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Photosensitive materials and potential of photo current mediated tissue regeneration

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Abstract

Photocurrent therapy with participation of light and electrical stimulations could be an innovative and promising approach in regenerative medicine, especially for skin and nerve regeneration. Photocurrent is generated when light irradiates on a photosensitive device, and with more and more types of photosensitive materials being synthesized, photocurrent could be applied for enhanced regeneration of tissue. Photosensitive scaffolds such as composite poly (3-hexylthiophene)/polycaprolactone (P3HT/PCL) nanofibers are fabricated by electrospinning process in our lab for skin regeneration in presence of applied photocurrent. This review article discuss on the various *in vitro*, *in vivo* and clinical studies that utilized the principle of ‘electrotherapy’ and ‘phototherapy’ for regenerative medicine and evaluates the potential application of photocurrent in regenerative medicine. We conclude that photocurrent therapy will play an important role in regenerative medicine.

Key words: Electrotherapy; Phototherapy; Photosensitive; Photocurrent;

Regenerative medicine

1. Introduction

Regenerative medicine is a recently developed, multidisciplinary field involving the development of biological substitutes that can help restore, maintain or improve body functions [1, 2]. Regenerative medicine has the potential to solve the problem of shortage of donor organs, as well as solve the problem of organ transplantation rejection [3]. Cell-based transplantation, tissue engineering and gene therapy are important therapeutic

strategies for present and future regenerative medicine [4]. The essential tools for regenerative medicine are: cells and specially designed materials. A large variety of cell types have been used for regenerative medicine, including the adult cells, resident tissue specific stem cells, bone marrow stem cells and embryonic stem cells (ESC) [5]. Natural biomaterials such as collagen has been used in skin, bone, cartilage and heart regeneration [6]. While synthetic polymers like poly(lactic-co-glycolic acid), poly-L-lactide acid, and polycaprolactone (PCL) have been used in nerve, cardiac and blood vessel regeneration [7-16]. The ultimate aim of regenerative medicine is to provide safe and efficient therapies for a large number of clinical conditions [17].

Basically there are two phenomena of generating photocurrent; by light irradiation and electron-hole generation. The techniques relative with these two phenomena have easily been applied in regenerative medicine and are named as electrotherapy and phototherapy.

Electrotherapy has been used in tissue engineering for a long time. The main signals used as electrotherapy are DC electrical signals, pulsed cathodal electrical signal and electromagnetic fields. Bai et al.'s study indicates that a DC electrical signal could be a directional cue that plays a significant role in the spatial organization of vascular structure [18]. Feedar et al. used pulsed cathodal electrical stimulation(ES) in chronic dermal ulcer healing and their results showed that pulsed ES has a beneficial effect on healing chronic dermal ulcer [19]. Carl et al. used three types of electromagnetic fields (capacitive coupling, inductive coupling, or combined electromagnetic fields) to stimulate MC3T3-E1 bone cells. These electromagnetic fields produced a significant increase in DNA content compared to the controls without electromagnetic field

stimulation [28]. According to the study by Serena et al. [20], ES plays a role in cardiac differentiation of human ESC, through mechanisms associated with the intracellular generation of reactive oxygen species. On the other hand, long-term ES of osteoblasts appears to alter the pattern of gene expression resulting in enhanced extracellular matrix (ECM) synthesis which promotes bone tissue formation [21]. Phototherapy is the therapy in the presence of light irradiation. The types of light sources are usually low-level lasers, light-emitting diodes and natural light. The wavelength of light used in phototherapy is from 470 nm to 1200 nm. Clinical application of phototherapy or the therapeutic application of light has been increased in recent years. Infrared (700 nm-1200 nm) and near-infrared (600 - 700 nm) light delivered through lasers or light-emitting diodes have been reported to significantly accelerate the repair of chronic skin wounds [22-24]. In skin tissue engineering (TE), it has been proved that phototherapy can reduce inflammatory reactions; enhance cell proliferation; increase basic fibroblast growth factor generation and accelerate cutaneous wound healing [20, 35, 62]. Phototherapy can also promote axonal regeneration, functional recovery and enhance spinal cord repair [81-86].

With electrotherapy and phototherapy having positive effects on regenerative medicine, it is also thought that the photocurrent (the current generated under light stimulation) can be used as a promising method in regenerative medicine. In this review paper, the potential application of photocurrent in regenerative medicine is evaluated by *in vitro*, *in vivo* and clinical studies which are relative to electrotherapy and phototherapy. The probable mechanism of photocurrent therapy with respect to molecular biology reactions is also explained. Innovative thoughts towards new material developments in this field with possibility towards tissue regeneration are also discussed.

