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ENHANCEMENT OF PROLIFERATION AND VASCULAR ENDOTHELIAL GROWTH FACTOR(VEGF) SECERETION IN OSTEOLAST BY PERIODIC BIPHASIC MICRO-CURRENT STIMULATION

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In this paper, we present a method for enhancing proliferation and VEGF(vascular endothelial growth factor) secretion in osteoblast by stimulating μ -level periodic charge balanced biphasic current. This method can be used to the dental implant or other implants using titanium and its alloy materials by the early osteointegration and increase in bone density.

We designed a current stimulator IC and a specific *in vitro* culture system so that biphasic μ -current pulses may stimulate the osteoblast. Each culture system is composed of an upper(channel electrode) and lower(reference electrode) conductive plate connected with a μ -current stimulator and specifically designed Teflon® well with a dimension of 30mm \times 30mm. In this culture system, two stimulating electrodes were entirely immersed in culture media to flow periodic current pulses between electrodes.

Rat calvarias osteoblasts inoculated at a density of 2,500 cells/cm² were exposed to electrical stimulation of 1.5 μ A/cm² at 1,000 Hz during 6-hours and 24-hours respectively. Control group was cultured in same culture system without electrical stimulation. At 1 and 2 days after stimulation, cells were trypsinized, and counted as live and dead cells with tryphan blue staining. The number of osteoblasts increased from 33,800 \pm 11,292 (mean \pm SEM) cells to 44,300 \pm 9,287, which represented a 31% increase in proliferation of osteoblasts. At day 4 after exposure to stimulation, mRNA expression was investigated for differentiation-promoting cytokines such as BMP-2, BMP-4, TGF- β 1, IGF-1, 2 and angiogenesis-promoting VEGF. mRNA expression of TGF- β 1 and BMP-2 remained in relative low level compared to BMP-4 of IGF-2. But induction of these three important cytokines did not follow stimulation. On the other side, mRNA level of two VEGF isoforms was significantly up-regulated in both groups of stimulation.

In conclusion, this study showed that periodic biphasic electrical current contributes to enhanced osteogenesis by increasing cell proliferation and by induction of angiogenesis, which are important for initiation of bone formation. Continuous stimulation by periodic biphasic electrical current was more effective than interrupted exposure. The results in this study indicate that biphasic electrical current can act as a potential osteogenic inducer. To evaluate this potential effect in bone formation, further studies are needed *in vivo*.