Biomimetic electroconductive scaffolds for muscle regenerative engineering

Xiaoyan Tang[1,2,3,4], Yusuf Khan[1,2,3,4,5], Cato T. Laurencin[1,2,3,4,5,6*]

[1]*Institute for Regenerative Engineering, Uconn Health, Farmington, CT 06030, USA*
[2]*Department of Orthopaedic Surgery, Uconn Health, Farmington, CT 06030, USA*
[3]*Raymond and Beverly Sackler Center for Biological, Physical and Engineering Sciences, Uconn Health, Farmington, CT 06030, USA*
[4]*Department of Material Science and Engineering, University of Connecticut, Storrs, CT 06269, USA*
[5]*Department of Biomedical Engineering, University of Connecticut, Storrs, CT 06269, USA*
[6]*Department of Chemical and Biomolecular Engineering, University of Connecticut, Storrs, CT 06269, USA*

[*]Corresponding author. Tel: +1 860 679 6544; Fax: +1 860 679 1255; E-mail: laurencin@uchc.edu

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Abstract

Conducting polymers are emerging as highly attractive materials since they can be used alone or in combination with other biomaterials to provide electrical stimulus for tissue regeneration. Here, we report the fabrication of a novel stimuli-responsive conducting polymer scaffold, which can be used to regulate muscle cell adhesion, proliferation and differentiation. Our goal in this study was to develop electroconductive nanofiber polymer scaffolds that can modulate the cellular physical microenvironment to increase electrical communication between cells and ultimately generate a more robust and functional construct for muscle regeneration. Matrices such as those designed here could have a significant impact in the clinical setting, where muscle atrophy and fatty infiltration prevent healing of common injuries such as rotator cuff tears. The bio-interface consists of a conducting polymer, poly (3,4ethylenedioxythiophene): poly (styrenesulfonate) (PEDOT: PSS), with a dopamine-polymerized biodegradable substrate made from poly (ε-carprolactone) (PCL) that is rationally assembled together based on the native structure of muscle fibers. XPS analysis confirmed that poly (dopamine) deposition on the PCL scaffolds was successful. The coating of PEDOT: PSS on the poly(dopamine) modified PCL scaffolds was stable as both representative peaks were shown. C2C12 cells, a myoblast cell line was cultured on conductive substrates with different concentrations. Biocompatibility and cellular proliferation of the conducting polymer scaffolds were assessed. It was found that conducting polymers scaffolds of all groups were biocompatible. PEDOT:PSS coating of a low and medium concentration(1% and 10%) showed stimulatory effect on C2C12 growth compared to the control groups. These results showed that the presence of PEDOT:PSS at optimum concentration might enhance C2C12 cell growth and proliferation. These conducting polymer scaffolds hold great promise as biomimetic platforms for skeletal muscle regeneration.

Keywords: Conducting polymers, muscle tissue regeneration, intrinsic conductivity, bioelectronics.

Introduction

Around 40% to 45% of the human body's mass consists of skeletal muscle. Remarkably, skeletal muscle has the ability to perform robust self-repair after an injury[**1, 2**]. However, when a large volume of muscle is lost as a result of trauma or due to chronic disease, the ability of skeletal muscle to regenerate is limited [**3**]. The current therapeutic approach for muscle volumetric loss is to transfer and engraft healthy, vascularized, and innervated autologous tissue. Yet, this approach does not lead to full recovery of the native muscle strength and functionality. In addition harvesting skeletal muscle may lead to donor site morbidity**[2**]. Here we propose to use regenerative engineering as an alternative approach for patients with large volume muscle loss to prevent scar tissue formation and restore muscle function in moderate injuries [**2**]. A more clinically viable scaffold would satisfy the following attributes: biocompatibility, mechanical stability, long term *in vivo* functionality such as electrical conductivity, complete cell residence throughout the three-dimensional structure, and cues to support and promote proliferation and differentiation of cells throughout the scaffold. Our goal in this study was to modulate the cellular physical microenvironments to allow the application of controlled electrical impulses via electroconductive nanofiber scaffolds to generate a more robust and functional muscle construct for muscle regeneration. The development of this system may have a significant clinical impact, where muscle atrophy and fatty infiltration prevent healing of rotator cuff injuries **[4**].

