fields (PEMF) affects induction of cytokines such as osteoinductive growth factor BMP-2, 4, transforming growth factor TGF-b1, growth factor that augments angiogenesis FGF-2 and induces bone mineralization and proliferation and differentiation of osteoblasts [8]. The type of capacitive coupling and direct current injection also derive release of cytokine such as BMP-2, 4, 7, TGF-b1, prostaglandin E2 [9]. However, each of stimulation type has some shortcomings. PEMF can promote tumor progress through angiogenesis and needs relatively big device [10]. Also PEMF is hard to deliver concentrated stimulation to local area cause of PEMF affects a wide region. Direct current injection requires careful surgery and can be arisen inflammation to the affected part. Besides when introducing charge injection, current balancing is required because of creation of undesirable faradic products including hydrogen peroxide, hydroxyl and oxygen ions, free radicals and other intermediates. Such products like hydrogen peroxide was reported which can modulate bone resorption [11]. Also unbalanced charge injection affects toxic results to cell and tissue due to the local pH elevation [12].

Charge balanced BEC can deliver adequate electrical stimulation to the local area and suppress the pH elevation and production of faradic products by inhibiting charge accumulation. Kim and et al, first reported that when BEC was applied, the proliferation of endothelial cell and secretion of VEGF which were not shown in other stimulation strategy were detected, showed BEC might play an important role on the bone metabolism because angiogenesis is essential for bone regeneration [13]. Also BEC inhibits the most representative markers for osteoblasts differentiation such as ALP and cbfa1 but augments msx2 which is induced largely in pre-osteoblast stage, BEC may have attractive effect for proliferation of osteoblast while prohibit osteoblast differentiation. Hence, on bone regeneration using cell therapy BEC stimulation can be favorable alteration of acquisition of abundant osteoblasts and activated cells through cytokine release good for bone formation.

We applied bio-favorable BEC to the 3D in-vivo bioreactor, created an implantable electrical bioreactor system combining in-vivo bioreactor and electrical stimulation system and executed preliminary study of about cell proliferation at in-vitro and in-vivo. Also through in-vivo study, cell viability when cells were transplanted was studied. Through this study, we suggest new alteration on cell proliferation adjusting host system and augmentation of cell viability due to cytokine induction for bone tissue grafts using electrical stimulation by deriving effective electrical stimulation to cell in the in-vivo bioreactor.

II. Methods

A. Design of Implantable Electrical Bioreactor

Electrical bioreactor was composed of three parts; electrode, chamber and stimulation chip. Electrode was designed using biocompatible, flexible polyimide which has been generally used as an electrode substrate material of various neural prosthesis devices. The electrode was made of two parts – one was the part which was put into the bioreactor chamber another was connection line which was connected to the electrical stimulation system. The part which was put into the bioreactor was a 15mm square shape, had two rectangular shaped gold patterns. The length and the width of gold pattern were 12mm and 3mm respectively. Two gold patterns were placed with opposite patterns facing when electrode was rolled up cylindrical to put the electrode into the bioreactor chamber, so electric current path was induced in the bioreactor chamber only. And except the area of gold patterns, electrode area was evenly holed sizing 300um in a diameter. Chamber, where electrode was inserted, was designed in a form of cylinder which had a length of 17mm, diameter of 8mm using Teflon and was holed as like in electrode. It was designed as one side blocked cylinder and had a Teflon cap to block other side after put into the stimulation electrode and scaffold. Implantable electrical stimulation system was composed by stimulation chip, chip PCB, and battery for running the stimulation chip and molded by silicon elastomer to implant into the organism. Stimulation chip was produced by MAGNA/HYNIX 0.35um process and the circuit configuration was same as our previous stimulation chip [14]. Electrical stimulation type which produced by stimulation chip was charge-balanced biphasic electric current pulse. The parameters of BEC can be selected the amplitude from 2uA to 300uA in 2-uA steps, the duration and the frequency from 16us to 512us in 16-us steps and from 32Hz to 1 kHz in 32-Hz steps respectively by attaching external components. Stimulation system without battery was a round shape of 1.2cm diameter and 1.5mm thickness. We used 1620 battery for supply power and connected the battery and the stimulation system with conductive epoxy. The entire stimulation system had 20mm diameter and 4mm thickness could deliver about seven day-electrical stimulation of 20uA, 100us and 100Hz used in in-vivo experiments. To package the system, silicon elastomer (MED-6215, Nusil, USA) was used. Then we could produce the implantable electrical bioreactor system by combining bioreactor chamber and stimulation system and used this system in in-vitro and in-vivo experiments.

