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Electroactive 3D Materials for Cardiac Tissue Engineering

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1. Abstract

By-pass surgery and heart transplantation are traditionally used to restore the heart's functionality after a myocardial infarction (MI or heart attack) that results in scar tissue formation and impaired cardiac function. However, both procedures are associated with serious post-surgical complications. Therefore, new strategies to help re-establish heart functionality are necessary.

Tissue engineering and stem cell therapy are the promising approaches that are being explored for the treatment of MI. The stem cell niche is extremely important for the proliferation and differentiation of stem cells and tissue regeneration. For the introduction of stem cells into the host tissue an artificial carrier such as a scaffold is preferred as direct injection of stem cells has resulted in fast stem cell death. Such scaffold will provide the proper microenvironment that can be altered electronically to provide temporal stimulation to the cells.

We have developed an electroactive polymer (EAP) scaffold for cardiac tissue engineering. The EAP scaffold mimics the extracellular matrix and provides a 3D microenvironment that can be easily tuned during fabrication, such as controllable fibre dimensions, alignment, and coating. In addition, the scaffold can provide electrical and electromechanical stimulation to the stem cells which are important external stimuli to stem cell differentiation. We tested the initial biocompatibility of these scaffolds using cardiac progenitor cells (CPCs), and continued onto more sensitive induced pluripotent stem cells (iPS). We present the fabrication and characterisation of these electroactive fibres as well as the response of increasingly sensitive cell types to the scaffolds.

2. Introduction

Myocardial infarction (MI), commonly referred to as a heart attack, is a leading cause of death worldwide with a high associated health care cost for survivors. After an MI the cardiac tissue damaged due to lack of oxygen causes a wound healing response that replaces the damaged cardiac tissue with non-contractile scar tissue. This results in reduced cardiac function, leading to reduced quality of life and further complications for the patient.

Cardiac stem cell therapy is an approach that aims to replace and regenerate new functional cardiac tissue, through the introduction of targeted stem cells that will differentiate into cardiomyocytes around the affected areas within the heart. Stem cell therapy has advantages over current therapies, such as by-pass graft surgery or complete organ transplantation, as it does not require donor organs or complicated open heart surgeries. However, current clinical trials of cardiac stem cell therapy have been unsuccessful; high stem cell mortality within the first few days after injection and low retention are major contributors to this lack of clinical efficacy.[1] The cardiac environment is also quite difficult for injected cells to survive due to immune responses, inadequate vascularization, fibrosis and inadequate access to nutrients.[2,3]

For these reasons, the direct injection of stem cells into cardiac tissue is not a beneficial approach; instead, delivering the stem cells on an implantable platform, or cardiac patch, would provide a more stable environment and allow the stem cells time to develop into effective tissue[4]. Grafting stem cells onto bio-engineered tissue scaffolds can address the majority of the issues that currently limit the efficacy of cardiac stem cell therapy. The use of electrospun fibres for the support of cardiac cells has an advantage due to similarity to extracellular matrix morphology, as well as the ability to tailor fibres in dimension, composition, and functionality. Different types of fibre materials have been used for stem cell graft materials, such as nano- and micro-sized fibres with different polymer compositions [5] and bio-functionalised fibres [6]. The 3-dimensional morphology provided by the fibres makes for a good basis for a cardiac patch.

The introduction of an electroactive material to the fibres provides another aspect to the influence and control of the stem cells on the fibres[7]. Electroactive polymers (EAP), such as polypyrrole (PPy), are conductive and when reduced/oxidized they will mechanically actuate [8]. This provides the possibility to stimulate stem cells both electrically and mechanically while growing on the cardiac patch. Electrical and mechanical stimuli have demonstrated in the past to

