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Electrical Stimulation in Tissue Regeneration

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1. Introduction

That human body generates biological electric field and current is a well-known natural phenomenon. In 1983, electrical potentials ranging between 10 and 60 mV at various locations of the human body were measured by Barker (Foulds & Barker, 1983), who also located the so-called epidermal or skin "battery" inside the living layer of the epidermis. Naturally occurred electrical field in human body was also reviewed in 1993 (Zipse, 1993). Bioelectricity is inherent in wound healing. An injury potential occurs in the form of a steady direct current (DC) electric field (EF) when a wound is created. This endogenous EF has been shown to guide cell migration to sprout directly toward the wound edge. On the other hand, wound healing is compromised when the EF is inhibited. McCaig et al. (McCaig et al., 2005) revealed that electrical events induced by injury potential could persist for a long time and present across hundreds of microns rather than be confined to the immediate vicinity of the cell membrane. Moreover, a voltage gradient called "action potential" across cell membrane is known to trigger cells to transmit signals and secrete hormones.

The electrical resistivity of biological tissues obviously varies due to the variation in tissue composition, such as tissue type and density, cell membrane permeability, and electrolyte content. The resistivity of these biological tissues has been measured by means of bioelectrical impedance analysis (BIA). When nutritional and metabolic disorders occur, the electrical properties of certain tissues become abnormal. BIA has therefore been used to diagnose human organ malfunctions. However, it remains difficult to delineate living tissue, such as bone tissue, because this tissue is a composite material that is anisotropic in structure and inhomogeneous in composition. For example, in 1975, Liboff (Liboff et al., 1975) reported a resistance ranging widely from 0.7 to 1×10^5 ohm/cm in human tibia. Recent advances in computed three-dimensional microtomography (microCT) now enable us to clarify the interrelationships between the electrical properties and the microstructures of human bone. The electrical property of bones varies widely caused by many factors such as the unevenly distributed and electrolyte-filled pores, moisture content, pH and conductivity of the immersing fluid. Nevertheless, it remains both essential and possible to normalize the resistance and the capacitance of different bone types. Electrical measurements provide a tool for the rapid quantitative diagnosis of bone

grafts or bone quality during such procedures as total joint replacement surgery. For example, high bone conductivity is associated with high marrow content, while low conductivity is related to high porosity, low bone mineral density (BMD), and low bone volume to total tissue volume (BV/TV) ratio (Sierpowska et al., 2006). Combined with the microCT technique, microstructural changes, such as the reduction of trabecular thickness and number, can be revealed by electrical and dielectric parameters (Sierpowska et al., 2006).

At cellular level, cells are responsive to the exogenous electric field. The discovery regarding the response of yeast (Mehedintu & Berg, 1997) and diatoms (Smith et al., 1987) to electromagnetic field (EMF) stimulation was such a milestone, as both yeast and diatoms are single cell organisms, which implies that the EMF effects occur at the cellular level. These studies also suggested that the control of cellular behaviours is feasible through a manipulation of the cytoskeleton proteins using external physical stimuli such as EF. Various biological systems respond to endogenous or exogenous ES, suggesting that physiologically relevant EF may serve as an efficient tool to control and to adjust cellular and tissue homeostasis. To date, a variety of cells have been used to study their responses to ES, including mesenchymal stem cells, bone and cartilage cells, neuronal cells, and cardiac cells. Numerous in vitro experiments have shown that EF affects important cellular behaviours such as adhesion, proliferation, differentiation, directional migration, as well as division.

2. How cells respond to ES: Possible mechanisms

The biochemical and biophysical mechanisms of how cells respond to ES are very complex and remain largely unknown. For example, how do cells sense ES? How and at what level do cellular behaviours begin to change in an EF? For now, these questions remain unanswered, as cell response to ES is not only complicated by the complexity of the cell signalling pathways, but also by the complex nature of EF in biological medium. However, despite the lack of knowledge regarding the effect of EF on cell properties, we are not completely in the dark, as important experimental evidences support the following possible mechanisms of electrical signal recognition and signal transduction, as well as the regulation of gene expression.

2.1 Recognition of electrical signals and signal transduction within individual cells

The sophisticated cell membrane is the first point of contact with the environment. The 4 to 10-nm-thick lipid bilayer membrane separates the interior of a cell from the drastically different external medium. Cell membrane surface is negatively charged due to the predominance of negatively charged chemical groups (e.g., carboxylates, phosphates) in membrane proteins and glycans. An electrical potential gradient in the order of -100 mV (negative inside the cell) (Bezanilla, 2008), namely, transmembrane potential, exists across the cell membrane and is generated by the uneven distribution of ions – in particular potassium – on both sides of the membrane.

Cellular activity is regulated by the distribution of soluble ions through various ion channels, pumps and transporters, which are mostly membrane proteins subjected to external stimuli, including electricity. Several membrane proteins, referred to as voltage-sensing proteins, such as ion channels, transporters, pumps, and enzymes not only sense but

use external electric field to regulate cellular functions (Bezanilla, 2008). The movement of the charge or the dipole within these proteins, i.e., the transient or gating current, may also be affected by external EF. Although physiological-level EF in the range of mV and μA is too weak to depolarize the lipid bilayer cell membrane, it is strong enough to activate transmembrane channels and transmembrane receptors (Aaron et al., 2004a). An ion flux caused due to the difference in ion concentration across the membrane triggers signalling pathways. The potential difference, in the order of 100 mV between the two sides of the membrane, plays an important role in signal transduction processes. For example, Aaron (Aaron et al., 2004a) observed that cellular response to ES may involve the calcium/calmodulin pathway. The change in transmembrane potential may rearrange the charged groups or dipoles within the membrane; thus, channel-mediated ions such as Na^+, and K^+ may conduct through the membrane. The charge movement, or gating current (I_g), has been detected by Armstrong (Armstrong & Bezanilla,1973). EF may also alter ligand binding as result of the modification of the density and distribution of receptors through polarizing membrane components, moving receptors, or alternating receptor conformation. For instance, a DC EF adjusted the assembly and distribution of actin filaments within the cytoplasm of endothelial cells (Li & Kolega, 2002). As part of the complex intracellular signalling pathways, cytoskeleton provides structural stability and elasticity to the cell undergoing multiple deformations without losing its integrity. The physical attachment between membrane and cytoskeleton actin takes place through linker proteins such as spectrin and ezrin/radixin/moesin (ERM). EF induced intracellular ATP depletion and consequently inhibited ERM function, resulting in altered membrane characteristics such as endo- and exocytosis, signalling, adhesion, and motility (Orr et al., 2006). This suggests that the ATP depletion may occur as a result of increased ATP transport to the exterior cell membrane and of increased ATP consumption because of the EF-stimulated high metabolic activity.

2.2 Gap junction intercellular communication

Gap junctions are specialized regions of the plasma membrane where protein oligomers establish contact between adjacent cells. The gap junctions in osteoblasts derived from newborn rat calvaria in culture measured between 0.10 and 0.91 μm, with a mean length of 0.43 ± 0.23 μm, and a distance of 2-4 nm between the neighbouring membranes. Gap junctions enable the rapid and efficient propagation of ions, nutrients, metabolites, secondary messengers, and small molecules under ~ 1,000 Da between adjoining cells ("coupling"). In bone cells, the gap junction enables different preosteoblastic cells to progress in a coordinated manner toward a mature phenotype and to maintain the expression of the phenotypic functions associated with differentiated bone tissue. For example, signalling molecules (~ kDa) such as calcium, cyclic nucleotides, and inositol phosphates, exchange through gap junction channels. Moreover, much evidence indicates that gap junction communication is necessary for the development and maintenance of a differentiated osteoblast phenotype, including the production of alkaline phosphatase, osteocalcin, bone sialoprotein, and collagen, as well as decreased mineralization (Stains & Civitelli, 2005). The junctional conductance between coupled cells revealed a high degree of voltage dependence through electrophysiological studies. Two regulation mechanisms were confirmed to respond to the voltage difference between two cells (transjunctional voltage, Vj), i.e., Vj-gating (fast) and loop-gating (slow). The connection composition of gap junction channels defines their unique properties, such as their

