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Review

Unraveling the mechanistic effects of electric field stimulation towards directing stem cell fate and function: A tissue engineering perspective

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ABSTRACT

Electric field (EF) stimulation can play a vital role in eliciting appropriate stem cell response. Such an approach is recently being established to guide stem cell differentiation through osteogenesis/neurogenesis/cardiomyogenesis. Despite significant recent efforts, the biophysical mechanisms by which stem cells sense, interpret and transform electrical cues into biochemical and biological signals still remain unclear. The present review critically analyses the variety of EF stimulation approaches that can be employed to evoke appropriate stem cell response and also makes an attempt to summarize the underlying concepts of this notion, placing special emphasis on stem cell based tissue engineering and regenerative medicine. This review also discusses the major signaling pathways and cellular responses that are elicited by electric stimulation, including the participation of reactive oxygen species and heat shock proteins, modulation of intracellular calcium ion concentration, ATP production and numerous other events involving the clustering or reassembling of cell surface receptors, cytoskeletal remodeling and so on. The specific advantages of using external electric stimulation in different modalities to regulate stem cell fate processes are highlighted with explicit examples, *in vitro* and *in vivo*.

1. Introduction

It is well known that nerve, muscle and glandular tissues make use of endogenous electric fields (EF) to transmit electric signals/impulses [1,2]. It is also well established that endogenously generated bioelectric currents play a critical role in important biological processes including embryogenesis, wound healing, tissue repair and remodeling as well as normal growth of organisms [3]. Endogenous EF exists in both the cytoplasm and extracellular space. Such EF can vary in strength from as small as a few mV/mm to hundreds of mV/mm [4]. It may be noted that electric stimulation of cells has been in practice for quite some time now. In clinical settings too, EF treatment is being extensively used, especially to revive the damaged or disabled tissues in the neuromuscular system (CNS – brain and spinal cord; PNS – sensory and motor neurons) as well as to accelerate healing of injured musculoskeletal tissues such as bone, ligament and articular cartilage. Taken together, such biophysical mechanisms suppress the progression of bone diseases like osteoarthritis and osteonecrosis [5–7]. Furthermore, EF is being proposed as a viable therapeutic option to minimize pain, to overcome tissue malfunction/impairment, to reduce muscle spasm, and to promote overall tissue/organ function [8]. Similarly, direct deep brain stimulation is reported to be beneficial in treating Parkinson's disease, by ameliorating symptoms through stimulation of basal ganglia [9].

The treatment of biological systems/cells with EF can evoke favorable biochemical and physiological responses, provided that

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the exposure duration and EF strength are within tolerance limits [10]. However, the predominant mechanism of EF interaction with biological systems still remains a mystery. Nevertheless, the biophysical changes upon EF exposure can be triggered at the cell surface, affecting membrane protein functions like enzyme activity (Na^+/K^+ATPase and Ca^{2+} ATPases), membrane-receptor complexes and ion-transporting channels by altering the charge distribution (i.e. the conformation) on these biomolecules (Fig. 1) [11,12]. Often, it is believed that a similarity exists in the signaling pathways triggered by mechanical stress and electric field [13].

In order to realize the underlying phenomenon at the cellular level, one needs to determine first, whether the electric field exerts its effect directly on the cell or indirectly through alterations of physical or chemical factors in the extracellular environment. There are three probable lines of action by which external EF can exert its effect. a) The EF may act intracellularly by influencing the movement and concentration profiles of charged cytoplasmic molecules [14]. b) It may perturb the transmembrane potential (TMP) that can alter the membrane responses and can activate growth-regulating ion transport across the plasma membrane [15]. c) It may also act along the plasma membrane, causing an electrophoretic accumulation of surface molecules or by modulating the conformational states of membrane proteins [16]. Such conformational changes are mainly induced by the interactions of protein dipole moments with electrically modulated membrane potentials [17].

The motivation for this review is to inspect various established approaches for the electro-manipulation of stem cells, in particular reference to the predominant mechanisms guiding stem cell response. Though such molecular mechanisms of EF interaction with stem cells are not explicitly understood, some compelling arguments are presented in the subsequent sections to explain the effects observed *in vitro*. The major objective of this review is to bring forth to the tissue engineering community, the need for a detailed investigation of the molecular mechanisms of EF stimulation of stem cells, which would eventually provide a rational starting point for future pre-clinical and clinical studies.