stimulate stem cells and to influence differentiation into cardiac type cells[9-11]. EAP coated fibres have been demonstrated to work as support for many types of cells, including neural, myogenic, and cardiac cells [12-15]. Following on from this foundation, we will produce EAP coated fibres using PPy to investigate the response from primary and stem cells. Our previous work has demonstrated the efficacy of using PPy materials prepared with dodecylbenzenesulfonate (DBS), as the polymer showed to be biocompatible with endothelial progenitor cells and cardiac progenitor cells (CPCs)[16]. CPCs are resident cardiac stem cells with the ability to generate cardiomyocytes, smooth muscle, and endothelial cells and have the potential to generate new functional cardiac tissue [17,18]. Hence, we begin this study observing PPy(DBS) coated fibre materials and the response from CPCs to observe how primary cells respond. We will then move onto iPS cells, which are generally more sensitive and difficult to culture successfully[19] but offer true pluripotency compared to the CPCs. Comparing the behavior of the two cell types will help elucidate the suitability of EAP coated fibres for cardiac tissue engineering.

3. Materials & methods

3.1. Scaffold fabrication

The electroactive scaffolds are prepared in a step-by-step process as shown in the scheme in Figure 1. 50:50 poly(lactic-co-glycolic acid) was prepared as a 17.5% wt/wt solution in chloroform. The PLGA solution was electrospun at a voltage of 20 kV with a flow rate of 0.5 mL/hour with a throw distance of 120 mm(Fig. 1A). The electrospun PLGA fibres were then collected and dried over night to evaporate any remaining solvent. The fibres were then coated with a solution of 5% wt/wt iron (III) chloride in methanol using a spincoater (WS-400B-6NPP/LITE, Laurell Tech. Corp., USA) with an initial step of 1000 RPM for 120 seconds, followed by 2500 RPM for 30 seconds (Fig. 1B). The FeCl₃ coated fibres were then dried over night to evaporate any remaining solvent. The fibres are then exposed to pyrrole (Py) vapour in a sealed vessel at 50°C for 60 seconds (Fig. 1C). An aqueous monomer solution of 0.1M Py and 0.1 M dodecylbenzenesulfonic acid (TCI) was prepared.

For electropolymerisation the aqueous pyrrole solutions were prepared with 0.1 M concentration of dopant (DBSA) and 0.1 M pyrrole. The VPP coated mesh was then placed into the aqueous pyrrole/dopant solution in a 3 point electrochemical cell (Fig. 1D) The counter electrode was a gold coated silicon wafer, and the reference a Ag/AgCl reference electrode. A constant potential of 0.67 V was applied to the electrochemical cell for 600 or 1800 sec. The ECP coated mesh was then lightly rinsed three times with DI water, dried gently with N₂ gas, and stored in a Petri dish. All chemicals are supplied from Sigma Aldrich unless indicated otherwise.