selectivity for small molecules and voltage-dependent gating. For example, Nakagawa et al. (Nakagawa et al. 2010) reported that the displayed potential in the Cx26 gap junction channel was in the range of -20kTe^{-1} to 20kTe^{-1}, therefore, gap junctions can even be gated by membrane voltage (Vm), termed Vm-gating. In another study, the boundaries of intercellular communication were altered by applied current, and the current applied to one cell revealed a voltage-current (V-I) relationship (Harris et al., 1983). Lohmann reported that the levels of connection of Cx43 protein were reduced in both MLO-Y4 cells and ROS 17/2.8 cells under low frequency EMF (Lohmann et al., 2003). When applying ES (interval 1 s, duration 100 ms, amplitude 10 mA) to myocytes, Nishizawa et al. (Nishizawa et al., 2007) used a high-speed confocal microscope to analyze cellular communication within the myocytes and discovered that cytosolic Ca^{2+} concentration had been upregulated, which indicates that gap junctions largely contributed to the propagation of intracellular signals. Gap junctions also play a crucial role in the response of cellular networks to extracellular signals through the integration and amplification of the signals.

2.3 The role of extracellular matrix (ECM)

ECM, an intricate 3D network of fibrillar proteins, proteoglycans, and glycosaminoglycans (GAGs), provides an electrochemical environment surrounding cells and conveying signals from the exterior of the cell to the interior and vice versa. Many ECM components can be affected by EF, including soluble ions and charged groups in GAGs and proteins. It was reported that proteins moved along EF to reach the binding sites on cell membrane receptors (Adey, 1993). ES also reportedly influenced the adsorption of serum proteins, specifically fibronectin (FN), onto electrodes (Kotwal & Schmidt, 2001).

2.4 Regulation of gene expression

Numerous studies have investigated how cell gene expression and protein production respond to electrical signals of varying amplitude and frequency. However, little is known regarding the signal transduction cascade that consequently alters many cellular events during/after exposure of bone cells to physiological ES. The release of growth factors and cytokines from bone cells is critical to both bone formation and turnover. Several studies (Zhuang et al., 1997) have suggested direct or indirect effect of ES on the gene expression and protein production of bone cells. For example, ES increased the gene expression of transforming growth factor-β (TGF-β), collagen type-I, alkaline phosphatase (ALP), bone morphogenetic proteins (BMPs), and chondrocyte matrix. According to Aaron (Aaron et al., 1997), an increase in TGF-β1 may cause phenotype autocrine and paracrine signalling that consequently regulates osteochondral cells to proliferate and to differentiate, followed by an increase in extracellular matrix deposition. Meng et al. (Meng et al., 2010) reported that following osteoblast stimulation with 200mV/mm for 6h, osteocalcin (OC) and ALP increased 20% compare to the non-ES control. It is of interest that following a multiple 6h ES at 200mV/mm intensity every two days over a period of 6 days, the gene expression of OC, ALP, Runx-2 and BMP2 increased more than two folds compare with the non-ES controls.

3. ES in bone healing and bone tissue engineering

3.1 ES in bone healing and clinical application

While the first report of a successful use of ES appeared as early as 1841, ES treatment on bone did not occur until 1953 when Yasuda et al. applied continuous electrical current to a

rabbit femur for three weeks and demonstrated new bone formation in the vicinity of the cathode (Ryaby, 1998). Their milestone research launched the age of ES treatment in bone healing applications. It appears that the adjunctive noninvasive or extracorporeal ES therapy could be beneficial for those with bone fractures or osteotomies by speeding up the healing of bone.

3.1.1 Animal experiment

The benefits of ES include increased fracture callus, faster fracture consolidation, increased resistance to refracture, cortical thickening, periosteal or endosteal bone proliferation, reduced incidence of disuse osteoporosis and joint ankylosis. An early review published in 1977 (Spadaro, 1977) showed that 71% of animal experiment involved direct current (DC) stimulation in the 0.1-100 microampere (μA) range, 15% used pulsed DC with frequencies from 0.5 to 500 Hz, pulse widths from 1-500 milliseconds, and peak amplitudes from 0.5-1000 μA, 9% used alternative current (AC) stimulation with frequencies from 5-50 Hz, and only 5% reportedly used EMF with frequencies from 0-60 Hz. The animals involved in the ES experiments included rabbit, rat, sheep, horse, deer, cat, mouse, chick and frog. Capacitive coupling electric field (CCEF) and inductive coupling EMF are used more frequently in recent years. Continuous DC stimulation has also been used. The most frequently used animal models are rabbit, dog, and rat. The principal endpoints include bone mineral content/density, and the content of calcium, phosphorus, and carbon, as well as mechanical properties. (Manjhi et al., 2009).

3.1.2 Clinical application

Electrical bone growth stimulators are categorized as invasive, semi-invasive, or noninvasive. Invasive and semi-invasive DC stimulators deliver DC internally via surgically implanted electrodes, whereby the cathode delivering energy to the bone is placed around the area requiring treatment. The power source of an invasive system is implanted in nearby soft tissue and is removed at the end of treatment. In the semi-invasive system, the cathodes are inserted percutaneously under fluoroscopic guidance, and the power source is attached with anodes placed anywhere on the surface of the skin. Osteogenesis is stimulated at the cathode with a current of 5-100 μA (Aaron et al., 2004b). Current below certain threshold was found to lead to bone formation, while that above the threshold caused cell necrosis. Noninvasive stimulators are based on either capacitive coupling (CC) or inductive coupling (IC). CC stimulators use the CC device to apply an electrical potential of 1-10 V at frequencies of 20-200 kHz. The EF strength generated in the tissue is in the range of 1-100 mV/cm. These stimulators are noninvasive and the electrodes are placed on the skin at the opposite side of the fracture site. IC stimulators use EMF. Noninvasive devices can use DC or AC, either constant or pulsed, and low frequency electromagnetic fields. The ES is delivered to the target site via externally mounted cathodes (in the case of DC or AC) or coils (in the case of EMFs). Most systems are equipped with monitors to assess system function and patient compliance. The applied electromagnetic fields vary in amplitude, frequency, and wave form. The order of magnitude of the magnetic field is in the range of 10 μT to 2 mT and the produced electric field strength is in the range of 1 to 100 mV/cm (Aaron et al., 2004b). Clinically, CCEF has been shown to induce osteogenesis, facilitate the consolidation of recalcitrant nonunions, and modify primary spine fusion (Goodwin et al., 1999) especially for the patients with posterolateral fusion and those with internal fixation.

One study showed that treating nonunions with CCEF achieved a success rate of 60-77% (Beck et al., 2008). Implantable DC/AC stimulators have the advantage of providing stimulation directly at the fracture site. Drawbacks include the risk of infection, tissue reaction, and superficial soft tissue discomfort caused by the protrusion of the device, possible pain caused by the electrode implant, and the stress associated with the operative procedures (Haddad et al., 2007a). No pain or surgery is involved with the noninvasive device which may even be conveniently used by the patient at home. The disadvantages of the capacitive and inductive coupled methods include skin irritation from the electrode disc, the relatively long treatment regimen, and the probability of promoting tumour jeopardy in unexpected regions.

Studies on electrical and electromagnetic stimulation to promote bone union have showed promising results in clinic, showing the significant effect of various types of ES on bone healing. However, a 2008 meta-analysis and systematic review found that evidence from the randomly controlled trials failed to conclude that EMF improves the rate of union in patients with a fresh fracture, osteotomy, delayed union, or non-union (Mollon et al., 2008). Although multiple randomised trials exist to support the various bone stimulation modalities, they are small and are limited primarily to radiological endpoints. Therefore, the universal acceptance of ES in clinical applications will require greater support from larger, more definitive trials. Nonetheless, in a 2008 survey of 268 tibial shaft fracture management cases, 16% of 450 Canadian orthopaedic surgeons reported using electrical bone growth stimulators to manage uncomplicated open and closed tibial shaft fractures, while 45% used them for complicated tibial shaft fractures (Busse et al., 2008).