Regenerative engineering strategies for repairing skeletal muscle defects so far can be classified into three classes, which are *in vitro* tissue engineering, *in vivo* tissue engineering and in *situ* tissue engineering [**2**]. The *in vitro* tissue engineering method mainly involves initially culturing the biomaterial with cells, allowing it to develop into mature and functional engineered muscle constructs followed by transplantation into patients. Major issues related to this approach include how to achieve proper myofiber packing density and alignment, and sufficient vascularization to ensure cellular viability. More importantly, the regeneration of physiologically relevant contraction forces is critical [**5**].

The *in vivo* strategy, which requires transplanting cells alone or in combination with a biomaterial scaffold to the site of injury, overcomes the problem of over-manipulating the cells during *ex vivo* culture. Thus the cells would influence muscle regeneration either by integrating into the host tissue or by stimulating the body's own regenerative mechanisms to promote new tissue formation. This method allows tissues to retain their functional properties [**6**]. However, this approach may cause certain immune responses, leading to decreased viability of the transplanted cells. So far neither method has been able to consistently show success in various preclinical models, which means that local biological conditions at the defect site and the natural cascades of endogenous regeneration at play may need to be taken into account for regenerative engineering strategies **[7**]. Here, *in situ* tissue engineering comes as a relatively promising approach where biomaterials alone are designed as guides to enable endogenous regeneration of injured tissues. These biomaterials are designed to degrade at certain ratios, provide cells with physiologically relevant chemical, electrical or mechanical properties, as well as present surface cues in order to activate, recruit, and reorganize host cell populations [**2, 8**].

There are a number of biomaterials to regenerate muscle such as electrospun fibrous meshes, hydrogels, patterned scaffolds, etc [**2**]. Among them, electrospun scaffolds are intrinsically advantageous as artificial matrices for muscle regeneration[9].Aviss et al. showed that aligned electrospun PLGA nanofibers can stimulate myotube formation [**10**]. Choi *et al.* showed that unidirectional oriented electrospun PCL/collagen nanofiber significantly induced muscle cell alignment and myotube formation as compared to randomly oriented nanofibers on Human skeletal muscle cells (hSkMCs) [**11**]. However, because synthetic polymers lack biological recognition cues, surface modification of those electrospun fibers plays a central role in biomedical applications as the fiber surface provides a direct interface to adherent cell [**12**]. In our study, we used poly(dopamine) deposition on PCL scaffold to change surface hydrophobicity. Such chemistry was inspired by the composition of adhesive proteins in mussels. Lee's group was the first to report a simple and straightforward method to form multifunctional polymer coatings through simple dip-coating of objects in an aqueous solution of dopamine [**13**].

Besides the dopamine surface modified poly (ε-carprolactone) PCL, a conducting polymer (CP) was also incorporated in the process, which is poly (3,4-ethylenedioxythiophene)-polystyrene sulfonate (PEDOT: PSS). This is because in addition to topographical cues adherent cells can also sense mechanical and electrical cues [**2**]. Electrical signals can provide important physiological stimuli that control the adhesion and differentiation of certain cell types [**14**]. In recent decades, a number of conducting polymer such as polypyrrole (PPy)[15],polyaniline (PANi) [**16,17**], polythiophene, and their derivatives [**18–21**] have been widely used in various biomedical areas such as neural probes, neural prostheses, and controlled release applications [**22–24**]. For example, neurite outgrowth can be enhanced in neuron cells cultured on polypyrrole-coated electrospun PLGA nanofibers [**25**]. Studies with polyaniline have demonstrated that this material supports the adhesion, proliferation and differentiation of skeletal myoblasts [**14, 26**]. PEDOT: PSS has captured a considerable amount of attention owing to its good electrical, chemical and environmental stability as well as improved conductivity and thermal stability over conventional polypyrrole (PPy) [**27**]. For example, Abidian et al. reported application of PEDOT traces with hydrogel nerve conduits for axonal regeneration for the first time [**28**].