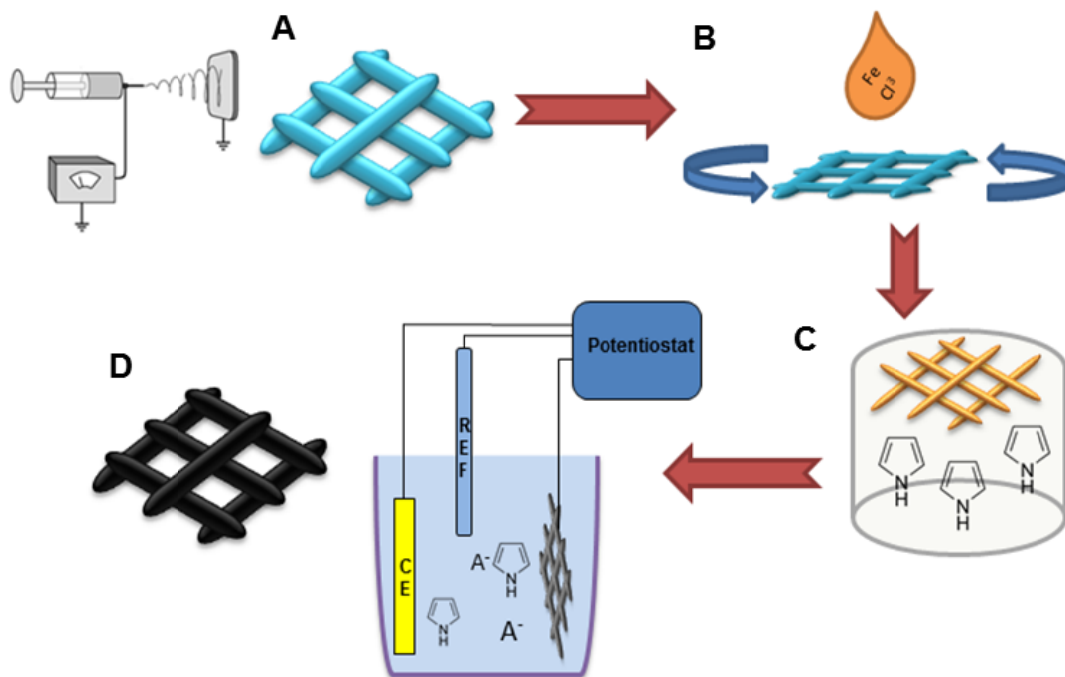


Figure 1: EAP fibre fabrication schematic; (A) electrospinning PLGA fibres, (B) spin-coating FeCl_3 onto PLGA fibres, (C) vapour phase coating PPy onto FeCl_3 coated fibres, and (D) electropolymerising PPy onto the VPP coated fibres where anion A^- is DBS.

3.2. Characterisation

3.2.1. SEM

The fibres were sputter coated with gold (30\AA) to improve conductivity for SEM. The fibres were examined in the LEO 1550 scanning electron microscope (Zeiss, Germany) with an electron beam energy of 5.02 kV. The iPS cells were prepared for SEM after being fixed to the fibre samples with formaldehyde. The fibre and iPS were then carefully rinsed with MilliQ water (18.1Ω), then dried via gradual ethanol dehydration and finally sputter coated with a gold layer (30\AA) for SEM imaging.

3.2.2. Electrochemistry

The input parameters were set at applied voltage range of -1V to 0.4V , 3 cycles, and scan rate 50mV/s . Before cyclic voltammetry (CV) VPP or ECP samples were soaked in the PBS solution for at least 30 minutes to allow the fibres become fully hydrated. The CVs were performed in 7.4 pH PBS (Sigma Aldrich tablet).

3.3. Cell culture

3.3.1. CPC

CPCs were isolated from the hearts of adult mice using a cardiac stem cells isolation kit (Millipore). The maintenance medium used was Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) (Sigma-Aldrich) supplemented with 10 % FCS, 1 % penicillin – streptomycin (Invitrogen), 1X Insulin-Transferrin-Selenium (ITS) (Invitrogen), 0.5 % DMSO (Sigma-Aldrich) and 20 ng/ml Epidermal Growth Factor (EGF) (Invitrogen).

All samples were firstly incubated overnight in 5 x concentrated penicillin-streptomycin solution followed by thorough washing with sterile PBS aqueous solution. The materials were then incubated for 24 h in sterile antibiotic-free medium to check the efficacy of the bacterial decontamination. If no microbial growth was observed, the samples were used for cell culture testing. All decontaminated samples were placed on the bottom of a 12-well cell culture plate and 1 ml of the cell maintenance medium was added. CPCs were collected by trypsinization and seeded at a density of 5×10^4 cells per well.

After 3 days of culture, once the control sample became confluent, a Live/Dead Assay (LIVE/DEAD® Viability/Cytotoxicity Kit (Life Technologies, cat. No. L-3224) was used to investigate cell adhesion, viability, and cell density and assessed with an inverted fluorescent microscope AXIO CAM ICm1 (Zeiss, Germany). Cell numbers were quantified using the cell count function in ImageJ (NIH).