3.2 Important factors in bone tissue engineering

There are major clinical reasons to develop tissue engineering strategies for bone regeneration. In bone tissue engineering, to summarize, all of the factors can be channelled into three essential elements: (1) osteoblasts or their precursors, (2) an osteo-compatible

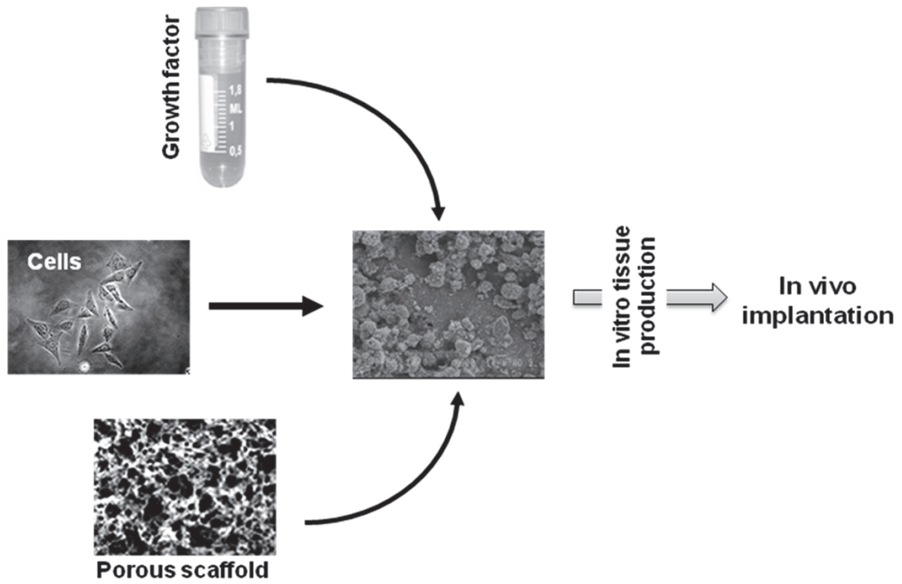


Fig. 1. Basic concept of tissue engineering

scaffold, and (3) biological cues such as growth factors. The functions of various cell types and their interactions with ECM during tissue repair and regeneration have been extensively explored, yet much remains to be investigated. It is apparently accepted that scaffolds with appropriate chemistry and morphological design and assisted by stimulation factors are able to promote cell adhesion, proliferation, and the formation of ECM. When these three essential factors are appropriately assembled together, living bone tissue is hopefully generated in culture and then transplanted into animal models in the hopes of integrating with the host and finally growing into functional tissue or organs, as showing in Figure 1. Because of the directional feature of EF and various biological systems responding to endogenous or exogenous ES, ES contributes to a supportive environment for tissue repair and regeneration.

3.2.1 Osteoblasts and their precursors

Four progenitor cells are commonly used for bone engineering. These include whole freshly collected bone marrow cells, purified and in vitro propagated mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), and stem cell differentiated osteoblasts. Bone marrow possesses a limited number of osteoprogenitor stem cells and the mean prevalence of colony-forming units with osteoblast phenotype is very low (Heliotis et al., 2009). The MSC approach relies on identifying and isolating adult stem cells with a sufficient quantity. This can be a lengthy process, especially with old patients, as cell numbers and proliferative capacities may be low. As for ESCs, ethics remains a major issue, particularly when embryos are used solely for research purposes. In addition, while stem cells may have the potential to be used to generate entire skeletal tissues, the long-term biological consequence of stem cells at the implant site, as well as issues of cell plasticity, remains largely unknown (Heliotis et al., 2009). Osteoblasts, which are active in bone development and bone remodelling as bone-forming cells, have the ability to synthesize and secrete collagen type I and glycoproteins (osteopontin, osteocalcin), cytokines, and growth factors into the unmineralized matrix (osteoid) between cell body and mineralized regions. Furthermore, they are rich in alkaline phosphatase activity (Kartsogiannis & Ng, 2004). Compared to marrow and stem cells, osteoblasts are plentiful and can be harvested from new bone lamellae which they have just secreted.

3.2.2 Scaffolds

The “ideal” scaffold, whether permanent or biodegradable, naturally occurred or synthetic, should firstly be biocompatible, then be osteoinductive or at least osteoconductive, and finally, mechanically compatible with native bone. Various currently available synthetic materials have served for the design of bone implants, including bioresorbable polymers such as polylactide, polyglycolide, copolymers based on lactide, glycolide and ε-caprolactone, trimethylene carbonate, or tyrosine carbonate. In order to gain the desired mechanical and biological properties, these synthetic polymers are often combined with bone components such as hydroxyapatite (HA). The porous 3D scaffold (> 100 micron diameter) provides sufficient opportunity for cell migration and expansion as well as enables the transport of nutrients and metabolic wastes. The porous structure can be generated through various techniques including temperature-induced phase inversion, salt leaching, and prototyping (Hutmacher, 2000). A wide range of porosity and pore size reportedly promotes cell growth and bone formation; for example, 70 to 95% porosity was achieved by adjusting the polymer–to-salt ratio, and the pore size was controlled

independently by varying the leachable particle size with standard testing sieves (Hou et al., 2003). 3D scaffolds displaying a high porosity and large pore size often weaken their mechanical properties. Nevertheless, a 3D silk biomaterial matrix prepared by salt leaching achieved a compressive strength up to 175 ± 3 KPa while maintaining porosity between 84 and 98%. , A compressive strength up to 280 ± 4 KPa was achieved at porosity between 87 and 97% when the silk scaffold was fabricated by gas foaming. When the porosity approached 99%, its maximum compressive strength was shown to be only 30 ± 2 KPa (Nazarov et al., 2004).

3.2.3 Growth factors

Tissue regeneration involves many promoters/factors. Cytokines are secreted by many cell types and function as signalling molecules. They also promote and/or prevent cell adhesion, proliferation, migration, and differentiation by up- or down-regulating the synthesis of proteins, growth factors, and receptors. The important signalling molecules in bone healing and development include TGF-β and BMPs. The use of growth factors in bone regeneration is becoming increasingly promising, as this strategy is direct, relatively simple, and highly efficient. These proteins are easily available yet are costly. Of interest is the fact that some of these proteins can be regulated through physical stimulation. Cultured cells and tissues subjected to different biophysical stimuli of varying intensities in both in vitro and in vivo settings have been reviewed (Chao & Inoue, 2003; Wiesmann et al., 2004). These stimuli include mechanical force, electrical and electromagnetic field, laser irradiation, heat shock, and ultrasound. Some of them are already applied in the clinical setting to treat tissue defects (Barrere et al., 2008). Studies have shown that osteoprogenitor cells and osteoblasts increased their proliferation and differentiation, as well as the production of ECM and growth and differentiation factors including TGF-β and BMPs by EMFs (Chao & Inoue, 2003; Dimitriou & Babis, 2007). Therefore, growth factors regulating bone formation can be endogenously induced through the appropriate extrinsic biophysical stimulation to bone cells. Due to the intrinsic instability of the growth factors toward chemical and physical inactivation, and the relatively long contact time between the growth factors and the target cells required to obtain the desired effect, direct injection of growth factors into the regeneration site or their simple dispersion into the porous scaffold may prove to be less effective. Consequently, endogenously induced bone-forming growth factors may provide a solution because of their steady secretion from cells.

3.3 ES methodology and cellular reactions

ES methods vary according to the type of ES used. There is no standardized methodology or setup. According to the methodologies reported in literatures, ES methods can be categorized into the following three groups: 1) DC/AC EF, 2) capacitive coupling-induced EF (CCEF), and 3) electromagnetic field (EMF). Various cell types have been studied and found to be responsive to ES under different experimental settings.