As an initial step towards regeneration of functional skeletal muscle, the present work investigated the effects of PEDOT: PSS coating on surface modified electrospun nanofibers on the biocompatibility and proliferation of skeletal myoblasts. It is hypothesized that conducting polymer scaffolds fabricated are biocompatible and may have a positive impact on cell attachment, proliferation and differentiation. Due to superior conductivity of PEDOT: PSS, we are trying to apply minimum but sufficient coating to effectively promote cell growth and differentiation. Using electrospinning techinique, we fabricated PCL scaffolds and used dopamine chemistry to modify the scaffold surface. We further compared and optimized coating conditions to generate scaffolds that were biocompatible and supported cell growth. These conducting polymer scaffolds hold great promise to treat muscle defect in the future.

Experimental

Materials/ chemicals details

The poly (ε-caprolactone) (PCL) was purchased from Sigma-Aldrich (average Mw 8000). Dopamine hydrochloride was purchased from Sigma-Aldrich. poly(3,4-ethylenedioxythiophene)-polystyrene sulfonate (PEDOT:PSS 1:2.5) was purchased from Sigma-Aldrich.

Material synthesis / reactions

Electrospinning

The substrate material was electrospun using optimized parameters of 15 % (w/v) PCL solution in ethanol and methylene chloride (15:85 ratio) with a 2.5 mL/min flow rate, and 1 kV/cm-1 potential at ambient temperature and humidity to obtain bead-free nanofibers.

Conductive scaffold fabrication

2mg/ml dopamine was applied to the surface for 24 hours at pH 8.5 to polymerize onto the surface of PCL fiber mats to form a polydopamine coating[2]. The modified DOPA-PCL surfaces were washed, dried, and subsequently coated with 1% PEDOT: PSS, 10% PEDOT: PSS, 33% PEDOT: PSS, and 100% PEDOT: PSS solution, where the 100% solution indicates a PEDOT: PSS 1.3wt% dispersion in H_2O and 33%, 10%, 1% are further diluted with PBS. The dried scaffolds were treated with mild acetic acid pH 2 to enhance its conductivity [**29**].

Fig. 1. Schematic illustration of the conducting polymer coated scaffolds [**30**].

Characterizations / device fabrications /response measurements

XPS analysis

After fabrication, the scaffolds were carefully washed with distilled water three times and dried under vacuum. Chemical composition of the dopamine polymerized PCL scaffolds and electroconductive scaffolds were then determined using X-ray photoelectron spectroscopy (XPS) (PHI 595 Multiprobe System, University of Connecticut, Storrs, CT, IMS).

Culture of murine skeletal muscle cells (C2C12) C2C12myoblasts

C2C12myoblasts (CRL-1772) (ATCC, Manassas, VA, USA) were used to study cell proliferation and differentiation on fibers. Cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin (p/s) under standard culture conditions (37 ^{O}C, 5% CO2). For cell culture, circular scaffold samples were punched out using 5" size puncher (scaffold diameter: 11.11mm in diameter) and sterilized with 70% ethanol and UV for half an hour. Cells were seeded onto the different groups at a density of 80 K per well and cultured under growth media for 3 days.

Live/dead cytotoxicity assay

Cytotoxicity of the 1% PEDOT: PSS, 10% PEDOT: PSS, 33% PEDOT: PSS, and 100% PEDOT:PSS as well as dopamine polymerized PCL and pure PCL scaffolds were determined with a live/dead cytotoxicity assay. CellTracker green and ethidium homodimer-1 (Molecular Probes) were added to one-day cultures of C2C12 cells on scaffolds to determine cell viability (green fluorescence) and cell death (red fluorescence) by using Zeiss Axio Scan.Z1 with chroma filters for each distinct fluorophore. The results represented the mean values of three individual samples for each type of scaffold.