3.3.2. IPS

Human induced pluripotent stem (iPS) cells were generated from primary human dermal fibroblasts by retroviral-mediated transduction of four transcription factors (Sox2, c-Myc Oct4 and Klf4) as described previously. IPS cells were maintained on mitomycin c treated mouse embryonic fibroblasts (MEFs) in serum-free Primate ES Cell Medium (ReproCELL) supplemented with 1 mM valproic acid (Sigma) and 10 μ M ROCK inhibitor (Y-27632, STEMCELL Technologies). For cardiac differentiation, colonies of IPS cells were detached from culture plates by incubating with 1 mg/ml collagenase/dispase (Roche) and transferred into ultralow attachment plates. Cells were then cultured in suspension in differentiation medium (80% DDM/F12, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 0.1 mM 2-mercaptoethanol, 50 U/ml penicillin and 50 mg/ml streptomycin, 20% fetal bovine serum) to initiate cardiac differentiation. After 4 days cells were plated onto the fibre samples (fixed to the bottom of the culture plate with a PDMS ring overlay) and cultured in differentiation medium (replaced every second day) for next 10 days.

4. Results and Discussion

The coating of the PLGA fibres through the dual-step coating process results in fibres with a consistent coating of PPy through the fibre mat sample. The initial VPP coating results in a smooth, continuous layer of PPy, and the secondary ECP coating displays the typical 'cauliflower' PPy morphology with every individual fibre coated (Fig. 2B,C). The coaxial nature of the fibres is clearly visible in Figure 2A and 1E, with a PPy thickness of approximately 200 nm.

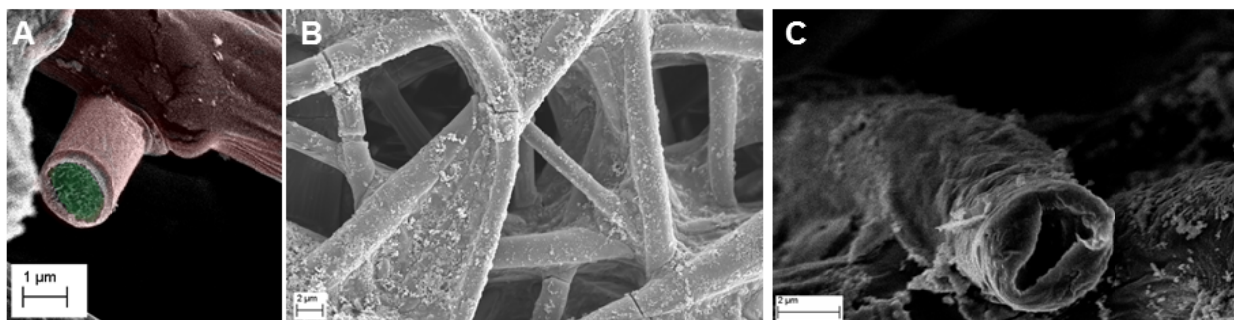


Figure 2: SEM pictures of PPy coated PLGA fibres. (A) Sheared coated fibre with (false colour coded) red indicating PPy coating and green the PLGA fibre core, (B) PPy/DBSA, and (C) Vapour phase coated sheath.

The fibre materials were characterized using cyclic voltammetry (CV) to observe their electroactivity (Fig. 3). PLGA is a non-conductive material, hence once the VPP is performed the material becomes electroactive (Fig. 3A). As expected, the conductivity and capacitance of the coated fibres increases from the VPP coating to the ECP coating; as more PPy is deposited the capacitance of the PPy layer increases (increasing peak height).