3.3.1 DC/AC EF

Following the discovery of ossification enhancement by DC EF in 1964 (Berg & Zhang, 1993), ES was viewed as a new therapeutic alternative and thus investigated using both DC and AC EF. DC and AC EFs in medical literatures often indiscriminately refer to either electrical potential gradient or electrical current established between two points of different

electrical potentials generated by either a DC or an AC power source. Electrical potential gradient and electrical current are related but two very different things, of which the biological consequences can be quite different. Nevertheless, potential gradient and current intensity of DC or AC electricity are probably the most frequently used ES approaches in research. DC/AC EF can be created between two electrodes immersed in electrolytes such as culture medium or tissue fluid. In such circumstances, the charge carriers are the ions in the electrolytes. DC/AC EF can also be established across a solid electrical conductor, such as by directly connecting a conductive polymer into an electrical circuit. Here, polarons/bipolarons serve as the charge carriers.
A variety of experimental setups are used in different labs, as described below.

3.3.1.1 Metallic electrode

Metallic electrodes are easily modified into different forms, connected with a power source, and inserted into culture medium or tissue. However, in order to convert ionic conductivity in the electrolyte into electron conductivity in metal, electrochemical reactions are known to occur on the electrode surface which generates chemical species often referred to as faradic products. The most common irreversible processes encountered with electrodes are water electrolysis and the resulting pH change and gas formation, electrode dissolution due to the oxidative formation of soluble metal complexes (typical of platinum (Pt) electrodes) over 320 μA, and the breakdown of passivity with subsequent pitting or transpassive corrosion (typical of stainless steel electrodes). Compared to salt bridges, the direct exposure of a titanium cathode and its faradic products led to a further lowering of the media calcium levels and also significantly increased the pH in culture medium (Bodamyali et al., 1999). Based on animal experiments and clinical studies, it is believed that electrical current can be injected as a constant or a pulsatile electric energy to treat fresh fractures and osteotomies, spine fusions, as well as delayed and nonunion fractures. A wide range of materials have been used as invasive electrodes, including stainless steel, tungsten, platinum, platinum-iridium alloys, iridium oxide, and titanium nitride in both animal and clinical experiments (Cogan, 2008). Experiments investigating the response of bone cells to ES based on metallic electrodes are rare. Kim et al. (Kim et al., 2006) found that the proliferation of rat calvarial osteoblasts on an Au anode increased 31% after 2 days under an ES of 1.5μA/cm².
When electrodes are used as implants in the clinical setting, reliability is crucial. In order to find ways to prevent electrode-related injuries, it is equally important to understand them. In 2007, Netherton (Stecker et al., 2006) discussed four factors that may influence the likelihood of electrode-related injury. The first factor is the heat generated by electrical current passing through resistive tissue, which consequently elevates the temperature of the surrounding tissue. Thus, the denaturation of cellular components, such as proteins, possibly appears. In addition, localized temperature increase due to the current may be significant near the edges of electrodes, near small electrodes, or near the regions where electrical conductivity changes rapidly. The second factor is electroporation, by which pores formed on cell membrane allow charged ions or large molecules to pass. Lee (Lee et al., 2000) suggested that electric fields as low as 60 V/cm may cause electroporation injury to muscle or nerve. Clinically, however, electroporation happens when the electric field is in the order of 500 to 3000 V/cm (Hofmann et al., 1996). The third factor is called electroconformational denaturation of cellular proteins. Because many proteins contain charged groups, their structure can be affected by a strong electric field which can denature proteins and subsequently cause cell damage. The last factor is overstimulation, such as, for

example, a phenomenon called excitatory neurotoxicity. Electrochemical injury can be prevented by selecting the appropriate electrode type and by minimizing and maintaining the total level of stimulation below a certain threshold (Stecker et al., 2006).

3.3.1.2 Salt bridge

A salt bridge contains a saturated solution of inert salt, usually $NaClO_4$, KCl, or KNO_3. The term inert refers to the reactivity relative to the reaction under study. When the circuit is closed, the cations (such as K^+) flow out of the salt bridge at the cathode, and the anions (such as Cl^-) flow out at the anode due to the electric field created by the potential gradient. Salt bridges are used to isolate metallic electrodes from direct contact with the cell culture medium during in vitro experiments. With these bridges, the electrolyte composition (culture medium) remains undisturbed when electrical potential is applied and current passes. The primary function of the salt bridge is maintaining the culture medium electroneutrality and avoiding electrochemical or redox product contamination from the metal electrode as it allows the current to flow.

Using a salt bridge system, Sun (Sun et al., 2006) reported that a small fraction (less than 10%) of rat MSCs responded to ES and became contracted, reoriented, and demonstrated changes in cellular morphology in a 3D collagen scaffold. A DC electric field of 2 V/cm affected the actin reorganization and morphology of hMSCs and osteoblasts. A reduced ATP level led to an inhibition of the linker proteins which are known to physically couple the cell membrane and cytoskeleton, causing cell membrane separation from the cytoskeleton. However, in Sun's study, the strong electrical strength (7 V/cm and 10 V/cm) failed to induce any significant MSC reorientation. Curtze et al. (Curtze et al., 2004) used agar bridges to induce ES to cells cultured on either cover slips or collagen-coated polyacrylamide gels. They observed that primary bovine osteoblasts and human osteosarcoma cells exposed to a DC EF of 10 V/cm realigned themselves with their long axis perpendicular to the EF. In short, the cells lost approximately 46% (12 of 26 μm) of their length along the electric field lines. Perpendicular to the electric field lines, the cells lost 16% (4 of 24 μm) in the first 20 minutes and grew 35% (from 20 μm to 27 μm) within 100 minutes. The cells reacted to the electric field in two phases: retraction, followed by elongation. The authors also noticed that when ES was turned off after 10, 30, or 60 minutes, the cells did not continue their process but rather spread in all directions. The cells in this study responded to EF sequentially in opposite ways, namely, retraction followed by elongation. As far as how the ES signal induced the cytoskeletal reorganization to be large enough to cause significant change in cell shape, the authors attributed it to the change of intracellular free calcium levels. In another study, Ferrier et al. (Ferrier et al., 1986) cultured rabbit osteoclasts and rat osteoblast-like cells on glass coverslips in field strengths of 0.1 and 1 V/mm and found that the osteoclasts migrated rapidly toward the positive electrode, while the osteoblast-like cells migrated in the opposite direction toward the negative electrode, showing that different types of bone cells respond differently to the same electrical signal. When salt bridges are used, the so-called faradic products, including hydrogen peroxide, hydroxyl and oxygen ions, free radicals, and other intermediates, are excluded from the culture medium. However, using agarose salt bridges may decrease calcium levels in the media. The authors (Bodamyali et al., 1999) did not look at the calcium on the salt bridge but rather measured the calcium concentration in the media with a spectrophotometer by examining the calcium's reaction with o-cresolphthalein, which forms a red complex at pH 10-12 with an absorbance maximum at 575 nm. Calcium uptake by mouse calvaria was

suspected due to the acceleration of the intracellular transport of calcium, as previously reported (Wang et al., 1998), and may increase the activation of the calcium/calmodulin signalling pathway (Lorich et al., 1998; Zhuang et al., 1997).

While the salt bridge technique has been extensively used to study the effect of ES on cultured cells, this approach can be problematic when applied for tissue engineering purposes. Because salt bridges are suspended in cell culture medium, the electrical current primarily passes through the medium rather than the non-conductive substrate. This may constitute a major concern when porous 3D scaffold is used in culture. As most scaffolding materials are not electrically conductive (dielectric), cells inside the porous scaffold or on its surface are not exposed to the same degree of electric field and current. Secondly, through Fickian diffusion, the salt simply escapes from the salt bridges into the culture medium whenever the circuit is open or closed. As a result, when salt bridges are used, the cell culture medium is not homogeneous. Furthermore, salt bridges as electrodes are not easily implanted in animals or used in clinical trials.

