Biologicals xxx (2017) 1–9

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Electrical stimulation of somatic human stem cells mediated by composite containing conductive nanofibers for ligament regeneration

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ARTICLE INFO

Article history:
Received 14 January 2017
Received in revised form
27 January 2017
Accepted 29 January 2017
Available online xxx

Keywords:
Poly (3, 4-ethylene dioxythiophene)
Electrospinning
Conductive scaffold
Stem cells
Electrical stimulation
Bioreactor

ABSTRACT

One of the advances in the field of biomedical nanotechnology, is conductive nanofiber fabrication and the discovery of its applications. Biocompatible flexible nanofibers that have a good biocompatibility, mechanical properties and morphology. Poly (3, 4-ethylene dioxythiophene) (PEDOT) is a conductive polymer that has recently been used in medical applications. In this study, the electrospinning technique and vapor phase polymerization combination method with freeze drying was used to produce Silk fibroin/PEDOT/Chitosan nanocomposite scaffold. The aim of our study was to develop a ligament construct of PEDOT/Silk bilayer nanofibrous scaffold, to mimic the aligned collagen fiber bundles and Chitosan sponge coating was done on these fibrous scaffolds, to mimic the glycosaminoglycans of ECM sheath. The developed constructs were characterized. The unrestricted somatic human stem cells (USSC), were cultured on the scaffold. Then, the effect of applying DC electric pulses to cells cultured on polymer was assessed. Cellular function was actively exhibited in scaffold with electrical induction, as evident by the high expression of collagen I, collagen III, decorin, biglycan and aggrecan genes. Novel scaffold plus electrical stimulation shows facilitating cell seeding and promoting cell proliferation, differentiation. This composites can be used in this new field for stem cells differentiation to target tissues.

1. Introduction

Ligament tears happens for people of different ages and its complete healing is a very challenging problem due to its hypo cellularity that decelerate regeneration of tissue injury. Surgery is required to repair the injured tissue due to the low intrinsic healing capacity and the limited vascularization [1]. To overcome the surgery problems of ligament repairs, the field of tissue engineering has attempted to mimic the ligament structure and function by using engineered scaffolds that optimizes the response of cell–biomaterial and mimic the native environment. However, no FDA approved tissue engineered ligament replacements produced yet [2–4]. Electrospinning techniques and hydrogels are useful to engineer the structure of ligament [1,5,6]. Electrospinning method has the ability to produce nanofibers that may mimic the natural extracellular matrix of tissues, and thus can support cell adhesion, proliferation, and extracellular matrix production [7–10]. Electrospun fibers sheets are difficult to handle and use in clinical applications so architectural modifications are in attempt to improve their handle ability. For example, fabricating different fiber arrangements or use of composite structures. Besides synthetic and natural polymers are popular candidates for electrospun scaffolds production. Many of them have been used for regenerative medicine research. Among these materials, silk fibroin (SF) is a promising material. Many researches indicated that SF has considerable properties such as satisfactory strength, satisfactory biocompatibility, optimal oxygen and moisture permeability [9,11–13]. Chitosan is also a favorable polymer for tissue engineering via its nontoxic, nonallergenic, mucoadhesive, biocompatible,

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http://dx.doi.org/10.1016/j.biologicals.2017.01.007
1045-1056/

biodegradable property and also as it accelerates cell proliferation [6]. It is not water soluble but can swell that play a very important role in tissue engineering due to its soft nature that provides tissue like environment for cell growth and allow diffusion of nutrients [13,14].

In other point of view, the effect of electric fields on cells, have been studied for many years [15–19]. Today we know that electricity is effective on cell migration. It can also mediate growth cone steering, induce cellular alignment, influence anatomical development and can be used in wound healing and regenerative medicine [20–23]. How to apply electricity varies depending on the final use. Designing a bioreactor that can be placed inside incubators and could well impose electricity is one of the challenges facing the research in this field [2,21,24]. Hicks et al. have designed a bioreactor that was used to apply electric pulses to cells and its results on Rat cortical neuron cells was neurite extension increase and neurite orientation enhancement [21]. Knowing that the human body generates biological electric field, In 1983, the electric potential about 10–60 mV was measured in different parts of the body by Baker and in 1988, Ikai et al. indicated that electricity enhanced the repair process of the ligament by causing a change in the ratio of collagen types [19]. The electrical resistance of biological tissue depending on its type and structure. Factors such as tissue type, tissue density, cell membrane permeability and electrolyte content impact on the degree of this resistance. Studies suggested that the control of cellular behavior is feasible through a manipulation of cytoskeleton proteins using external physical stimuli such as electrical field [25]. Some studies have reported that ES can improve patient outcomes after the surgical repair of ACL injuries [16,19,26]. Some studies also have reported that ES can effect on stem cell behavior with the scaffold surface [10,11,13,15,22,27,28]. For example, Pierce et al. cultured ReN neural stem cells on the surface of PEDOT: PSS scaffold that was coated with laminin and check their results over a period of applying a DC electric pulses for 12 days. The results showed that the population of neurons obtained under electrical stimulus was higher than in its absence. Neurons obtained under electrical stimulus present higher elongations and longer neuritis [23]. Zhang et al. prepared an electrically conductive PPY/PCL scaffold. Human adipose-derived mesenchymal stem cells were cultured in the PPY/PCL scaffold and subjected to 200 μA of direct current for 4 h per day for 21 days, the amount of calcium deposited was 100% higher than that without ES. They showed that the conductive PPY/PCL scaffold with application of ES has good potential in bone defect therapy [28]. These researches created a hypothesis that use of conductive biopolymers with combination of electrical induction could be a new way to stimulate stem cells to differentiate.