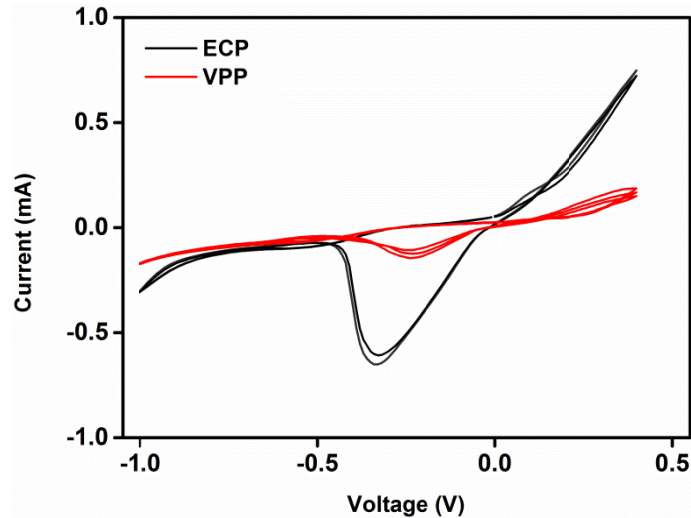


Figure 3: Representative CVs of a fibre sample with (A) VPP and (B) successive ECP PPy(DBS) coating.

Once the morphology and electroactivity was confirmed, the fibre materials were then tested for biocompatibility with CPC and subsequently iPS cells.

The CPCs were seeded onto plain uncoated PLGA fibres, VPP coated fibres, and ECP coated fibres at both 10 min and 30 min electropolymerisation time. The cell density of the CPCs was calculated and compared in Figure 4A.