3.3.1.3 Carbon nanotube (CNT) electrode

CNTs possess high tensile strength, formidable thermal and chemical stability, and excellent electrical properties. The conductivity of a CNT bundle approaches 1×10^4 S/cm. Despite the non-homogeneous distribution of CNTs with respect to length, diameter, and doping level, CNT networks maintain a conductivity ranging from 10-10^3 S/cm. Their superior physical properties make them ideal for the manufacture of conductive composites at very low concentrations (Sandler et al., 2003). Implantable CNTs may prove useful in bone tissue engineering applications. For instance, CNTs showed a powerful ability to absorb various proteins, including adhesion factors, growth factors, and differentiation-inducing factors. In cell culture, CNTs were found to increase osteonectin, osteopontin, and osteocalcin gene expression. ALP/DNA and total protein/DNA levels on carbon nanotubes were also found to increase. These results indicate that CNTs may facilitate cell adhesion and induce osteogenic maturation of osteoblasts by adsorbing specific proteins (Akasaka et al., 2009). CNTs were also shown to display the ability to inhibit osteoclastic differentiation, suppress a transcription factor essential for osteoclastogenesis in vitro, and inhibit osteoclastic bone resorption in vivo (Narita et al., 2009). Khang et al. cultured human chondrocytes on a film made of CNT/polycarbonate urethane composite. An ES of 3 and 6 h at a frequency of 10 Hz and a current of 10 μA was applied after seeding and was found to significantly enhance chondrocyte functions. Initial fibronectin adsorption, chondrocyte adhesion, and long-term cell density were enhanced by more than 50% (Khang et al., 2008). With polylactic acid/carbon nanotube (PLA/CNT) as the substrate, ES was also reported to promote various important osteoblast functions, notably cell proliferation, collagenous and noncollagenous protein gene expression, and calcium deposition in the extracellular matrix. Specifically, when osteoblasts cultured on the surface of PLA/CNT were exposed to an AC ES with a 10 μA current, cell proliferation increased 46% after 2 days, while extracellular calcium increased 307% after 21 days, and collagen type-I mRNA expression upregulated after both 1 and 21 consecutive days. In the experiments mentioned above, the electrodes were set parallel in either the horizontal or vertical position, and in both cases, the electrodes were in culture medium and produced ionic current.

CNTs are, however, non-biodegradable in nature and may be inherently cytotoxic. Since 2003, studies have shown CNTs to display cytotoxicity. An in vitro evaluation of the inflammatory potential of CNT on peritoneal and alveolar macrophages was reported by Jia (Jia et al., 2005).

At the threshold dose of cytotoxicity, single-walled carbon nanotubes (SWNTs) induced serious damage to alveolar macrophages at a low exposure dose of 0.38 μg/cm²(Zhu & Li, 2008). Cheng et al. (Cheng et al., 2009) found that MWNTs entered the macrophages through the plasma membrane into the cytoplasm and the nucleus, which may result in necrosis and degeneration. Apoptotic cell death was found with a higher dose at 3.06 μg/cm². The mechanisms responsible for CNT cytotoxicity are not yet fully understood. Cells exposed to CNTs resulted in accelerated oxidative stress (increased free radical and peroxide generation as well as depleted total antioxidant reserves), loss in cell viability, and morphological alterations to the cellular structure. CNTs have also been shown to block potassium channel activities in heterologous mammalian cell systems when applied externally to the cell surface, which also suggests a degree of cytotoxicity. A variety of factors are involved in CNT cytotoxicity, including species, impurities, length, aspect ratio, and chemical modification (Zhu & Li, 2008). In general, as long as the CNTs structures are hydrophilically modified, contain no trace metals, as well as do not form fibrous aggregates that frustrate the phagocytic cells, CNTs should show less or mild cytotoxicity. A recent study reported about the toxicity and biodistribution of hydrophilic CNTs showed that PEGylation-CNTs were not acutely toxic and primarily accumulated in the liver and spleen (Berlin et al., 2010).

3.3.1.4 Conducting polymers

Conductive polymers are softer and more compliant than metals or other inorganic conducting materials, such as silicon and carbon fibre. Hence, they represent a unique and useful way to improve the intimate bioelectronics/tissue connection. In addition, conducting polymers may hopefully improve the performance of implantable medical devices by facilitating mechanical compliance, electrical conductance, and the biological affinity between devices and cells. It has indeed been suggested that the inflammation caused by the tissue/conducting material stiffness mismatch will be alleviated.

PPy is the most widely researched conducting material to interact with mammalian cells since the first study in Langer's lab in 1994 when Wong et al. (Wong et al., 1994) demonstrated the effect of EF on cell growth and differentiation through PPy. The biocompatibility of PPy can be improved by choosing different dopants (such as dodecylbenzenesulfonate sodium or heparin (Meng et al., 2008)) and by immobilizing biologically active molecules including enzymes, antibodies, receptors, and even whole living cells to manifest its biological affinity (Ateh et al., 2006). Conducting polymers such as polyaniline (PANi), polythiophene (PT), and their derivatives also have shown sufficient stability and biocompatibility. However, the applications using polyaniline (PANi) in tissue engineering are not as numerous as those with PPy. It was reported that the electrochemical degradation of PANi may occur at a potential greater than 0.6 V in an aqueous solvent and 0.7 V in organic solvents (Mazeikiene & Malinauskas, 2001). The conductivity of PT is too low (10^{-3}-10^{-2} S/cm) to enable the manufacture of a conductive composite with a low PT content (Guimard et al., 2007). By manipulating external electrical potential, the electrical property of the PPy devices can be modified through dopant in- and out-flux. Moreover, PPy is an excellent substrate for the attachment, proliferation, and differentiation of bovine bone marrow stromal cells (BMSC). With a PPy film as substrate and a working electrode, and Au and Ag wires as reference and counter electrodes respectively, a constant EF of 20 V/m ES for 1 hour induced a statistically significant increase in the osteogenic differentiation of BMSC on the thin PPy film substrates. Compared to a conductive indium tin oxide (ITO) glass control, the authors (Shastri et al., 1999) claimed that the increased osteogenic differentiation of BMSC on PPy was attributed to

the chemistry of the underlying PPy substrate rather than the electrochemical byproducts formed in solution by the ES. The conducting polymer films may be used to manipulate physiologically relevant ionic interactions with living cells. The chromic transition of conductive polymer caused by the reaction between immobilized ligands and biomolecules has been observed in real time (Englebienne, 1999).

However, the highly conjugative chain structures render genuine conducting polymers generally insoluble and non-fusible, signifying very poor processibility. Conductive polymer composites were thus blended with other processable polymers in order to address this processability issue (Meng et al., 2008). It takes as low as 5wt% of conductive polymer in the composite to reach a percolation threshold at which the conductive phase disperses in the non-conductive matrix and becomes continuous, resulting in a surface conductivity in the range of 0.3-5.3×10^3 Ω/square when different amount of heparin was used as dopants. Although the presence of heparin reduced the PPy conductivity, it promoted cell adhesion to PPy (Figure 2) and enhanced its electrical stability as well. When these conductive composites are used as scaffolding materials, the cells cultured on it can receive electrical signals directly from the substrate rather than from the culture medium.