2. Molecular biological reactions related to photocurrent

Photocurrent is the current that flows through a photosensitive device, such as a photodiode, as a result of exposure to radiant power or via photoelectric effect. When visible light irradiates matter (metals and non-metallic solids, liquids or gases), electrons are emitted from the matter as a consequence of their absorption of energy from the visible light. Similar to photosynthesis which converts carbon dioxide into organic compounds, especially sugars, using the energy from sunlight. In the light reactions of photosynthesis, one molecule of the pigment chlorophyll absorbs one photon and loses one electron and in the phenomenon of photocurrent, when photons drop on the dye sensitive solar cell, the dye generates electrons. Both protons and electrons participate in photosynthesis and cause some molecular reactions in chloroplast. Hence it is also possible to induce some molecular biological reactions when photocurrent is applied in regenerative medicine. Light is an electromagnetic radiation which comprises of electric and magnetic field. When photocurrent is generated, electromagnetic field gets generated too. Electromagnetic field has been found to increase the cytosolic Ca^{2+} level and Carl et al. [25] used three types of electromagnetic fields (capacitive coupling, inductive coupling, or combined electromagnetic fields) to stimulate MC3T3-E1 bone cells. After three electromagnetic stimulations, there was an increase in cytosolic Ca^{2+} and an increase in activated cytoskeletal calmodulin compared to controls without electromagnetic stimulation. The mechanism of this effect is as follows: The initial event with capacitive coupling is Ca^{2+} ion translocation through cell-membrane voltage-gated calcium channels, whereas the initial event with inductive coupling and with combined electromagnetic fields is the release of Ca^{2+} from intracellular stores.

Transduction of a capacitively coupled electrical signal is by means of Ca^{2+} ion translocation through voltage-gated calcium channels (blocked by verapamil) leading to an increase in phospholipase A_2 (blocked by bromophenacyl bromide) and to an increase in cytosolic Ca^{2+} . The increase in phospholipase A_2 leads to an increase in prostaglandin E_2 synthesis (blocked by indomethacin), and the increase in cytosolic Ca^{2+} leads to an increase in activated (cytoskeletal) calmodulin (blocked by W-7). Activated calmodulin is known to promote nucleotide synthesis and cellular proliferation [26, 27], while prostaglandin E_2 acts as an autocrine and/or paracrine factor to stimulate bone cell proliferation and possibly to increase intracellular calcium [28].

Transient response of mitochondria to light stimulation has also been found in wound healing. The process of wound healing is believed to consist of three stages: the inflammatory phase, the proliferative phase, and the remodeling phase [29]. In the inflammatory phase, the red blood cells and macrophages migrate to the wound and activate fibroblasts. Furthermore the fibroblast increases in its proliferative phase and produces extracellular matrix and collagen in the remodeling phase. The mechanism of phototherapy has been studied based on these two stages. In the inflammatory phase, according to Albertini et al. [30], the low power laser irradiation possibly exerts anti-inflammatory effects by modulating the release of adrenal corticosteroid hormones. The reduction on the duration of inflammatory phase may result in a faster entry into the proliferative stage of healing, when granulation tissue is produced [24]. The mechanism by which low intensity lasers induce biomodulation of cell activity has been described by Karu [31]. Laser irradiation is postulated to intensify the formation of a transmembrane electromechanical proton gradient in mitochondria [32]. Friedmann et al. considered

photosensitization in which energy of the photoexcited porphyrin (referred to as porphyrin*) is transferred to molecules of oxygen in the ground state $O_2(^3\Sigma)$ (referred to as O_2), transforming them into singlet oxygen 1O_2 , ($^1\Delta_g$) (referred to as 1O_2) .

Replacing ground state O_2 by singlet oxygen 1O_2 a potent oxidizer [33] , stimulates the redox activity of the respiratory chain. In addition to the production of the long-lived 1O_2 molecules, laser light also activates the redox reactions of the respiratory chain by exciting the mitochondrial flavins and cytochromes. Thus, the efficiency of proton-motive force (pmf) is increased and more calcium is released into the cytoplasm from the mitochondria. At low laser doses, this additional calcium gets transported into the cytoplasm and triggers mitosis and enhances cell proliferations. In addition to the mitogenic action of calcium release into the cytoplasm by the pmf, a short-term rise in the intracellular pH by creation of the electromechanical proton gradient triggers mitogenic signals in the cells. Moreover, it is well known that pmf increase ATP production, which activates Na, K-ATPase, and other ion carriers. Thus, the intracellular K^+ level is increased and the Na^+ concentration and membrane potential are decreased And these factors also influence cell proliferation [32, 34] . In 1984, Passarella et al. [35] observed the promotion of adenosine triphosphate synthesis by He-Ne laser irradiation of mitochondria derived from rat hepatocytes. They concluded that the mitochondria had a point of action of light. In 1999, Karu [36] deduced that the mechanism of the transient response of mitochondria to light stimulation involved redox properties, activation of the

