Effect of pulsed electromagnetic fields on endothelial cell growth

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INTRODUCTION

Endothelial cells (ECs) covering the blood-contacting surface of a prosthetic material potentially enhance the subsequent nonthrombogenicity of the surface. Autologous seeding techniques have been tried to improve the patency of small caliber vessels during operation. However, intra-operative seeding techniques are limited by the relatively low number of harvested ECs and by poor retention of adhered ECs.

Many investigators have tried to facilitate cell proliferation and migration on artificial surface. Recently, Yen-Patton et al. [1] and Greenbaum et al. [2] reported that a pulsed electromagnetic field (PEMF) could induce cell migration of human umbilical vein endothelial cells (HUVECs) in a wounded layer of the umbilical vein.

In the present study, we investigated the effect of PEMF on the HUVEC's growth in culture system. The aim of this study is to verify the effect of PEMF on the cell proliferation and DNA synthesis. We also tried to find out the optimal PEMF conditions which stimulate the HUVEC growth.

MATERIALS AND METHODS

CELL CULTURE Endothelial cells were isolated from umbilical cord vein and inoculated to 25 cm^2 T-flasks containing M-199 supplemented with 20 mM Hepes (Sigma), 20 % fetal bovine serum (HyClone), 2 mM glutamine (Sigma), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma). The medium was changed every two days and the cells were grown at 37 °C in a 95 % air and 5 % CO_2 environment.

CELL GROWTH AND DNA SYNTHESIS To perform experiments, endothelial cells were removed from the 25-T flasks by treatment with 0.125 % trypsin and 0.01 % EDTA. HUVECs were seeded at a density of 3 x 10^4 cells/cm^2 onto 24-well culture plate and exposed to PEMF stimulation. Cells were cultured in presence and absence of PEMF stimulation for 5 days. Cells were counted using a Coulter counter and the amout of synthesized DNA was measured. DNA synthesis was evaluated by 5-bromo-2'-deoxy-uridine labelling and detection kit III (BrdU, Boehringer Mannheim Biochemica) in order to evaluate the rate of cellular proliferation.

The level of BrdU incorporation into cellular DNA was analyzed by ELISA. The morphologic change of cells was observed by an image analysis system attached to light microscope.

PULSED ELECTROMAGNETIC FIELD We developed the PEMF generator which gives the PEMF of rectangular shapes to each 24-well of the culture plate (Falcon®). The frequency and magnitude of PEMF can be adjustable in the range of 0 - 1,000 Hz and 0 - 50 gauss, respectively. After seeding HUVECs on 24-well culture plate, the plate was placed on the PEMF generating device located inside a CO_2 incubator. Two levels (3 and 10 gauss) of PEMF were comparatively applied at 50 Hz.

RESULTS AND DISCUSSION

We investigated whether PEMF could facilitate the endothelial cell growth. The growth curve of HUVECs was shown in Figure 1. The higher growth rate was observed at the condition of 3 gauss. The growth rate at 10 gauss decreased rapidly on the fourth day after seeding. The present results showed that the degree of growth stimulation on HUVECs was related with the magnitude of PEMF and 3 gauss of PEMF was effective in HUVEC growth. The kinetic profile of BrdU incorporation at 3 gauss and 50 Hz of the PEMF was shown in Figure 2.

This study demonstrated that applied PEMF could

promote the cell growth. Several cell types including neurons, muscle cells, and fibroblasts have been shown to respond to electromagnetic fields. However, exact mechanism of cellular interaction with PEMF has not been determined yet. We are also trying to observe the effect of PEMF frequency on the growth rate of HUVEC.

REFERENCES

1. Yen-Patton et al., J. Cell. Physiol., 134, 37, 1988
2. B. Greenebaum et al., Annual International Conference of the IEEE Engineering in Medicine and Biology Society, 13(2). 1024, 1991

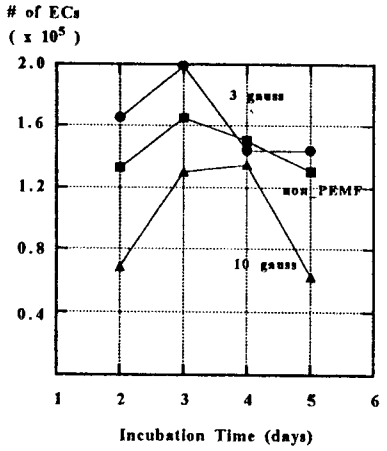

Figure 1.The Growth rate of the HUVECs under the PEMF at 50 Hz

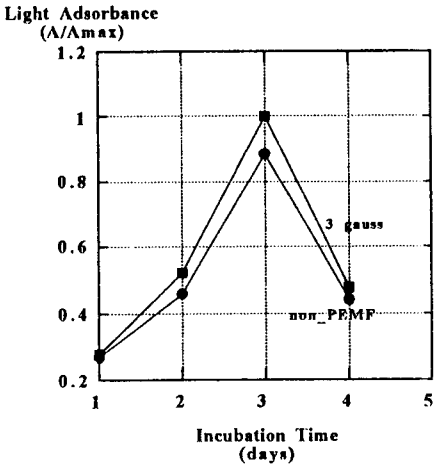

Figure 2. Proliferation of the HUVECs expos the PEMF of 3 gauss and 50 Hz