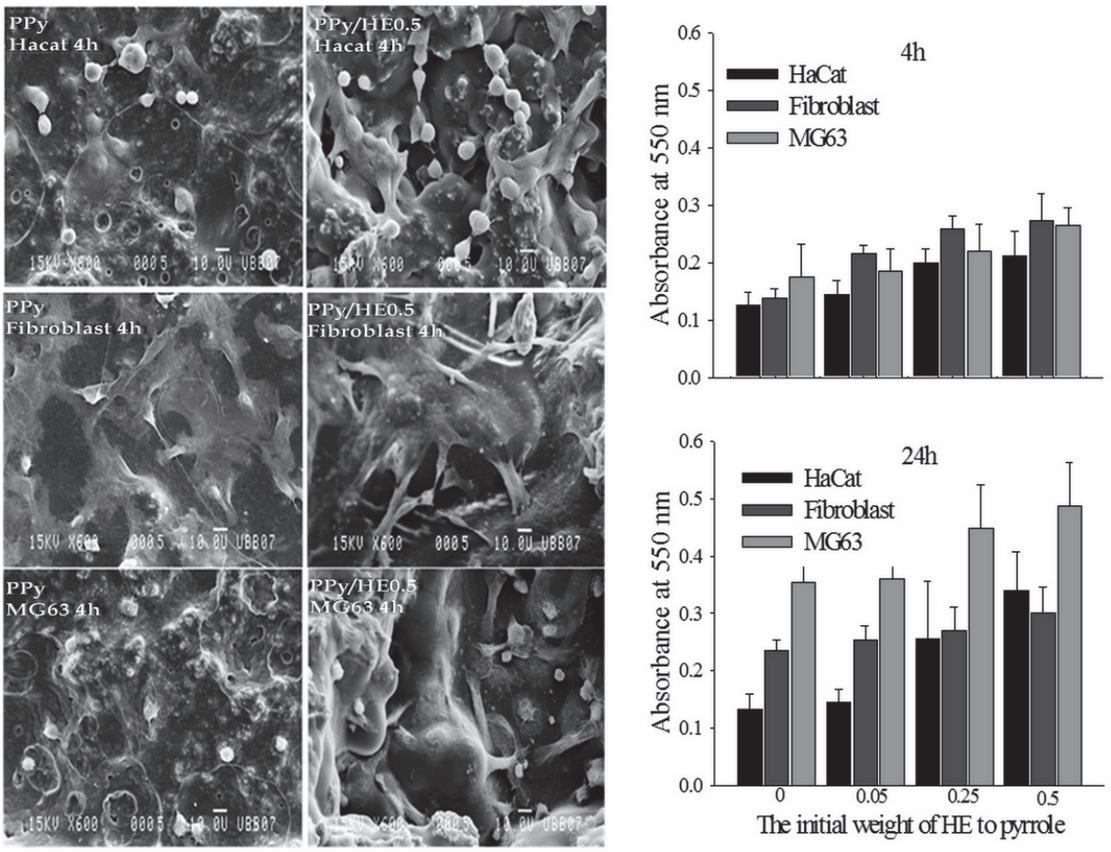


Fig. 2. The morphology of HaCat, fibroblasts and MG63 cells on PPy/PLLA membranes with or without heparin as dopant to PPy after 4h cell culture (left); and the cell viability after 4 and 24h culture on PPy/PLLA membranes with different amount of heparin in PPy (right).

One of the major issues in electrically stimulated cell cultures is to determine the optimal ES parameters including intensity, duration, frequency, and form. These parameters are likely cell type and indicator specific. For example, an effective ES "window" to osteoblasts-like cells Saos-2 was determined to be 6 h of ES at 200 mV/mm (manuscript to be published). When the potential gradient increased to as high as 300 and 400 mV/mm, Saos-2 cell proliferation was suppressed, regardless of the duration of the ES. On the other hand, a weak ES, such as 100 mV/mm, showed insignificant impact on cells. When an EF of 200mV/mm was applied across the surface of the PPy/PLLA membranes, a μA level current was generated. This model barely disturbs the culture medium by avoiding electrode reactions and ionic current, as shown in Figure 3. After an initial multiple ES (merely 3 times) to the osteoblast-like cells, Saos-2, followed by 4 additional weeks of culture, the deposited Ca and P ratio became 1:1.54, comparing the 1:1.46 for the control. The Ca/P ratio in polycrystalline HA varies between 1.5 and 1.71. Thus, the Ca/P ratio in the minerals of the ES group agrees well with the Ca/P ratio in the standard HA. WAXD, a gold-standard method used to identify the mineral phase, demonstrated that the crystalline structure of the mineral deposits on the conductive substrate was almost identical to that of the HA standard, meaning that ES indeed accelerated the formation of bone-like mineralization on the PLLA/PPy/HE substrate. Using these conductive composites, 2D or 3D conductive scaffolds of various shapes and sizes may thus be manufactured.

In summary, the invasive DC ES with an electrode implant provides intimate contact between electrode and cells/tissues, whereby the cells directly respond to the stimulation from the embedded electrodes. When ES signals in the range of 1.0-1.5 mV and 5-20 μA are applied, the electric field is highly localised and decays rapidly as a function of the distance from the electrode. Biochemical reactions, such as decreased local oxygen tension, hydroxyl radical production, corrosion, and electrolysis, occur close to the electrode, even in the presence of the salt bridge. These limitations become even more significant when a large area electrode is required.

3.3.2 Capacitively coupled electric field (CCEF)

As a noninvasive modality, the two capacitor plates of a CCEF are electrically insulated from the culture medium or tissue. No potential problems such as electrochemical reactions or electrode injuries are associated with the capacitors. When a voltage is applied across the two capacitors, the potential gradient between them generates an electric field. When an alternating potential is applied, the polarity of the plates continuously reverses, thereby generating an alternating EF between the plates. In vitro, CCEF has been shown to significantly enhance osteoblast proliferation, maturation, and ECM protein synthesis. For example, in 1984, Dannon et al. cultured rat embryo calvarial bone cells with medium containing radioactively labelled calcium and observed an increase in calcium uptake by counting the ^{45}Ca following stimulation with a CCEF of 12-54 V/cm and 3-100 Hz (Danon & Korenstein, 1984). In 1988, Brighton et al. cultured rat calvarial bone cells under a CCEF of 2.62 mV/cm for 2.5-30 min and found a 59% increase in cAMP (Brighton & McCluskey, 1988). Other experiments showed that CCEF signal transduction induced an increase in cytosolic Ca^{2+} via voltage-gated calcium channels, resulting in activated calmodulin, as well as an increase in TGF-β1 mRNA (Brighton et al., 2001; Zhuang et al., 1997). Lorich (Lorich et al., 1998) explored the mechanisms behind enhanced cell proliferation, showing that Ca^{2+} translocation through voltage-gated calcium channels (inhibited by verapamil) resulted in

elevated intracellular Ca^{2+} levels and concomitantly increased phospholipase A_2 (PLA2) activity (blocked by bromophenacyl bromide, BPB). The latter increase led to cyclooxygenase (COX)-dependent prostaglandin E2 (PGE2) synthesis (blocked by indomethacin), which in turn brought on an activation of calmodulin (blocked by N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride, W-7). Brighton's group reported that CCEF significantly upregulated BMP-2 mRNA expression, BMP-2 protein production, and alkaline phosphatase activity (Wang et al., 2006). In 2001, Wiesmann et al. reported that an electric field of 6-21kV/m with a saw-tooth shape signal, 63 ms width and 16 Hz frequency, affected the formation of newly formed mineral crystals of osteoblast-like cells in vitro (Wiesmann et al., 2001). A study on chondrocytes showed an upregulation of aggrecan and type II collagen mRNAs of bovine articular chondrocytes following a 30-min stimulation of 60 kHz sine wave signal with an output of 44.81 V (Wang et al., 2004).