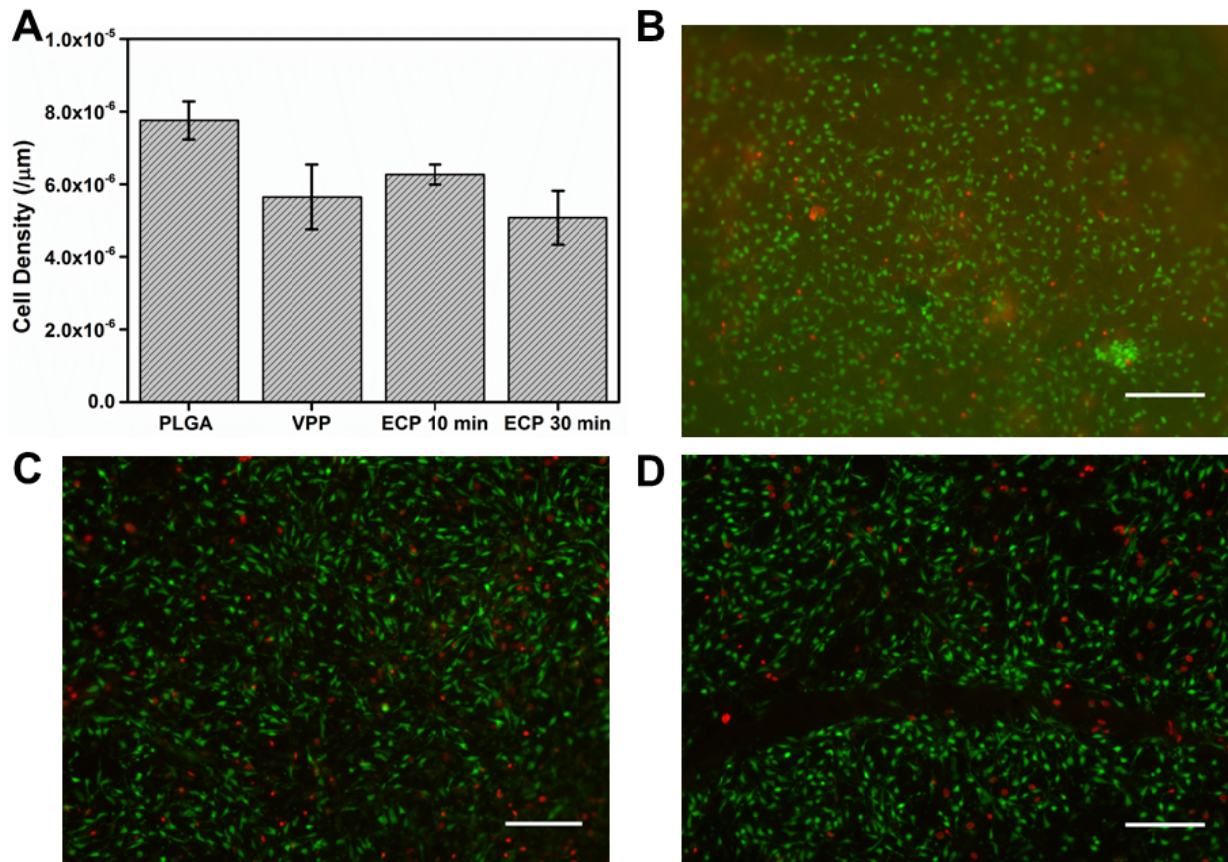


Figure 4: (A) Cell density of CPC on fibre materials, and live/dead CPC staining on (B) PLGA, (C) VPP, and (D) ECP 10 min fibre materials. Scale bars are 100 μm .

The uncoated PLGA fibres show the highest cell density ($7.8 \pm 0.5 \times 10^{-6} \mu\text{m}^{-1}$) and has a 94% live cell percentage, an expected result due to the biocompatible nature of PLGA and its many applications as a supportive biomaterial. The cell density for the PPy coated fibres shows that the 10 min ECP coated fibres have the highest cell density ($6.3 \pm 0.3 \times 10^{-6} \mu\text{m}^{-1}$) while the VPP and 30 min ECP fibres had similar cell densities ($5.7 \pm 0.9 \times 10^{-6} \mu\text{m}^{-1}$ and $5.1 \pm 0.7 \times 10^{-6} \mu\text{m}^{-1}$ respectively). However when taking into account the percentage of live cells calculated, the VPP fibres have a live cell percentage of 78% compared to the 10 and 30 min ECP fibres both with 86%. The FeCl_3 dopant in the VPP step is determined to be a detriment to the biocompatibility of the material, and once this VPP layer is coated with a more biocompatible layer of PPy(DBS) the cell viability improves. Cell density values may also be impacted by the 3D morphology of the fibre materials; some cells may not be clearly visible if they are not present on the surface of the material and hence reduce the overall cell count.

The fibre materials were then tested with iPS, and stained for Live/Dead to observe their morphology and viability on the fibres.