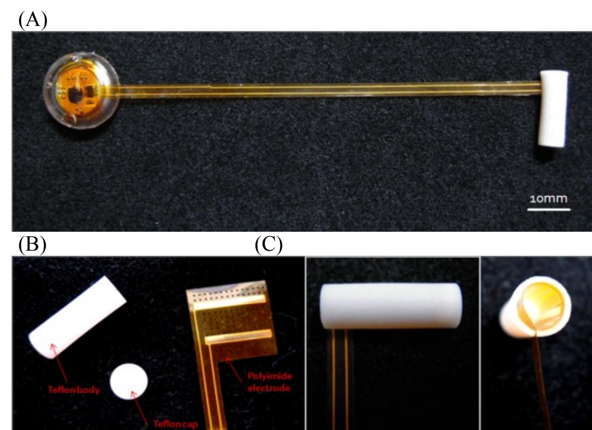

Fig. 1. The feature of in-vivo electrical bioreactor system. (**A**) The entire feature of in-vivo electrical bioreactor. 2cm diameter current stimulator was packaged with silicon elastomer for implantation and connected by 12cm polyimide connection line to the bioreactor chamber. (**B**) Bioreactor chamber and polyimide electrode. Bioreactor chamber consisted of Teflon body and Teflon cap. Teflon body was 17mm length and 8mm diameter. Many of 300um holes on the Teflon body and polyimide electrode help blood exchange. (**C**) Rolled up polyimide electrode could be put into the bioreactor chamber. Exposed two rectangular gold patterns were placed with opposite patterns facing in the bioreactor chamber.

B. In-vitro Experiment

Before in-vivo experiments, we composed in-vitro experiment to confirm the operation of in-vivo electric bioreactor system and to set up the stimulation parameters suitable for cell proliferation. Polyimide electrode was rolled up and put into the bioreactor chamber and two scaffolds with hMSCs were set in the bioreactor chamber (n = 4). Bioreactor chamber with electrode and scaffolds was immersed in culture media. Then connection line of the electrode was connected to electrical stimulation system. This stimulation system was same as previous research and delivered BEC

pulses into the scaffolds in the bioreactor chamber [15]. Electrical stimulation was delivered by the type of biphasic electric current stimulation at the amplitude of 40uA, duration of 100us and frequency of 100Hz. After seven days stimulation, we tested cell proliferation. In case of control group (n = 4), we performed experiments as same as experimental group except connecting electrical stimulation system and tested cell proliferation after seven days electrical stimulation.

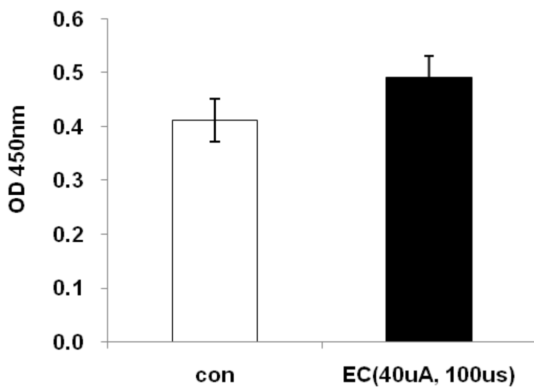

Fig. 2. Cell proliferation result of in-vitro experiment with hMSCs. Electrical current pulses of 40uA amplitude, 100us duration and 100Hz period were applied to hMSCs of experimental group for a week, and no stimulation to the control group. Cell proliferation of experimental group was 19% higher than control group ($p<0.05$).

C. *In-vivo Experiment*

Every in-vivo experiments were performed in accordance with the "Recommendations for Handling Regulation for Laboratory Animals for Biomedical Research" compiled by the Committee on the Safety and Ethical Handling Regulation for Laboratory Experiments in the School of Dentistry at Seoul National University. Male SD rats (6 week, n = 4) were used in this study. For preparing implantation, two collagen scaffolds with hMSCs and polyimide electrode connected to implantable electrical stimulation system were set into the in-vivo electrical bioreactor as same as in-vitro. After an anesthesia, the thigh of animal was incised and bioreactor chamber was placed upper thigh and stimulator was placed lower thigh, then sutured. After surgery electrical stimulation was performed for 1 week, after additional period of 1 week, the animal was sacrificed and bioreactor and scaffolds were extracted and tested cell proliferation and gene expression. In case of control group, all process was performed as same as experimental group but exception of stimulator implantation.

D. *Cell Proliferation Test and Data Analysis*

Cell proliferation was examined by MTT assay. After preparing MTT solution from extracted collagen scaffold, optical density (OD) of the wells determined at a test wavelength of 450nm and reference wavelength of 650nm. Also, to examine mRNA expression, RT-PCR was executed. Extracted cells were trypsinized, sedimented, and RNA was extracted. Extracted RNA was subjected to cDNA synthesis. Then each cDNA subjected to PCR. Data are expressed as the mean-standard error of the mean (SEM) of triplicate repeats over three independent samples (n >3) for the analyses of proliferation and RT-PCR. The results were statistically analyzed by Students' t-test (two-tailed) using SigmaPlot (version 8.0) statistical analysis software. A p-value < 0.05 was considered statistically significant.