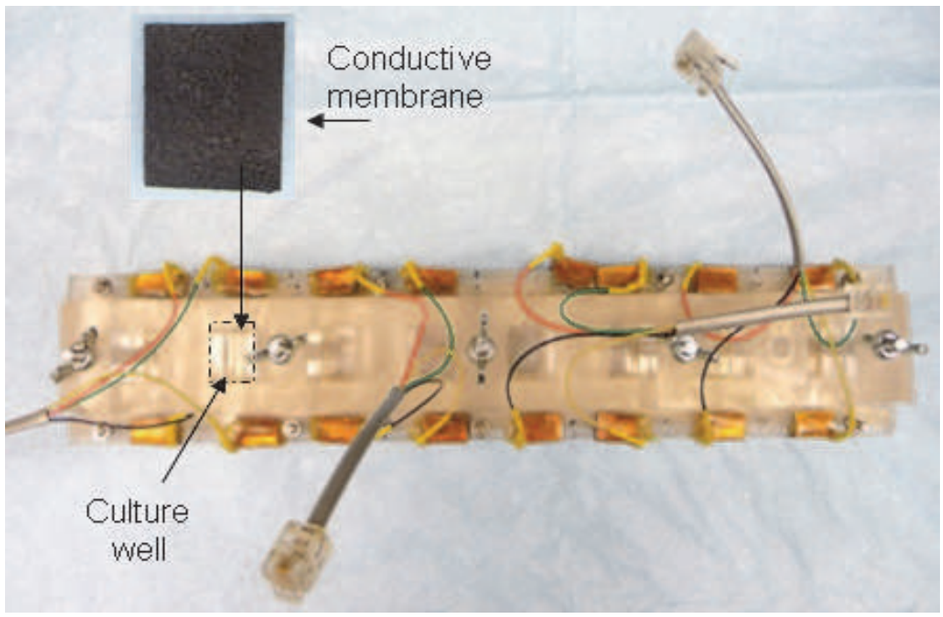


Fig. 3. Home-made multi-well electrical cell culture plate designed for ES

A major disadvantage of CCEF is the need for a high voltage power source (> 1000 V), as this EF energy decays quickly along the EF direction. Because the electrical resistance of an air gap and a cell culture Petri dish is much higher than that of the medium and the cell membrane, the external electric field produces a major voltage drop across the air gap and the culture dish so that only weak electric field acts on the cells. For example, peak-to-peak amplitude of 100 V at the capacitor only led to a 100 μV voltage drop across a cell monolayer. Brighton (Armstrong et al., 1988) calculated that for a peak-to-peak amplitude of 500-1000 V between the capacitors, and using relative dielectric constants of 1.0, 2.25, and 6.0 for air, plastic, and water, respectively, the electric field was only 1.5 to 3.0×10^{-2} V/cm (1.5-3.0 mV/mm), which is very weak compared to most values reported in the literature. In addition, in their device the distance between two opposite capacitor plates was only 1 cm. Another issue is that the EF generated in culture medium disturbs the homogeneity of the medium composition, e.g., the calcium migrated toward the anode under CCEF (Danon and Korenstein, 1984).

3.3.3 Electromagnetic field (EMF)

This technique is characterized by its low frequency electric and magnetic fields, and its noninvasive nature. The interaction of low frequency (< 1 MHz) and low magnetic field intensity (< 10 mT) electromagnetic field with human tissue has generated considerable interest. Based on calculations, an external 50-60 Hz EF in the order of kV/m induces an EF in situ in the order of mV/m or less. And, an external 50-60 Hz magnetic field of μT induces an EF in situ of a few tens of μV/m or less (Foster, 2003). At the cellular level, EMF reportedly promotes bone mineralization, osteoblast proliferation and differentiation, and cytokine gene expression and production. EMF was shown to stimulate osteoblast growth, cytokine release (prostaglandin E2 (PGE_2) and transforming growth factor β1 (TGFβ1)), as well as increase in ALP activity. The EMF signal can also enhance short-term NO production, with a concentration and time pattern consistent with chondrocyte proliferation. EMF-stimulated chondrocyte proliferation also involved calcium/calmodulin, nitric oxide synthase, nitric oxide, and cGMP (Fitzsimmons et al., 2008). In addition, Schnoke reported that EMF may be involved in bone anabolism, in part through the activation of proteins such as insulin receptor substrate-1 (IRS-1), S6 ribosomal subunit kinase, and endothelial nitric oxide synthase (eNOS) (Schnoke & Midura, 2007). Fitzsimmons reported a maximum potential gradient of 10^{-5} V/m capable of increasing the net calcium flux in human osteoblast-like cells (Fitzsimmons et al., 1994).

One major issue in EMF-related medical research is the contradictory results reported. For example, regarding gene expression, Berg reviewed that no less than 7 laboratories reported discrepancies with regard to *c-myc, c-fos* histone 2B, α-actin, and URA-3. This review focused on experiments using frequency < 100Hz, amplitude < 12mT and treatment time < 2h, and found the reproducibility of the results to be unsatisfactory (Berg, 1999). The parameters most often mentioned in EMF are frequency, amplitude, and treatment time. One would expect that changing one of these parameters would result in altered bone cell activity. However, Hannay et al. (Hannay et al., 2005) reported that the duration of EMF stimulation did not affect Saos-2 cells (rat osteoblast-like cell line) following four treatments at 15 Hz and < 1.3mT. In order to avoid such discrepancies, measurements using well-defined physical and physiological conditions and experimental parameters must be carefully performed. Another challenge to understand the effect of EMF is that it is difficult to link the biological effect of EMF exposure to the low power density of the EMF signal that is even lower than the power associated with the electromagnetic radiation of home electronics at radiofrequency, microwave, and visible light frequencies. It is also much lower than the power density of EMF used for other medical applications such as neurostimulation. In addition, the frequency-dependent Gauss rate of change is too weak to drive a useful current through the tissues of high impedance. It is unlikely that the mechanism of EMF therapy is solely the impact on transmembrane potentials, as the potentials generated by EMF are much lower than those of the cell membrane (Haddad et al., 2007b). Compared to CCEF, EMF also produces much lower EF strength and lower electric-to-magnetic field ratio.

4. ES, PPy-based scaffolds and other tissues

4.1 Nerves

Due to the intrinsic ability of neurons to transmit electrical signals within the nervous system, they are highly responsive to electrical stimuli. Neurons also grow well on

conductive polymers such as PPy. PPy-mediated ES and electrical recording are therefore unique approaches in nerve tissue engineering, including neural probes, nerve conduits, and scaffolds to support neurons for nerve regeneration. Under cell culture conditions, Schmidt et al. (Schmidt et al., 1997) reported a significant increase in neurite length of rat pheochromocytoma 12 (PC-12) cells cultured on PPy films following a steady potential of 100 mV stimulation for 2 h. The median neurite length for the electrically stimulated PC-12 cells was almost twice that recorded in the non-ES controls. ES has also dramatically enhanced the expression and secretion of the NGF and BDNF of Schwann cells compared to the control cells without ES.

To maintain an efficient level of electrical communication between nerve cells and an electronic element, such as neural probes, it is crucial that the PPy-based interface successfully connects to neurons. To take advantage of the anionic dopants in PPy, negatively charged glycosaminoglycans (GAGs) such as hyaluronic acid, heparin, and chitosan were incorporated into PPy as dopant ions. Because GAGs are involved in a number of complex cell signalling events, including migration, attachment, and neuronal sprouting (Serra Moreno et al., 2009), doping with GAGs is expected to increase the bioaffinity of the PPy-coated neural probes. To improve cell attachment and migration and to enhance neurite growth, synthetic peptides and silk-like polymers containing fibronectin fragments (SLPF), laminin fragment p31(CDPGYIGSR), p20(RNIAEIIKDI), and YIGSR sequences (DCDPGYIGSR) (Cui et al., 2003) were also successfully doped into PPy. Other methods include chemical modification such as immobilization of nerve growth factors (NGFs) or neurotrophin-3 (NT-3) (Richardson et al., 2007) on the surface of PPy. All of these strategies have reportedly increased affinity and therefore the communication efficiency between neural probes and neurons. Since substrate morphology regulates cellular behaviours, PPy has been manufactured into various structures to facilitate electrical communication between neurons and neural probes. For example, PPy nanotubes in a narrow range of conductivity was found to promote the outgrowth of neuritis, resulting in a decrease in the number of growth cones and an increase in cell body area (Zheng et al, 2003). The longitudinally implanted electrodes were shown to have stable stimulating and recording characteristics as well as good electrical response (Zheng et al, 2003). When cultured on aligned electrospun poly(lactic-co-glycolic acid) (PLGA) nanofibres, PC-12 stimulated by a 10 mV/cm potential gradient exhibited 40-50% longer neurites and 40-90% more neurite formation than those generated by the non-stimulated cells on the same scaffolds (Lee et al., 2009). Scaffolds to guide nerve regeneration are often tubular in shape. PPy tubes ranging from 25 μm to 1.6 mm of inner diameter as well as multichannel tubes were fabricated by electrodeposition. One- and 2-μm-wide PPy microchannels were fabricated using patterning technology (Gomez et al., 2007). Commercially available patterned junctions of conducting PPy have microelectrode arrays displaying typical dimensions of 200 μm between the electrodes, with each electrode measuring 30 μm in diameter (Simon & Carter, 2006). A low impedance electrode/tissue interface is important to signal transfer quality. Martin et al. (Abidian & Martin, 2008) fabricated nanostructure-conducting polymers that decreased the impedance of microelectrodes typically by two orders of magnitude and increased the charge transfer capacity by three orders of magnitude.