electron transfer system, and superoxide production. They speculated that the reaction site was cytochrome c oxidase (COX) because the reaction occurred at the near-ultraviolet to infrared region of the absorption spectrum. COX is a membrane protein complex present in the mitochondrial inner membrane and is involved in cellular respiration. Redox active iron and copper ions are present in the active center of the enzymes. The complexes of these metal ions are said to have an absorption range in the near-infrared radiation range [36]. In 2005, Riley et al. [37] used the COX inhibitors such as tetrodotoxin and potassium cyanide in their experiment and verified the effect of irradiation of near-infrared light using LED due to COX. However, COX is difficult to extract or crystallize and much of its reaction mechanism and inner structure have not yet been elucidated in detail [38].

3. Potential applications of photocurrent for regenerative medicine

Based on the molecular biological reactions caused by photons and electromagnetic field, photocurrent might play a significant role in regenerative medicine. In the late 1960s, Endre Mester began a series of experiments on the carcinogenic potential of lasers by using a low-powered ruby laser (694nm) on mice. The results showed that the laser did not cause cancer but improved hair growth. This was the first demonstration of “photo-biostimulation” with low-level laser therapy (LLLT) [39]. In the middle 1970s, Thomas Dougherty successfully treated skin cancer in patients and performed the first clinical studies in humans. In the same time, Greek and Roman physicians used electric fish (capable of generating 100-150 V) to relieve headache, arthritis, and hemorrhoids [40, 41]. Phototherapy has been increasingly used since 1990 [42] and is mainly employed to

treat patients with superficial non-melanoma skin cancer and dysplasia. And the Golden age of medical electricity arrived, by which time most American doctors possessed at least one electrotherapeutic machine [40, 41] . In the 1990s, the therapeutic of electricity in medicine and diagnostic is gained widespread application [43] . However, the two promising methods have not been used together for regenerative medicine till now, though phototherapy and ES have been used in regenerative medicine for a long time. In order to evaluate the potential application of photocurrent, the application of phototherapy and ES are discussed with further application of photocurrent in regenerative medicine.

3.1 Electrotherapy

3.1.1 Skin

The skin is the outer covering of the body. In human, it is the largest organ of the integumentary system, made up of multiple layers of ectodermal tissue, and it guards the underlying muscles, bones, ligaments and internal organs. As skin gets exposed to sunlight directly, it provides opportunities to use photocurrent in skin regeneration. The mechanism of photocurrent therapy is probably the combination of electrotherapy and phototherapy. ES has been widely used in a variety of populations for rehabilitation including those with disabilities and athletes as an adjunct to exercise for stimulation of paralyzed muscle [44] or non-paralyzed muscle [45]. ES is also used to increase the tissue blood flow [46], destroy bacteria [47, 48] and for wound healing [49-51] .Weak electrical currents applied to wounds have been reported to improve wound healing by as much as 20% per week [52]. Studies on induced wounds *in vivo* have reported that ES

can improve the survival of skin flaps [53] and significantly increase the rate of wound epithelialization [54] and contraction [55] and proliferation of fibroblasts [56]. Falanga et al. [57] have demonstrated that dermal fibroblasts in culture, stimulated with pulsed current at 100 pulses/sec and 100 V, increased the expression of receptors for TGF- β to six times greater than those of control fibroblasts. The natural ECM of skin is mainly composed of collagen and elastin. Collagen synthesis and growth factors play an important role in wound healing, and TGF- β has a fundamental role in collagen synthesis. Morris et al [58] developed the Ahn/Mustoe lapine wound model for systematic investigation of the effects of ES on ischemic wound therapy. In their study, transcutaneous blood gas levels, histology, total RNA content were measured and $\alpha 2$ (I) collagen (COL-I), type IV collagen (COL-IV), $\alpha 1$ (V) collagen (COL-V), and vascular endothelial growth factor (VEGF) expressions were measured. All markers for stimulated wounds showed increased activity relative to non-stimulated control wounds. Both COL-I and COL-V showed significantly increased activity between day 7 and day 14 for longer duration of electrical pulse treatment, potentially indicating a continued effect on matrix remodeling. Houghton et al. [59] investigated if electrical stimulation therapy (EST) administered as part of a community-based, interdisciplinary wound care program accelerates healing of pressure ulcers in people with spinal cord injury (SCI). They assigned adults (N=34; mean age $\pm$ SD, 51 $\pm$ 14y) with spinal cord injury and stage II to IV pressure ulcers to receive either standard wound care (SWC) or EST along with standard wound care (SWC) using a concealed, random process that involved opening an opaque envelope prepared by an independent person with random number generation. All subjects were evaluated on a monthly basis for at least 3 months. Wound healing was