In the other hand, use of conjugated polymers allows versatile interactions between cells and flexible processable materials, while providing a platform for electrical stimulation, which is particularly relevant when targeting differentiation of stem cells [10,11,13,27,29]. Conjugated polymers are considered from three points of view: First is a structural point of view due to the many ways of establishing an extended pi-conjugation. Second is a functional point of view due to their electronic and optical properties and third is a research point of view due to their potential of fostering cross-disciplinary research [19,27]. Jin et al. developed a novel facile method to fabricate PEDOT nanofiber mats by electrospinning combined with insitu interfacial polymerization. They cultured human cancer stem cells on the surface of the PEDOT and within three days, cellular morphology and proliferation was evaluated. The results showed that the level of PEDOT biocompatibility is similar to TCPS (Tissue Culture Plates) [22]. Esrafilzadeh et al. produced a coaxial fiber by wet spinning that the fiber core was PEDOT: PSS plus antibiotics and polypyrrole was as its shell. The effect of the electric force on drug release was studied. Results showed no cytotoxicity effects and drug release profile was acceptable [20].

In conclusion, the application of electrical current on cell behavior is evaluated in many researches with so many polymers and has shown its significant impact on the in vitro adhesion, differentiation, directional migration and division [17–20,25]. But research on conductive scaffolds applications in ligament regeneration and the differentiation behavior of cultured stem cells under the influence of electrical induction need more investigation and must be taken into consideration [2,15,17,24]. In addition, less attention has been paid to PEDOT as a conductive biopolymer in the field of tissue regeneration. In this study, a new design PEDOT/Silk double layer nanofibrous scaffold embedded in chitosan sponge was produced by electrospinning, vapor phase polymerization and freeze dry sublimation methods then the effect of DC electrical induction on stem cell culture and differentiation has been studied in vitro.

2. Materials & methods

2.1. Nanofiber fabrication, surface treatment and characterization

B. Mori silk cocoons were purchased from the Gilan Silk Production Factory, Iran. Na2CO3, CaCl2, ethanol, methanol, formic acid and acetic acid were purchased from Merck (Germany). Polyvinylpyrrolidone (PVP, 1300000 g/mol) as well as acetonitrile (99%, anhydrous), a solution of iron(III) **p**-toluenesulfonate (FeTos) 40 wt % in butanol and 3,4-ethylenedioxythiophene (EDOT) were purchased from Sigma-Aldrich (USA). Chitosan (molecular weight: 100–150 kDa, degree of deacetylation-85%) was purchased from Sigma-Aldrich (USA).

Silk fibroin (SF) nanofibrous scaffold fabricated according to our previous work [12,30]. The PVP powder was dissolved in butanol solution together with a small amount of pyridine (0.5 mol/mol FeTos) by magnetically stirring overnight in a closed vial at 50 °C. Small amounts of pyridine were added to the FeTos solution as a base inhibitor in order to hinder acidic side reactions during the subsequent polymerization. The polymer solution was then filled into a syringe. A stainless steel substrate covered with SF scaffold was used as a monolayer substrate in order to make a final double layered composite mat. The distance between the needle and the substrate was fixed at 15 cm and the voltage at 30 ± 1 kV. Relative humidity (RH) in the electrospinning chamber was set to 10 ± 2% in order to prevent the nanofibers to liquefy by humidity uptake. The temperature was 30 ± 2 °C. The electrospinning process was very stable and could typically run for hours. Vapor-Phase Polymerization was done after electrospinning, the nonwoven mats were immediately placed in a glass reactor under active vacuum for 15 min and then closed under passive vacuum for the desired polymerization time with the liquid EDOT at the bottom of the reactor. They were then soaked in methanol for 30 min and dried under vacuum at room temperature for 18 h [29,31]. Methanol soaking also reduce the water solubility of Silk fibroin layer of final scaffolds and increase their strength. Chitosan aqueous solution (2% wt.) was prepared in a very low acidic condition. Double layered SF/PEDOT scaffold was placed inside of the chitosan solution and freeze dried 48 h immediately to achieve a uniform bilayer mat embedded sponge. The final mats were then soaked in methanol for 60 min and dried under vacuum at room temperature for 24 h for chitosan crosslinking.

A low pressure-low temperature plasma generator (30 W), Diener Ltd. co (Nano, Germany) was used for surface treatment of the scaffold SF side to improve its hydrophilicity before embedding in chitosan gel. Applied radio frequency was 40 kHz. Samples were

Please cite this article in press as: Dodel M, et al., Electrical stimulation of somatic human stem cells mediated by composite containing conductive nanofibers for ligament regeneration, Biologicals (2017), http://dx.doi.org/10.1016/j.biologicals.2017.01.007