In this review, the different facets of electrical stimulation in the context of guiding stem cell fate and function are discussed. This review encompasses the physiological origin of endogenous bioelectric fields and the experimental approaches to simulate endogenous electrical signals by exogenous EF stimulation. Furthermore, the modulation of stem cell proliferation, migration and differentiation to multiple lineages (osteogenic, neurogenic, cardiomyogenic and angiogenic) by manipulating EF stimulation parameters are exemplified. Also, a critical analysis of the possible mechanisms of EF dictated stem cell response such as biochemical signaling pathways, calcium transients, cytoskeletal reorganization, ATP synthesis, reactive oxygen species and heat shock proteins is provided. Finally, the utility of exogenous EF for deep brain stimulation, cardiac pacing and defibrillation, *in vivo* is illustrated.

2. Endogenous vs exogenous EF

Endogenous EFs are considered to be essential for maintaining cellular homeostasis and are invoked in many biological events, from embryonic development to healing of the wounded tissues. EFs of detectable magnitude have been reported to occur in tissues and embryos of different origin, such as in Xenopus, chicken, and mouse [19]. Endogenous EF of around 20 mV/mm were measured in a 2–4 days old chick embryos and disruption of such field affected tail development structures. A similar EF was recorded in axolotl embryo, interference of which caused developmental

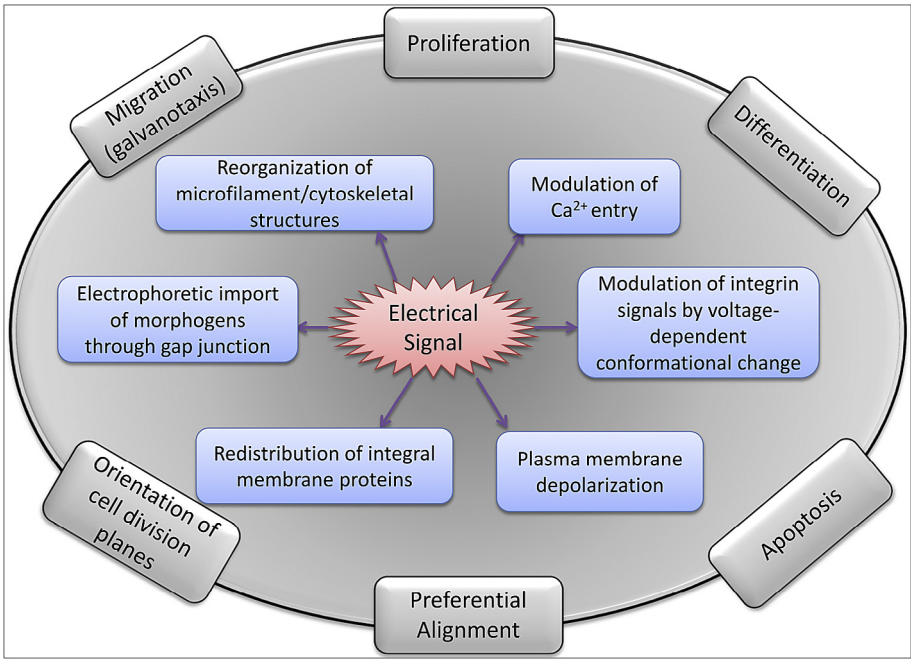

Fig. 1. Bioelectrical control mechanisms at cellular level (clock-wise): Electric field (EF)modulates levels of intracellular calcium, a secondary messenger that drives numerous cellular processes and signaling cascades that govern proliferation and differentiation of stem cells. Endogenous EF guided embryogenesis implicates the key role of integrin receptors, a group of transmembrane linkers of the cell membrane to the extracellular matrix (ECM). The integrin receptor conformation is sensitive to alterations in membrane potential, thus affecting differentiation. Tumor treating oscillating EFs induce cell apoptosis via plasma membrane depolarization. Numerous cell surface receptors (CSRs) such as epidermal growth factor-like receptor (EGFR) undergo redistribution on the cell surface under the influence of an applied EF. This leads to a preferential alignment of the cell axis and orientation of cell division planes during mitosis. The import of morphogens and neurotransmitters through longitudinal gap junctions driven by electrophoretic mechanisms as in embryonic morphogenesis to the three germ layers is central to the migration of stem cells. Electrotaxis/galvanotaxis of cells occurs by reorganization of the cytoskeletal structures such as tubulin and actin microfilaments, wherein actin nucleation and polymerization is induced in the direction of the applied EF by actin-related protein (ARP2/3) complex. Adapted from Ref. [18] Copyright © 1999, FASEB Journal.