measured by reduction in wound size and improvement in wound appearance at 3 months of treatment with EST+SWC or SWC. The percentage decrease in wound surface area (WSA) at the end of the intervention period was significantly greater in the EST+SWC group (mean $\pm$ SD, 70 $\pm$ 25%) compared to the SWC group (36 $\pm$ 61%; P=0.048). The proportion of stage III, IV, or X pressure ulcers improved by at least 50% WSA and was significantly greater in the EST+SWC group compared to the SWC group. These results demonstrate that EST can stimulate healing of pressure ulcers of human with SCI.

3.1.2 Nerve

Nerve provides a common pathway for the electrochemical nerve impulses that are transmitted along each of the axons. It conveys information in the form of electrochemical impulses (known as nerve impulses or action potentials) carried by the individual neurons that make up the nerve. The impulses travel from one neuron to another by crossing a synapse, the message is converted from electrical to chemical and then back to electrical. When the impulses travel along axons, external signals can affect the impulses. Both of electrotherapy and phototherapy have been applied in nerve tissue with positive results. ES is one potential intervention that is increasingly being studied for use in nerve injury settings. Many studies demonstrated that ES enhanced both neurite outgrowth *in vitro* [60-62] and nerve regeneration *in vivo* [63-65]. Based on the advantage of permitting external control over the level and duration of stimulation, electrically conducting polymers, such as oxidized polypyrrole and polyaniline, have been investigated for use in nerve tissue engineering. Schmidt et al. [60] demonstrated the application of ES to PC-12 cells (a neuron-like cell line) cultured on

oxidized polypyrrole, it significantly enhanced the neurite extension by 90% compared to cells grown on oxidized polypyrrole without ES. Ghasemi et al. [66] prepared PANI/PG conductive nanofibers by electrospinning polyaniline (PANI) with poly (ε-caprolactone)/gelatin (PG) solution for nerve tissue engineering. They found that electrical stimulation through conductive nanofibrous PANI/PG scaffolds showed enhanced cell proliferation and neurite outgrowth compared to the PANI/PG scaffolds that were not subjected to electrical stimulation. In Kotwal et al.'s study [67], the conductive polymer oxidized polypyrrole (PP) surfaces were adsorbed with fibronectin from purified fibronectin solutions or serum-containing solutions with or without ES, respectively. They found that ES of PP increased the adsorption of fibronectin from homogeneous fibronectin solutions, as well as from serum. Fibronectin is an ECM adhesive glycoprotein that is crucial for cell attachment to substrates. Hence, enhanced ECM protein adsorption with ES likely increased the neuronal attachment and neurite outgrowth. Huang et al. [68] studied the interaction between Schwann cells and a biodegradable conductive composite made of PPy (2.5%) and chitosan (97.5%), and further examined the possible regulatory effect of ES on Schwann cells through the conductive polymer. They demonstrated that ES enhanced proliferation of Schwann cell; increased nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) mRNA levels; increased beta-NGF and BDNF protein levels. *In vivo* study by Devyani et al. used rats to evaluate the effect of ES on facial nerve functional recovery from a crush injury [69] where nine rats underwent ES and eight were sham stimulated until complete facial nerve recovery. Their results have demonstrated that ES significantly reduced the recovery time for semi-eyeblink reflex, the earlier sign of facial nerve function. Tagami

et al. [70] investigated the effect of ES on axonal regeneration of retinal ganglion cells (RGCs) after optic nerve (ON) crush in adult rats. The mean number of regenerating axons significantly increased at 250 μm distal from the lesion and increased IGF-1 immunoreactivity was observed in retinas treated daily with transcorneal electrical stimulation.