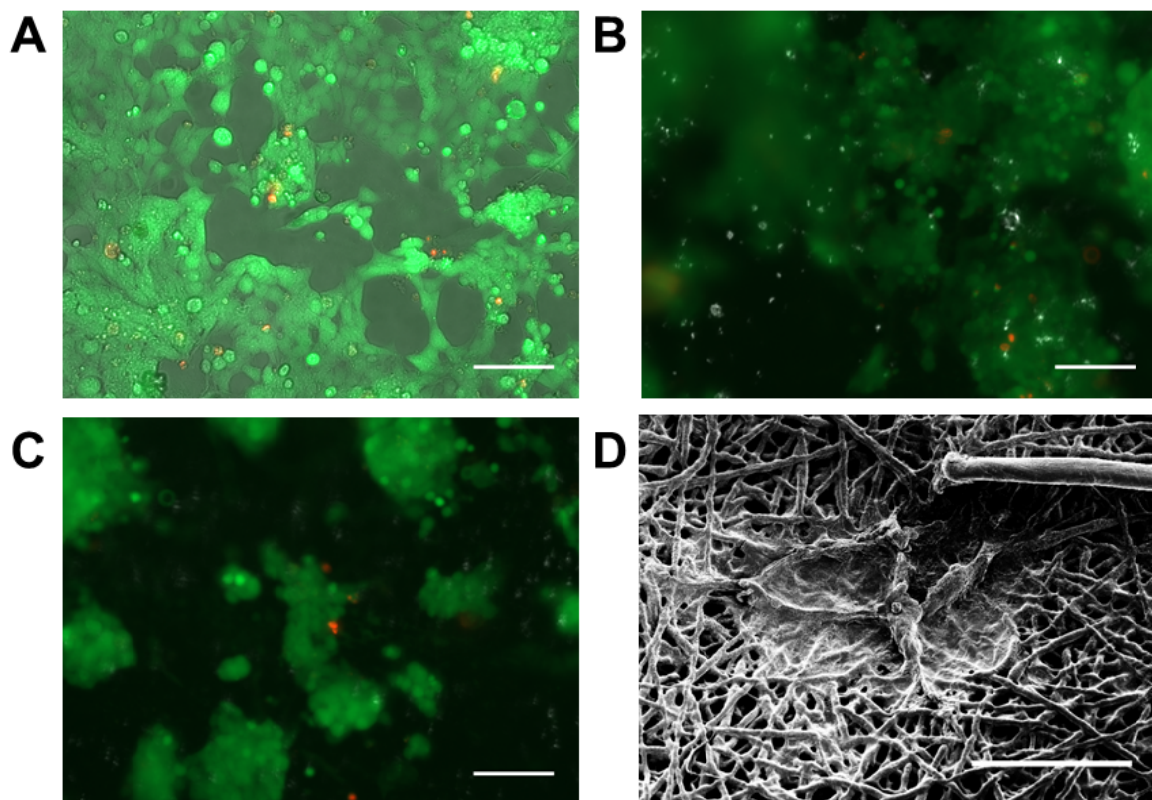


Figure 5: Live/dead staining of iPS on (A) cell culture dish, (B) ECP 10 min, (C) ECP 30 min, and (D) SEM micrograph of iPS on 30 min ECP fibres. Scale bars are 100 μm .

Due to the 3D nature of the fibre materials and the ‘clumping’ of iPS cells when seeded, imaging discrete cells for a detailed analysis was not possible in this instance. Visually comparing the iPS morphology on the 2D cell culture dish (Fig. 4A) with iPS on the scaffolds does provide a strong indication that the iPS are viable on the PPy coated fibre materials. The live/dead staining of the iPS show that the cells are spreading and growing on the fibres (Fig. 4B and C), and SEM imaging of fixed cells (Fig.4D) shows the iPS spreading along and around the individual fibres within the material.

5. Conclusion

This study demonstrates a new approach to creating new functional fibre materials, specifically fibres coated with an electroactive material. These EAP fibres are designed for use in new cardiac tissue engineering research, to provide the possibility of electrical and mechanical stimulation alongside the 3-dimensional morphological advantage of the fibres. The presence of the EAP coating on the fibres does result in slightly lower CPC cell density than plain PLGA fibres, but overall the viability is good with a high live cell percentage and density. The iPS cells display the ability to grow and spread on the fibres after 10 days in culture without severe apoptosis, indicating that they are also compatible with the EAP fibres. This study leads the way to introducing external stimulus via the electroactive coating in the future, to provide further control and direction over stem cell fate for cardiac tissue regeneration.