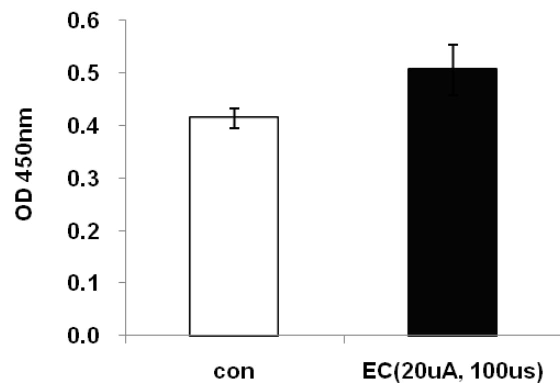

Fig. 3. Cell proliferation result of in-vivo experiment with hMSCs. Electrical current pulses of 20uA amplitude, 100us duration and 100Hz period were applied to hMSCs of experimental group for a week, and no stimulation to the control group. Cell proliferation of experimental group was 22% higher than control group ($p<0.05$).

III. RESULTS

Proliferation of hMSCs was assessed by MTT assay at OD 450 nm comparing stimulated group with unstimulated group. After one week BEC stimulation with a magnitude of 40uA, 100us, and 100Hz to the scaffold inside of bioreactor in culture media, the proliferation of electrically stimulated hMSCs increased 19% ($p<0.05$) compared to proliferation of unstimulated hMSCs. (Fig. 2) After implantation of in-vivo electrical bioreactor with hMSCs seeded in collagen scaffold and one week BEC stimulation and additional one week, the proliferation of stimulation group increased 22% ($p<0.05$) compared to proliferation of control group (Fig. 3) Also from RT-PCR results, collagen type-I expression which is the gene related to bone formation of stimulation group was observed higher than control group at the same GAPDH condition. (Fig. 4) The expression of actin, the important component of extra cellular matrix was observed higher in experimental group.

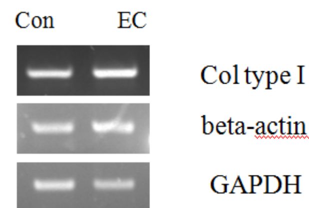

Fig. 4. The mRNA expressions of Collagen type I, beta-actin and GAPDH

IV. DISCUSSION

In this study, we designed an implantable electrical bioreactor which can deliver BEC stimulation into the bioreactor chamber on in-vivo circumstance. After seeding hMSCs to the collagen scaffold using implantable electrical bioreactor we executed 3D cell culturing in-vivo. We developed an alteration for in-vivo 3D cell culture method that using electrical stimulation which is known as an effective tool for cell proliferation and vitalization. In-vivo electrical bioreactor system may be used an alteration of 3D cell culture method promising amount of cell acquisition for cell therapy and augmentation of cell survival after cell injection to the host body. Because purpose of this study was 3D cell culturing inducing electrical stimulation to the cell in the bioreactor, adequate parameter setting of electrical stimulation for cell proliferation was necessary. In the previous study, BEC of 15uA/cm2 amplitude was induced to the osteoblast on the culture plate; the proliferation was decreased by two fold compare with control group [15]. But, in case of BEC of 1.5uA/cm2 amplitude, which was smaller by one order than former case, the proliferation was increased by 31% compare with control group. In this study, we conducted 3D cell culturing using cell seeded collagen scaffold and the area of electrode site was smaller than 2D cell culturing on the culture plate. So the total charge released from electrode site was much smaller than 2D cell culturing at the same amplitude condition. To supply sufficient charge to the cell inside of scaffold, more large amplitude parameter should be used compare with the case of 2D cell culturing. Also, because in-vivo electrical bioreactor supplied electrical power by battery, stimulation parameter should be selected carefully to deliver electrical charge to the scaffold stably during one week stimulation period. We estimated previously the parameter of 20uA amplitude, 100us duration and 100Hz period was suitable for one week stimulation with the battery. After one week electrical stimulation and additional period of one week, cell proliferation was estimated for cases of in-vitro and in-vivo experiments using MTT assay. Cell proliferation was increased only in using electrical stimulation. Every condition except electrical stimulation was same, from these results electrical stimulation affected cell proliferation increase directly as like as former researches. Also these results showed that 3D cell culturing using electrical stimulation in the in-vivo circumstance is possible. Electrical stimulation to the cell induces cytokine release like VEGF, BMP-I, prostaglandin which drive bone formation and angiogenesis. These cytokine play optimistic role to the improvement of cell viability after cell injection and bone formation. We assessed gene expression with RT-PCR to examine in case of 3D cell culturing using implantable electrical bioreactor could be achieved the same effect for bone formation. After total amount of RNA estimation using RT-PCR at the same GAPDH condition, collagen type I expression increase was observed in-vivo with electrical stimulation. Collagen type I is the main component of bone possesses 90 percent portion of bone. So bone graft obtained by using implantable electrical bioreactor may mix well with bone substrate and have high cell survival of injected cell. Because beta actin also expressed more in the electrical stimulated group, this result showed electrical stimulated hMSCs formed more extra cellular matrix on the scaffold and could make a stable cell fixation. These results showed that the produced graft using implantable electrical bioreactor may have higher cell survival cell injection, be adaptable to the host tissue cause of much better cell fixation.

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