3.1.3 Muscle and Bone

Electrotherapy has been applied in bone tissue engineering and muscle regeneration. ES has been employed to induce osteogenesis in clinic for a long time and a successful treatment of the tibia nonunion with direct current has already been documented [71]. Especially after the discovery of electromechanical properties of bone in the 1950s [72], the development of this treatment method was accelerated both in theoretical and experimental ways [73]. New bone formation was observed in animal models with the application of different amplitudes of electric currents [74, 75]. Carl et al. have reported that some electrical fields increased the (TGF)- β_1 mRNA in MC3T3-E1 bone cells. Moreover, this study demonstrated that ES delivered by capacitive coupling induced an increase in (TGF)- β_1 mRNA in osteoblastic cells by a mechanism involving the cytosolic Ca^{2+} /calmodulin pathway, which results in cell proliferation [25]. Itoh et al. [76] used a wrist-bridging external fixator coupled with alternating electric current stimulators for the treatment of comminuted intraarticular fractures of the distal radius. The results of their study suggested that alternating electric current stimulation increased the rate of callus maturation after fracture of the distal radius and reduced the required duration of external fixation. Park et al. [77] used an animal model to evaluate the effect of neuromuscular

electrical stimulation therapy on fracture healing and demonstrated the application of neuromuscular electrical stimulation therapy to the venous congested limb during the early fracture healing period with enhanced callus formation and mineralization. Phototherapy has also been used in bone tissue engineering. In Fujihara et al.'s study, rat calvaria osteoblast-like cells were previously treated with or without dexamethasone, respectively and then, they were irradiated with or without a GaAlAs diode laser (wavelength of 780 nm, 10 mW, 3 J/cm²), respectively. Results showed that phototherapy acted as a proliferative stimulus on osteoblast-like cells, even under the influence of dexamethasone [78]. Renno et al. investigated the effects of 670 nm, 780 nm, and 830 nm laser irradiation on cell proliferation of normal primary osteoblast (MC3T3) and malignant osteosarcoma (MG63) cell lines *in vitro*. They found that the 830 nm laser irradiation (at 10 J/cm²) promoted osteoblast proliferation significantly and did not promote osteosarcoma cell proliferation. However a recent study done by Renno et al. demonstrated that laser irradiation (830 nm) produced a 13% decrease in osteoblastic MC3T3 cell proliferation on glass-ceramic discs (mean $\pm$ SD = 0.192 $\pm$ 0.002) compared with control (non-irradiated) discs (mean $\pm$ SD = 0.22 $\pm$ 0.002). Although there have been some promising results of phototherapy in bone tissue engineering, the exact effect of phototherapy on bone is not clear yet.

Neuromuscular electrical stimulation devices have been successfully used in sports medicine and physical therapy to increase muscle strength, maintain muscle mass during prolonged periods of immobilization, and control edema after injury [79, 80]. Colson et al. have investigated the feasibility, safety, and effectiveness of neuromuscular electrical

stimulation (NMES) strength training in facioscapulohumeral muscular dystrophy (FSHD) patients [81]. Patients underwent five months of strength training with NMES bilaterally applied to the deltoideus, trapezius transversalis, vastus lateralis, and vastus medialis muscles for five 20-minute sessions per week. The results showed that most of the muscle functions (shoulder flexion and extension and knee extension) assessed by manual muscle testing were significantly increased. Maximal voluntary isometric contraction of shoulder flexion and abduction and 6-minute walking tests distance were also improved.

3.2 Phototherapy

3.2.1 Skin

Phototherapy has been widely used in regenerative medicine and Table 1 shows the summarized literature of different phototherapy systems applied in regenerative medicine. In skin regeneration, the main light sources of phototherapy are lasers and Light emitting diode (LED). Damante et al. [82] used continuous diode laser to irradiate human gingival fibroblasts twice (6 h interval) to study the relation of laser phototherapy on the release of growth factors by human gingival fibroblasts. They found that basic fibroblast growth factor (bFGF) was significantly greater (1.49-times) in groups treated with infra-red laser than groups without laser phototherapy, and bFGF accelerated granulation tissue formation and induced re-epithelialization [83, 84]. Medrado et al. [85] applied an AlGaInP diode laser (670 nm-9 mW) on 72 Wistar rats and cutaneous wounds were inflicted on the back of rats. The rats were divided into three groups. Rats in group 1 were irradiated at a fluence of 4 J/cm^2 with 31 seconds exposure time. Rats in group 2 were irradiated at 8 J/cm^2 with the same exposure time, while the third group consisted of