Cell proliferation

To measure cell proliferation, 80,000 cells were seeded on each scaffold. After 3 days, the cell proliferation was determined quantitatively by utilizing Cell Titer-Blue cell viability assay (Promega). This assay is based on the ability of metabolically active cells to reduce of resazurin to purple resorufin. A standard curve of known cell concentrations was made.

Scaffolds seeded with cells were incubated with 300 µl reagent for 45 minutes at 37 °C. The optical density of the solution in the 96 well plates was measured at 530 nm and 590 nm using BioTek Synergy HTX Multi-Mode Reader.

Statistical analysis

Statistical analysis was carried out using one-way analysis of variance (one-way ANOVA). Different groups were compared using Turkey's pairwise comparison. The star (*) sign were used to indicated the specific group was statistically significant when competed with another test group.

Results and discussion

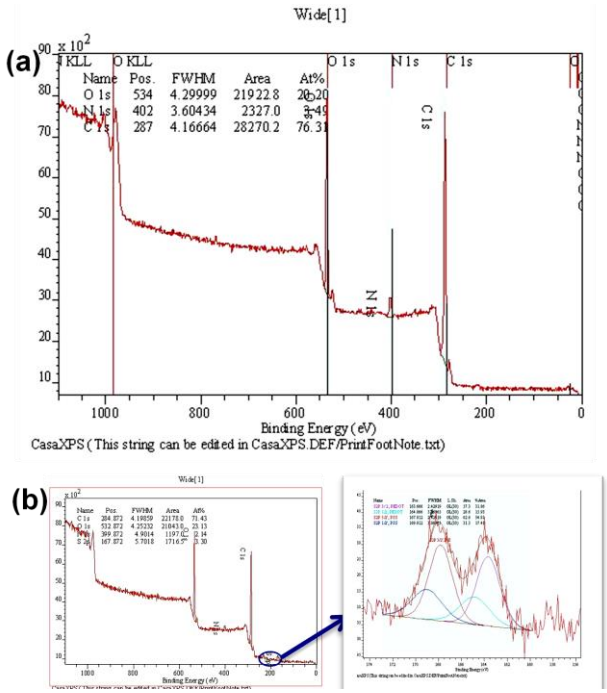

Fig. 2. XPS analysis on (a) Dopamine polymerized PCL scaffolds. (b) PEDOT:PSS coated dopamine polymerized PCL scaffolds.

Fig. 2(a), it shows that dopamine treatment was successful by the presence of nitrogen on the surface of the composite nanofibers as confirmed by XPS, where the N 1s peak was observed at 399.872 eV for the dopamine polymerized PCL scaffolds. According to the literature, there was no nitrogen peak being found in the PCL samples [**31, 32**]. (b) Both sulfer in PSS and sulfer in PEDOT were shown. The percentage are 52% against 48%, which indicated that acid treatment did take away excessive PSS [**29**], as the original PEDOT:PSS ratio was 1:2.5. XPS analysis confirmed that poly(dopamine) eposition on the PCL scaffolds was successful,which was

consistent with previous reported literature [**33**]. The additon of PEDOT:PSS was stable as both representative peaks were shown. Besides, acid treamtent took away excessive PSS as the final PEDOT:PSS ratio was almost 1:1 compared to intial 1:2.5. We have successfully coated PEDOT:PSS onto surface modified PCL scaffold. This scaffold fabrication method was straightforward yet effecitve. It provided an alternative approach as coducting polymers are conventionally electrochemically deposited on hard surfaces and the fabrication process invovled several steps **[34, 35**].

After seeding 80K C2C12 myoblasts on each treatment group of the nanofiber scaffolds, cell viability was qualitatively assessed using a Live/Dead Cell Viability assay kit. As shown in **(Fig. 3)**, between the six groups, after 1 day, the vast majority of the cells were viable, suggesting that the developed scaffolds were cytocompatible and can provide topographical cues to for cell growth.