6. References

- [1] Terrovitis, J. V., Smith, R. R. & Marbán, E., "Assessment and optimization of cell engraftment after transplantation into the heart," *Circulation research* 106 (3), 479-494 (2010).
- [2] de Muinck, E. D., Thompson, C. & Simons, M., "Progress and prospects: cell based regenerative therapy for cardiovascular disease," *Gene Ther* 13 (8), 659-671 (2006).
- [3] Mummery, C. L., Davis, R. P. & Krieger, J. E., "Challenges in using stem cells for cardiac repair," *Science translational medicine* 2 (27), 27ps17-27ps17 (2010).
- [4] Leor, J., Aboulafia-Etzion, S., Dar, A., Shapiro, L., Barbash, I. M., Battler, A., Granot, Y. & Cohen, S., "Bioengineered Cardiac Grafts: A New Approach to Repair the Infarcted Myocardium?," *Circulation* 102 (suppl 3), Iii-56-Iii-61 (2000).
- [5] Zong, X., Bien, H., Chung, C.-Y., Yin, L., Fang, D., Hsiao, B. S., Chu, B. & Entcheva, E., "Electrospun fine-textured scaffolds for heart tissue constructs," *Biomaterials* 26 (26), 5330-5338 (2005).
- [6] Yu, J., Lee, A. R., Lin, W. H., Lin, C. W., Wu, Y. K. & Tsai, W. B., "Electrospun PLGA fibers incorporated with functionalized biomolecules for cardiac tissue engineering," *Tissue engineering. Part A* (2014).
- [7] Bendrea, A.-D., Cianga, L. & Cianga, I., "Review paper: Progress in the Field of Conducting Polymers for Tissue Engineering Applications," *J. Biomater. Appl.* 26 (1), 3-84 (2011).
- [8] Gandhi, M. R., Murray, P., Spinks, G. M. & Wallace, G. G., "Mechanism of electromechanical actuation in polypyrrole," *Synth. Met.* 73, 247-256 (1995).
- [9] Rowlands, A. S. & Cooper-White, J. J., "Directing phenotype of vascular smooth muscle cells using electrically stimulated conducting polymer," *Biomaterials* 29 (34), 4510-4520 (2008).
- [10] Park, J. S., Chu, J. S., Cheng, C., Chen, F., Chen, D. & Li, S., "Differential effects of equiaxial and uniaxial strain on mesenchymal stem cells," *Biotechnol. Bioeng.* 88 (3), 359-368 (2004).
- [11] Kurpinski, K., Park, J., Thakar, R. G. & Li, S., "Regulation of vascular smooth muscle cells and mesenchymal stem cells by mechanical strain," *Mol. Cell. Biomech.* 3 (1), 21 (2006).
- [12] Breukers, R. D., Gilmore, K. J., Kita, M., Wagner, K. K., Higgins, M. J., Moulton, S. E., Clark, G. M., Officer, D. L., Kapsa, R. M. I. & Wallace, G. G., "Creating conductive structures for cell growth: Growth and alignment of myogenic cell types on polythiophenes," *J. Biomed. Mater. Res., Part A* 95A (1), 256-268 (2010).
- [13] Lee, J. Y., Bashur, C. A., Goldstein, A. S. & Schmidt, C. E., "Polypyrrole-coated electrospun PLGA nanofibers for neural tissue applications," *Biomaterials* 30 (26), 4325-4335 (2009).
- [14] Bolin, M., Svennersten, K., Wang, X., Chronakis, I. S., Richter-Dahlfors, A. & Berggren, M., "Nano-fiber scaffold electrodes based on PEDOT for cell stimulation," *Sensors and actuators B* 142, 451-456 (2009).
- [15] Borriello, A., Guarino, V., Schiavo, L., Alvarez-Perez, M. & Ambrosio, L., "Optimizing PANi doped electroactive substrates as patches for the regeneration of cardiac muscle," *J. Mater. Sci.: Mater. Med.* 22 (4), 1053-1062 (2011).
- [16] Gelmi, A., Ljunggren, M. K., Rafat, M. & Jager, E. W. H., "Influence of conductive polymer doping on the viability of cardiac progenitor cells," *J. Mater. Chem. B* 2 (24), 3860-3867 (2014).
- [17] Karam, J.-P., Muscari, C. & Montero-Menei, C. N., "Combining adult stem cells and polymeric devices for tissue engineering in infarcted myocardium," *Biomaterials* 33 (23), 5683-5695 (2012).
- [18] Beltrami, A. P., Barlucchi, L., Torella, D., Baker, M., Limana, F., Chimenti, S., Kasahara, H., Rota, M., Musso, E., Urbanek, K., Leri, A., Kajstura, J., Nadal-Ginard, B. & Anversa, P., "Adult cardiac stem cells are multipotent and support myocardial regeneration," *Cell* 114 (6), 763-776 (2003).
- [19] Yoshida, Y. & Yamanaka, S., "Recent Stem Cell Advances: Induced Pluripotent Stem Cells for Disease Modeling and Stem Cell-Based Regeneration," *Circulation* 122 (1), 80-87 (2010).