untreated control animals. The results showed that treated ulcers were smaller than untreated ulcers until the seventh day and were completely healed by fourteen days. The group that received 4 J/cm^2 laser treatments exhibited significantly more action/desmin-marked cells in correlation with its more marked vascular proliferation than the group treated at 8 J/cm^2 . Laser therapy reduced the inflammatory reaction, induced increased collagen deposition and a improved proliferation of myofibroblasts in experimental cutaneous wounds. Rezende et al. [24] used forty-eight male rats to investigate the effect of a single laser irradiation on the healing of full-thickness skin lesions in rats. An 830 nm near-infrared diode laser with an intensity of 53 mW/cm^2 was applied. They also found that low-intensity laser therapy may accelerate cutaneous wound healing in a rat model even if a single laser treatment is performed. Light emitting diode (LED) is widely used as light source of phototherapy because it has many advantages related to safety and superior portability, a phototherapy using LED has already shown some promising results [86-88]. Erdle et al. [89] evaluated the wound healing effect of 670 nm LED light on incisions and burn injuries in hairless mice and suggested that red light exposure may be helpful in postoperative wound repair. Their results show that 670 nm LED red light sources do accelerate healing in skin of hairless mice with incisions though not effective for burn injuries. Adamskaya et al. [90] recently found that blue light significantly influenced wound healing based on rat animal models. Rats were divided into three groups ($n = 6$) according to the therapy applied: Group 1 treated with blue LED (470nm), Group 2 treated with red LED (629nm), and Group 3 was not illuminated. Rates in group 1 and 2 were illuminated on five consecutive days for 10 min each. Keratin-10 mRNA level was elevated in both light treated group compared

to control. The size of excisions illuminated by blue light significantly decreased on day 7 post-operative, which correlated with enhanced epithelialisation. Treatment of the wounds with blue but not red light led to a significant decrease in the wound area. In order to further study the role of LED in phototherapy, Tada et al. used polarized light from light emitting diode (LED) to irradiate fibroblasts. They found that the right circularly polarized light and linearly polarized light promoted the process of wound healing by increasing the proliferation of fibroblasts, and the right circularly polarized light increased the expression of type 1 procollagen mRNA [38].

Many positive results have been achieved by electrotherapy and phototherapy in skin tissue engineering. Until now, the mechanism of these two therapies are related to the amount of photons participate in the molecular reaction in the cytoplasm and the electromagnetic field cause Ca^{2+} ion translocation. Both photons and electromagnetic field can be found during photocurrent phenomenon and hence photocurrent can probably be applied for skin regeneration.

3.2.2 Nerve

Similar to the results of ES on nerve tissue, phototherapy is also found to be profitable in the nerve regenerative medicine. The study of Byrnes et al. showed that 810 nm laser light applied transcutaneously penetrated to the depth of the spinal cord and promoted axonal regeneration and functional recovery in rat hemisection spinal cord injury model [91]. Rochkind et al. [92, 93] found that a composite implant followed by a 780 nm laser irradiation enhanced axonal sprouting and spinal cord repair in a completely transected spinal cord injury model. Xingjia et al. [94] used a laser diode with an output power of

150 mW and a wavelength of 810 nm for the treatment of spinal cord injury. After light treatment, the average length of axonal re-growth in the rats with the hemisection (6.89-0.96 mm) and contusion (7.04 - 0.76 mm) injuries was significantly longer than the comparable untreated control groups (3.66-0.26 mm, hemisection; 2.89 - 0.84 mm, contusion). Belchior et al. [95] used low-power laser (660 nm) to study its influence on functional and histomorphological recovery of the sciatic nerve in rats. They also found that the utilization of low-power laser (660 nm) showed positive results with regard to the functional recovery in sciatic nerve of rats following crushing lesion. In clinical study, Rochkind et al. [96] applied low-power laser irradiation (wavelength, 780 nm; power, 250 mW) for treatment of patients suffering from incomplete peripheral nerve and brachial plexus injuries for 6 months up to several years. For patients with long-term peripheral nerve injury, noninvasive 780 nm laser phototherapy was found to progressively improve nerve function, lead it to significant functional recovery. Other studied phototherapy of nerve tissue engineering also demonstrates promising results [97-99]. The process of peripheral nerve regeneration in rats was accelerated and reinforced by phototherapy. Based on the published results of using both electrotherapy and phototherapy in nerve tissue engineering, we can confirm that both therapies have shown positive results in nerve regeneration. Therefore photocurrent comprised of light irradiation and current generation can be applied for enhanced nerve regeneration.

3.2.3 Bone and Muscle

Recently, Hayworth et al. [100] used a sensitive method for enzyme histochemistry of cytochrome oxidase to examine the rat temporalis muscle 24 h after *in vivo* low-level

light therapy (LLLT). The findings suggested that LLLT might enhance the oxidative energy metabolic capacity of different types of muscle fibers, and that LLLT may be used to enhance the aerobic potential of skeletal muscle. Compared to skin and nerve regeneration, photocurrent is not applied much in bone and muscle regeneration. Phototherapy if applied, is hard for light to reach the area around bone fracture by penetrating soft tissue. In muscle regeneration, although light can penetrate skin and reach muscle defects, the intensity of light might be weak. The optimistic point of view could be to use photocurrent in this area by adding auxiliary facilities.