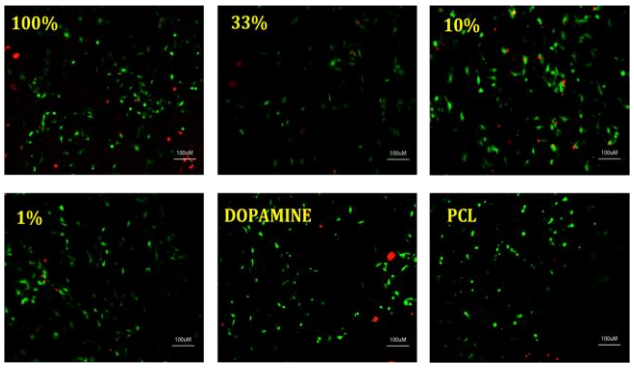

Fig. 3. Day 1 C2C12 cells live dead (100% indicates 100%PEDOT:PSS, 33% indicates 33% PEDOT:PSS etc).

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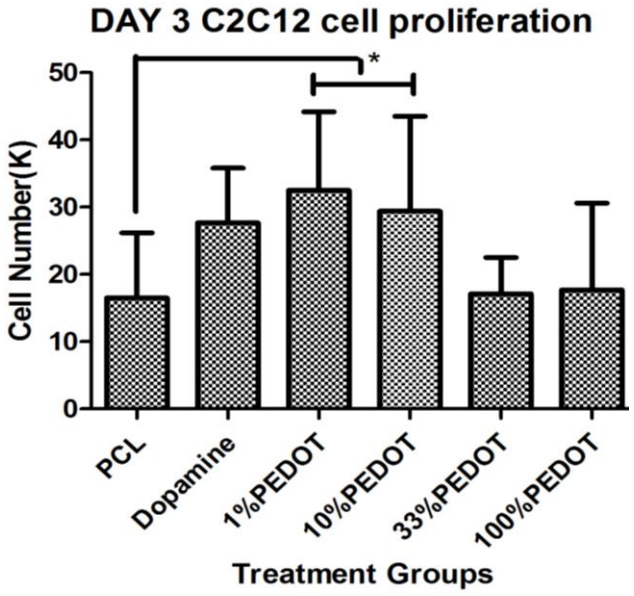

Fig. 4. C2C12 cells three day proliferation study.

80K C2C12 cells were seeded on scaffolds on each of the 6 test groups. From the proliferation study on day 3 on (**Fig. 4**), Statistically, PEDOT:PSS of high concentration groups (33% and 100%) showed similar effect on cell viability compared to the control group. Interestingly, PEDOT:PSS of a low and medium concentration(1% and 10%) showed stimulatory effect on C2C12 growth compared to the control groups as there is statistically significance between these groups. This results showed that the presence of PEDOT:PSS at optimun concentration may enhance C2C12 cell growth. It confirmed our hypothesis that conducting polymers may have a positive effect on cell growth, proliferation or even differentiation,which needs more investigation. This platform may further be optimized for tissue engineering or bioelectric applications when combined with small molecules, growth factors and along with electrical stimulation.

Conclusion

In this study, we have developed a novel method to incorporate the conducting polymer, poly (3,4ethylenedioxythiophene): poly (styrenesulfonate) (PEDOT: PSS), onto surface modified electrospun nanofiber matrices. The biological assessment of the C2C12 cells seeded on the scaffold was performed using live/dead assay and cell titer blue assay. It has been shown that the presence of PEDOT: PSS in the scaffolds enhanced the muscle cell viability, attachment and proliferation. Analyzing all the experimental data from different types of scaffolds, we concluded that the incorporation of conducting polymer might facilitate muscle cell growth.

From these studies we conclude that there is a combination of topographical and electrical guidance cues that have positive effects on C2C12 growth and differentiation, yet further investigation is needed.

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Author's contributions

Conceived the plan: XT, YK, CTL; Performed the expeirments: XT; Data analysis: XT; Wrote the paper: XT. Authors have no competing financial interests.

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