Editorial

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BIOELECTRONICS IN MEDICINE

Electrical stimulation–fracture treatment: new insights into the underlying mechanisms

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Non healing fractures, caused by injury or resection of infected bone or tumors, are a major challenge in trauma and orthopedic surgery. Bone is one of the few tissues whose natural response to injury is to regenerate, thus restoring its form and function back to its original state, instead of healing and forming a scar. Whether and to what degree bone regenerates or heals and scars is dictated by many local and systemic factors, including species, age, type and extent of injury, and physical and chemical signals. Among the latter, electrochemical signals are known to play an important role in regulating regeneration and embryonic development.

Cell function during embryonic development and regeneration is regulated by electrochemical signals that result from positively and negatively charged ions passing through their membranes. This passage of charged ions generates transmembrane voltage gradients or V_{mem}. The V_{mem} values on the surface of cells that are highly proliferative, like embryonic stem and cancer cells tend to be more positive and are depolarized, whereas V_{mem} values of cells in low proliferative states, like neurons, fibroblasts, skeletal muscle and fat cells are more negative, and are hyperpolarized [1–5]. During embryonic development V_{mem} values are in constant flux, and these changes have been shown to play an important role in regulating growth throughout development. This was clearly demonstrated in a series of landmark experiments conducted by Shi and Borgens in which they measured distinct bioelectric patterns on the surface of chick embryos during development. They found that by short circuiting these bioelectric current flows, with a copper wire implanted beneath the skin, newborn chicks hatched without lower extremities [6]. The presence of bioelectric signals during regeneration was demonstrated, by the same investigators, in a separate set of experiments. They measured endogenous bioelectric current emanating from the stumps of amputated, regenerating newt limbs, and found distinct patterns of current irradiating from the limb stumps. In these experiments they describe higher intensities of bioelectric current 4 days after amputation that gradually subsided over the course of a week [7].

Externally applied electrical stimulation (EStim) has been used successfully in clinical settings to improve bone healing for many years. The earliest mention of using EStim to treat fractures in patients was in the mid-1800s in which Garrat used metallic needles to deliver EStim directly into broken bones, and reported improved healing [8]. More recently EStim has been used in clinical settings, primarily as an adjunct to other treatments, to treat recalcitrant fractures [9]. In these cases, EStim is administered using three different approaches; direct current, pulsed electromagnetic field (PEMF) and capacitive coupled (CC). Direct current EStim is applied via a surgically implanted EStim power source and electrodes, at intensities between 10 and 100 μA of current [10]. CC and pulsed PEMF are both administered externally. In CC, an alternating voltage is applied via electrodes placed on the skin on opposite sides of a fracture, generating an electrical field of 0.1–20 G [11]. In PEMF, alternating currents, in an electric coil placed over the fracture site, generate a pulsed electromagnetic field, ranging between 3 and 10 V peak-to-peak [12].

In spite of demonstrated success promoting bone healing, only recently have we begun to understand the mechanisms by which EStim produces this pro-healing effect. The results of several recently published *in vitro* studies suggest that EStim's pro-healing effect is due, at least in part, to its effect on the behavior of bone-forming stem cells. Along these lines, we, and others have shown that EStim improves bone stem cell migration, proliferation, alignment, differentiation and attachment to scaffold materials [13–16]. In addition to these effects on cell behavior we and others have also shown that exposing these cells to externally applied EStim increases mineralization, extracellular matrix deposition and enhanced expression of several osteogenic genes [17,18], all behaviors/functions

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known to be central in both bone healing and regeneration. In *in vitro* studies, we exposed bone-forming cells, in culture to externally applied EStim and observed significant increases in metabolic activity and calcium deposition (osteogenic differentiation). We found that exposure to EStim caused the highest metabolic activity at 3 days of culture, and that EStim caused distinct changes in cell morphology and increased osteogenic differentiation at 7 and 14 days. These changes in cell behavior were accompanied by important changes in osteogenic gene expression patterns, with a significant increase in mRNA levels of Runx2, osteopontin and Col1A2 [18,19].

In other *in vitro* experiments, Wiesmann *et al.* [20] reported, that stimulating osteoblasts with CC EStim accelerated mineralization, and speculated the cause to be 'electrochemical processes' occurring during EStim. In a separate study, Nguyen *et al.* [21] showed that EStim changed calcium distribution within Matrigel and Schwann cells seeded onto gels, a week after stimulation, demonstrating that EStim can have a long-term effect on extracellular matrix deposition. Fredericks *et al.* [22] showed, for the first time, that DC EStim caused a sustained increase of multiple osteogenic genes, suggesting that the biologic mechanism for the observed increase in the rate and extent of bone formation, was mediated by the upregulation of these osteoinductive factors.

In a separate set of *in-vivo* studies, in a rat limb amputation model, we found that EStim stimulates bone marrow-derived stem/progenitor cells to generate highly vascularized osseocartilaginous centers in the zone of injury. We observed that the bone marrow cavity of EStim treated limb stumps remained open for 7 days, whereas in non-EStim sham and control animals the cavity was closed with a thin layer of newly formed fibrous scar-like tissue at day 7 [23]. We hypothesized that this delay in closure of the bone marrow cavity was due to EStim shifting the 'balance' from healing and scarring (seen in control stumps) toward continued proliferation and regeneration. This is an important observation that may provide a hint into the mechanisms by which EStim promotes bone regeneration over healing and scarring.

In another set of *in vivo* experiments, this time in a rat large bone defect model, we exposed defects treated with MSC and scaffold material with EStim and measured the rate and quality of healing [24]. As in our limb amputation experiments, we found that EStim further improved the healing, already provided by the stem cell + scaffold treatment. Of note, by 8 weeks, we found that defects in the control and sham animals contained greater amounts of newly formed fibrous tissue and less new bone and cartilage, than in the EStim-treated defects. In the latter, large areas of the defect were replaced with newly formed bone and cartilage. Although at this 8-week end point, complete bone healing was not observed in any of the EStim-treated samples, significant new bone growth into the center of the defect was evident. This observation supported our hypothesize that EStim's positive effect on bone healing results from reducing fibrous tissue formation, in favor of new bone and cartilage formation. In other words, shifting the balance from healing and scarring toward regeneration.

The exciting new field, and recent advancements in regenerative medicine technologies, hold tremendous potential for completely changing how we practice medicine. For the first time we may be able to actually cure disease, rather than just treat the symptoms. Endogenous bioelectric signals are known to play an important role in regeneration. Externally applied electrical stimulation can be used to manipulate these bioelectric signals to promote regenerative behaviors and functions in cells. A better understanding of the mechanisms by which endogenous and externally applied electrical currents regulate regenerative processes is needed. The many tools we now have at our disposition for probing these mechanisms, at a cellular and molecular level, are starting to reveal previously unknown secrets that could be used to develop powerful new approaches for unleashing the full potential of regenerative medicine.

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