3.3 Photocurrent therapy

Photocurrent is the current that flows through a photosensitive device, such as a photodiode, after exposure to radiant power. When visible light irradiates matter (metals and non-metallic solids, liquids or gases), electrons are emitted from the matter as a consequence of their absorption of energy from the visible light. As electrotherapy and phototherapy have shown positive results in regenerative medicines, the combination of these two therapies could be more helpful in regenerative medicine. When light irradiate on photosensitive fibers, electrons are generated in the photosensitive polymer molecules and when the electrons flow in the same direction, an electrical current is formed. Meanwhile, an electromagnetic field gets created and when the cells in the electromagnetic field get stimulated, Ca^{2+} ion translocates through the cell membrane voltage-gated calcium channels. Ca^{2+} ion translocates through voltage-gated calcium channels leading to an increase in cytosolic Ca^{2+} . This increase in cytosolic Ca^{2+} leads to an increase in activated calmodulin. Activated calmodulin is known to promote nucleotide synthesis

and cellular proliferation. Light stimulation also has a similar effect on cell proliferation. Porphyrin will absorb the energy of photons upon light irradiation and becomes photoexcited porphyrin (referred to as porphyrin*). The photoexcited porphyrin can increase the proton-motive force (pmf) by transferring molecules of oxygen in the ground state $O_2(^3\Sigma)$ into singlet oxygen $^1O_2(^1\Delta_g)$ which is a potent oxidizer [33]. Thus more calcium is released into the cytoplasm from the mitochondria. Figure 1 shows the schematics illustration of photocurrent stimulation in regenerative medicine.

All these reactions in the cell will lead to an increase in cell proliferation and the healing time gets decreased greatly. Based on this theory, phototherapy is actually the combination of electrotherapy and phototherapy. This conclusion corresponds to the *in vitro* and *in vivo* studies which are mentioned in skin, nerve and bone regeneration.

4. Prospective photosensitive materials for regenerative medicine

Photosensitive materials have been widely applied in energy field, especially in solar energy developments. Different types of dye materials have been used in dye sensitized solar cells and most of them have shown positive results. Conducting polymers have also been used in dye sensitized solar cells, such as poly(p-phenylene vinylene), poly(3-hexylthiophene) and polypyrrole. However, the photosensitive materials are rarely applied in regenerative medicine. Now Dr. Seeram Ramakrishna group in NUS is currently focusing research on the application of P3HT for skin regeneration. Here we

discuss on the variety of photosensitive materials that are being studied in our lab for regenerative medicine, especially for skin tissue engineering.

4.1 Prospective applications of electrospun photosensitive materials in tissue engineering

4.1.1 Poly (3-hexylthiophene)

Poly(3-hexylthiophene) or P3HT, has been the focus of great interest in organic solar cells [101] because of its optical and conductive properties. It has been used as an electron-donating polymer in bulk heterojunction (BHJ) solar cells [102]. Poly(3-hexylthiophene) can be synthesized by the chemical polymerization of the 3-hexylthiophene monomer by using iron(III) chloride as a catalyst [103]. Poly(3-hexylthiophene) is known for its high mobility, good solubility and good film giving properties [104]. When light irradiates on P3HT, it will absorb the energy of photons with a certain wave length and if the energy of photons is greater than the electron binding energy of P3HT polymer, P3HT molecular will release electrons after absorbing the energy from the photons. This is the general mechanism of photosensitivity of P3HT.

P3HT is the most promising photosensitive materials for regenerative medicine and Dr. Seeram Ramakrishna group in NUS have found that human bone marrow-derived mesenchymal stem cells can attach on electrospun P3HT-PCL blend nanofiber. The biocompatibility of P3HT nanofibers is studied and Figure 2 shows the SEM pictures of cell morphology on P3HT nanofibers. After 7 days cell culture, the cells can attach on the

nanofiber. Based on the results of cell culturing study, we found that cells can attach on the nanofiber with P3HT molecule on its surface. Therefore P3HT is biocompatible and it can be applied in tissue engineering. Being biocompatible, together with its photosensitive properties, P3HT nanofibers could be a suitable substrate for cell attachment and improved tissue regeneration.

4.2 Other photosensitive materials

Other photosensitive polymers that have been used in organic solar cells are mainly ruthenium complex dyes such as N3, K19 and C104 dye and organic dyes such as chlorophyll, cyanine [105, 106] and merocyanine [107].. These materials have the ability to convert light energy into electrical energy and could possibly be applied in photocurrent therapy.