An important issue is mechanical property compliance, i.e., matching the elastic module or stiffness of the PPy implant with the surrounding tissues. In situ PPy polymerization in

biodegradable PLLA, hydrogel, or elastomeric silicone may reduce the mechanical mismatch between implant and tissue, resulting in enhanced biocompatibility and long-term performance.

4.2 Cardiac tissues

Coordinated excitation-contraction coupling activation of the ventricular myocardium from apex to base is an electrical stimulated chemical-mechanical behaviour. The ventricular myocardium has long been recognized as an anisotropic tissue with tensile mechanical properties strongly affected by cardiac muscle fibre orientation. Although micropatterning and microabrasion technologies may engineer precise cardiac patch anisotropy to match that of the surrounding host tissue, ES is also considered as an important stimulatory factor. ES to myocardium may progressively enhance the excitation-contraction coupling and improve cell function. For example, Radisic (Radisic et al., 2007) reported that ES to native heart tissue resulted in the progressive development of conductive and contractile characteristics of cardiac tissue, including cell alignment and coupling, which increased the amplitude of synchronous construct contractions and achieved a remarkable ultrastructural organization. At molecular level, ES raised the levels of all of the measured cardiac proteins and enhanced the expression of the corresponding genes. Radisic (Radisic et, 2007) also found that with increasing culture time, the ratio of mature and immature forms of myosin heavy chain (α-MHC and β-MHC, respectively) decreased in non-stimulated constructs and increased in stimulated ones. Moreover, the electrical stimulated cardiac cells showed modulated ion channel expression (Feld et al., 2002). Despite these and many other observations showing the beneficial effect of ES to myocardium and cardiac muscle cells, the application of conductive polymers in cardiac tissue engineering remains very limited. In 2007, Nishizawa et al. (Nishizawa et al., 2007) applied external ES to cultured cardiac myocytes using a microelectrode made of PPy-coated polyimide substrate. In their experiment, the myocytes were cultured on the PPy-coated anodic electrode, with the cathodic Pt immersed in the culture media over the cells. Through confocal fluorescence Ca^{2+} imaging, the authors observed that the cytosolic Ca^{2+} concentration was evoked by the electrical pulses delivered by the electrode (interval 1 s, duration 100 ms, and amplitude 10 mA) and that the cardiac myocytes were electrically conjugated through gap junctions. Using the same system, this group also obtained the threshold conditions from 0.5 to 10 mA using a different pulse duration for the excitation of the myocytes that showed a synchronized beating upon pulsation. The longer pulsed duration exerted an effective stimulation, regardless of current amplitude.

5. Future developments and challenges

Stem cells have been the focus of cell sourcing for a long time. Theoretically, embryo stem cells can differentiate into every cell lineage and eventually develop into every tissue and organ, including bone marrow, peripheral blood, umbilical cord, and adipose tissue. However, the use of human embryos for research purposes remains a highly sensitive ethical issue. On the other hand, adult stem cells are much easier to obtain from bone marrow or other tissues, such as adipose tissue and dental pulp. Studies have shown that adult stem cells have the potential to develop into various important cell phenotypes, including osteoblasts, under appropriate conditions such as growth factors. Cell

differentiation may also be promoted by EF. Indeed, considering the role of EF in embryo development, adult stem cells may represent important candidates to study the potential of ES to differentiate stem cells into osteoblasts and other phenotypes.

Because of its critical role in maintaining the long-term survival of engineered tissue, vascularisation is extremely important in tissue engineering. Therefore another cell lineage, namely, endothelial cells, should be co-cultured with osteoblasts to form viable bone. Different cells may react differently when subjected to the same ES in co-culture. Furthermore, because of the crosstalk between the two cell populations, the ES parameters for the co-cultured cells may differ from those acquired in single cell cultures and must therefore be carefully studied.

ES as a distinct feature may be integrated into the design of bioreactors. Currently available well-designed and versatile bioreactors already provide physical stimuli such as shear stress and mechanical force. Integrating ES into the design may either make the conditions in a bioreactor similar to those in vivo, in the case of embryo or stem cell development, or add another dimension to manipulate cellular behaviour to facilitate tissue regeneration.

While materials science has made significant strides in bone regeneration, the ideal substitute has yet to be developed and large bone defects continue to represent a major challenge for orthopaedic and reconstructive surgeons. PPy-based conductive composites can be made elastic, biodegradable, and electrically conductive. This unique feature thus makes them ideal candidates in the process of engineering bone tissue in bioreactors through both mechanical and electrical stimulations.

In an integral biosystem, how do cells differentiate in the precise location, at the right time, and into the devoted phenotype? Many questions remain unanswered. To large extent, the answers depend on the progress in developmental biology, from which cell genetics and the relationships between cells and their surrounding environment can be better understood. Such knowledge is vital to understand how to simulate the “natural environment” with the appropriate biological and physical cues. The fundamental challenge of the next generation of tissue engineering will be to fully elucidate the molecular energy absorption processes that may explain physical and biochemical changes in cells, and to provide cells with the right cues to predictably form functional tissues in various tissue development steps. ES continues to define its roles and should find its place in this increasingly exciting era.

The fabrication of PPy-based conductive scaffolds must take advantage of the most recent technologies and integrate biological components. These technologies include patterning, self-assembly, and 3D prototyping. Natural materials include collagen, coralline, aminoglucosan, chondroitin-sulfate, and fibrin. The ideal technology must be able to control the composition and distribution of these natural materials within various synthetic biodegradable polymers, such as polylactic acid (PLA), poly-4-hydroxybutgrate (P4HB), and copolymers of PGA and PLA (PGLA). The ideal porous structure of a biomaterial must enhance cell attachment, adhesion, growth, nutrition and metabolic exchange, and neovascularity development.

Because osteoprogenitor cell proliferation, differentiation, and matrix mineralization are heterogeneous, one important area of ES research is the optimization of parameters such as intensity, frequency, direction, and duration of the applied electric field in order to

accommodate the various bone formation steps. This work is expected to be significantly extensive yet directly relative to potential clinic applications.

6. Conclusions

Although ES has been attempted in clinic setting for a long time, its official authentication and comprehensive acceptation by physicians still need much support from further academic and clinic research. The mechanism of how the smallest organism unit, cell, responds to ES is still very much to be revealed. A physiological level of ES, in the order several tens to hundreds of mV and μA, is believed appropriate to activate voltage-sensitive proteins like transmembrane channels and transmembrane receptors, to modify gene expression, to affect cellular communication, as well as to affect the adhesion of ECM components. The most frequently reported parameters involved in ES experiments such as potential gradient and current density are probably oversimplified, ignoring frequency, wave form, duration and magnetic field. Various conducting materials like metals, CNTs, polymers have been used to prepare conductive scaffolds. From those conductive scaffolds, the adhered cells sense and respond to ES. Up to now, significant amount literatures have validated the role of ES in tissue regeneration, showing the high promise of using conductive materials and ES in tissue engineering, particular for the repair and regeneration of bone, nerve and cardiac tissues.

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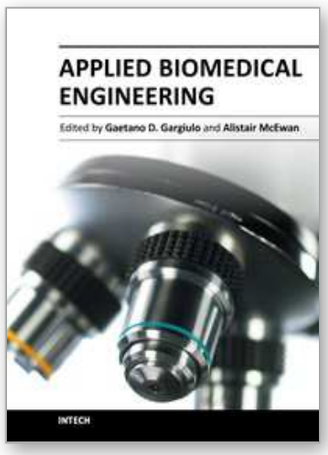
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