4.2.1 N3 [4,40-dicarboxylic acid-2,20-bipyridine) ruthenium(II)] or (N3dye)

N3 [4,40-dicarboxylic acid-2,20-bipyridine) ruthenium(II)] is the first high-performance polypyridyl ruthenium complex which was reported in 1993 by Nazzaruddin et al [108]. Later, the combination with guanidinium thiocyanate, an additive that increased the cell open circuit voltage, the performance of N3 has been improved [109] and hence N3 tops in its rank of level. Based on the high number of photon-electron conversion performance of N3 dye, it has a great scope in regenerative medicine.

4.2.2 Chlorophyll

Chlorophyll is a green pigment found in all plants and it absorbs light most strongly in the blue portion of the electromagnetic spectrum, followed by the red portion. Chlorophyll is the pigment responsible for light absorption in photosynthesis. It consists of porphyrin ring structures linked to a hydrocarbon tail [110]. Chlorophyll A has been extensively explored for photovoltaic applications, since the 1970s. Chlorophyll is a natural pigment and the toxicity of chlorophyll is much lower than synthetic dyes.

With the photosensitive ability and low toxicity, chlorophyll could be applied in regenerative medicine. Figure 3 shows the chemical structure of poly (3-hexylthiophene), N3 dye and chlorophyll.

5. Conclusion and Remarks

Photocurrent has been intensively studied in solar energy in recent years and currently silicon solar cells or dye sensitive solar cells can be used convert solar energy to electrical energy and other forms of energy. The phenomenon of photocurrent comprises of light illustration and electron generation. Photon and electromagnetic field have induced positive biology reactions by *in vitro* and *in vivo* studies. Both electrotherapy and phototherapy can increase Ca^{2+} concentration in the cell cytoplasm by a couple of molecular biological reactions. The increase of Ca^{2+} concentration leads to an increase in cell proliferations. Phototherapy and electrotherapy both have been used separately in regenerative medicine for a long time. In skin regeneration or wound healing, ES can improve the survival of skin flaps and significantly increase the rate of wound epithelialization, contraction and proliferation of skin fibroblasts. Phototherapy was explained to promote fibroblast proliferation, collagen synthesis, and growth factor

release. In nerve regeneration, ES was also found to enhance the proliferation of Schwann cell, increased production of nerve growth factor and regenerating axons; while phototherapy promoted axonal regeneration and functional recovery too. In bone and muscle regeneration, using ES and phototherapy showed favourable results, such as increase in cell proliferation of cells and promoted the secretion of proteins and cytokines. Relatively little is reported on “photocurrent therapy” but it can be referred as the combination of phototherapy and electrotherapy. In conclusion, photocurrent might play a significant role in regenerative medicine in the near future.

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Figure 1: Schematic illustration of photocurrent stimulation in regenerative medicine

Figure 2: SEM images of (A) electrospun P3HT-PCL nanofibers and (B) bone marrow-derived Mesenchymal Stem Cells on P3HT-PCL nanofibers

Figure 3: Chemical structure of (A) poly (3-hexylthiophene), (B) N3 dye, (C) Chlorophyll

Table 1: Phototherapy systems applied in the field of regenerative medicine

Light type	*Source (wavelength)	Energy	Result	Ref
Infrared	LLL (830 nm)	53 mW/cm ²	Low-intensity laser therapy accelerated cutaneous wound healing in rat model	[24]
Infrared	LLL (810 nm)	150 mW/cm ²	Average length of axonal re-growth in rats with hemisection (6.89-0.96 mm) and contusion (7.04-0.76 mm) injuries was longer than untreated controls	[94]
Infrared	LLL (780 nm)	250 mW/cm ²	Laser irradiation enhanced axonal sprouting and spinal cord repair	[92,93]
Infrared	LLL (780 nm)	250 mW/cm ²	Patients with long-term peripheral nerve injury, noninvasive 780-nm laser phototherapy improved nerve function	[96]
Red	LLL (670 nm)	4 J/cm ²	Laser treated rat ulcers were smaller than untreated ulcers	[82]
Red	LED (670 nm)	-	Red light exposure helpful in postoperative wound repair	[89]
Red	LLL (660 nm)	4 J/cm ²	Positive results on functional recovery in sciatic nerve of rats following crushing lesion	[95]
Red	LED (660 nm)	9 mW/cm ²	Light therapy enhanced the oxidative energy metabolic capacity of different types of muscle fibers	[100]
Blue	LED (470 nm)	-	Keratin-10 mRNA level elevated in light treated group compared to control group in rat model.	[90]

*LLL: Low Level Laser, LED: Light Emitting Diode