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Optimizing PANi doped electroactive substrates as patches for the regeneration of cardiac muscle

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Abstract In scaffold aided regeneration of muscular tissue, composite materials are currently utilized as a temporary substrate to stimulate tissue formation by controlled electrochemical signals as well as continuous mechanical stimulation until the regeneration processes are completed. Among them, composites from the blending of conductive (CPs) and biocompatible polymers are powerfully emerging as a successful strategy for the regeneration of myocardium due to their unique conductive and biological recognition properties able to assure a more efficient electroactive stimulation of cells. Here, different composite substrates made of synthesized polyaniline (sPANi) and polycaprolactone (PCL) were investigated as platforms for cardiac tissue regeneration. Preliminary, a comparative analysis of substrates conductivity performed on casted films endowed with synthesized polyaniline (sPANi) short fibres or blended with emeraldine base polyaniline (EB-PANi) allows to study the attitude of charge transport, depending on the conducting filler amount, shape and spatial distribution. In particular, conducibility tests indicated that sPANi short fibres provide a more efficient transfer of electric signal due to the spatial organization of electroactive needle-like phases up to form a percolative network. On the basis of this characterization, sPANi/PCL electrospun membranes have been also optimized to mimic either the morphological and functional features of the cardiac muscle ECM. The presence of sPANi does not relevantly affect the fibre architecture as confirmed by SEM/image analysis investigation which shows a broader distribution of fibres with only a slight reduction of the average fibre diameter from 7.1 to 6.4 μm. Meanwhile, biological assays—evaluation of cell survival rate by MTT assay and immunostaining of sarcomeric α-actinin of cardiomyocites-like cells—clearly indicate that conductive signals offered by PANi needles, promote the cardiogenic differentiation of hMSC into cardiomyocite-like cells. These preliminary results concur to promise the development of electroactive biodegradable substrates able to efficiently stimulate the basic cell mechanisms, paving the way towards a new generation of synthetic patches for the support of the regeneration of damaged myocardium.

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1 Introduction

Conducting polymers (CPs) constitute an attractive class of materials for electronic, magnetic, and optical applications. Research on CPs for biomedical applications gathered momentum with the discovery in the 1980s that these materials were compatible with many biological molecules, such as those used in biosensors. Conducting polymers such as polypyrrole (PPy), polythiophene (PT) and polyaniline (PANi) have widely been utilized in the microelectronics industry, including battery technology, photovoltaic devices, light-emitting diodes and electrochromic displays [1]. Among them, PANi has received increasing attention due to its environmental stability, controllable electrical conductivity, and interesting redox properties. In the emeraldine oxidation state, PANi can be reversibly switched between

electrically insulating and conducting forms according to its proton doping state. This excellent processability, together with the presence of multiple intrinsic redox states, have substantially enhanced the potential applications of aniline polymers for their use in several application fields [2–6].

Recently, PANi has also been shown to modulate cellular activities, including cell adhesion and migration, DNA synthesis and protein secretion [7, 8] but, there is also a growing interest in conductive polymers for different biomedical application, i.e., tissue engineering. In particular, it has been demonstrate the efficacy of conductive polymers to stimulate a multitude of cell functions, such as attachment, proliferation, migration, and differentiation through a modulation of transferred electrical stimuli from the external support to the cell. Furthermore, recent in vivo studies showed that significant levels of electrical conductivity, albeit decreasing with time, are maintained for at least 100 h in a physiological environment.

To date, several studies investigated the biological response of polyaniline based systems only in film, bulk, or polymer blend form. However, the main shortcoming of using PANi in a biological environment concerns its deficiencies in workability and biodegradability, which generally induce chronic inflammation over long implant times and, ultimately, the need of second surgery for removal [9].

Hence, an interest to synthesize *conducting* and *biodegradable* polymers is forcefully emerging. For example, polypyrrole has been recently blended with poly(D,L-lactide) (PDLLA) [10, 11], or PANi has been blended with the natural polymers collagen [12] and gelatin [13, 14]. In other studies, biodegradabile segments have been introduced into the main chain of conducting polymers [15], and conducting heterocyclic oligomers have been joined together via degradable ester linkages [16, 17], so introducing degradable polyactide (PLA) into a backbone structure which also incorporates aniline units. Nevertheless, it remains a considerable challenge to synthesize the ideal electroactive polymer which also exhibits tailored requisites of biocompatibility and biodegradability able to minimize the inflammatory reaction raised by the non-degradable particles in the host tissue.

In this work, it has been proposed to integrate short conductive camphorsulfonic acid (CSA)-doped PANi nanoneedles into polycaprolactone (PCL) substrates. PCL matrix with slow degradation rates should play a protective action which assure an efficient transfer of the electrical signal due to bridged PANi needles, also preventing the unascertained release of PANi fragments potentially able to catalyze undesired inflammatory responses. Here, cast films of these materials have been prepared in order to investigate any improvement in conductivity offered by the inclusion of electroactive phases. Moreover, a preliminary study on PANi/PCL electrospun membranes was assessed to evaluate the influence of PANi content on the fibre morphology and hMSC differentiation with the ultimate goal of creating scaffolds with percolative electroactive phases which are able to support the transfer of electric stimuli to cells during the regeneration of myocardium tissue.

2 Materials and methods

2.1 Materials

Aniline monomer, Ammonium Peroxy Disulphate (APS), Camphor Sulphonic Acid (CSA), Chloroform ($CHCl_3$), emeraldine base polyaniline (EBPANi, M_w 10 kDa), 1,1,1,3,3,3-hexafluoro-2-Propanol (HFP), Poly ε-caprolactone (PCL, molecular weight (M_w) 65 kDa) were purchased from Sigma-Aldrich, Italy. All chemicals were of analytical grade and used as received.

2.2 sPANi synthesis

Ultrafine short fibres of polyaniline doped with camphorsulfonic acid (CSA) were prepared as follows: 0.8 mmol Ammonium peroxydisulfate initiator and 3.2 mmol amount of aniline monomer were separately dissolved in a 1 M CSA-doped acid solution and rapidly mixed together all at once. The appearance of bluish green droplets of polyaniline was noticed within 30 min of the start of the reaction and the synthesis was left to proceed for 2 h at room temperature. The as-prepared nanofibers can be purified by common solid–liquid separation techniques by centrifugation. Polyaniline nanofibers are obtained in powder form after drying at 80°C under vacuum. The dry powders of polyaniline have been re-dispersed in $CHCl_3$ solvent using mild sonication

The morphology of sPANi short fibres was preliminary analysed through Transmission Electron Microscopy (Jeol, JEM 1220) by dispersing PANi samples in Chloroform and depositing subsequently one drop of the dispersed solutions on a Copper grid covered with Celludione.

2.3 sPANi/PCL films

2.3.1 Preparation

Two different typologies of samples were prepared, in turn, by endowing synthesized polyaniline (sPANi) short fibres or blending EBPANi (Fig. 1) by imposing different PCL-PANi ratios (100/0, 99/1, 95/5 94/6, 90/10 and 80/20 wt/wt). All films were prepared by solvent casting for a preliminary characterization of chemical and electrical properties.

As for the PCL/sPANi composite, polyaniline short fibres were dispersed in $CHCl_3$ as previously reported. Afterwards, PCL pellets were added to the sPANi solution up to form an homogeneous solution. As for PCL/EBPANi

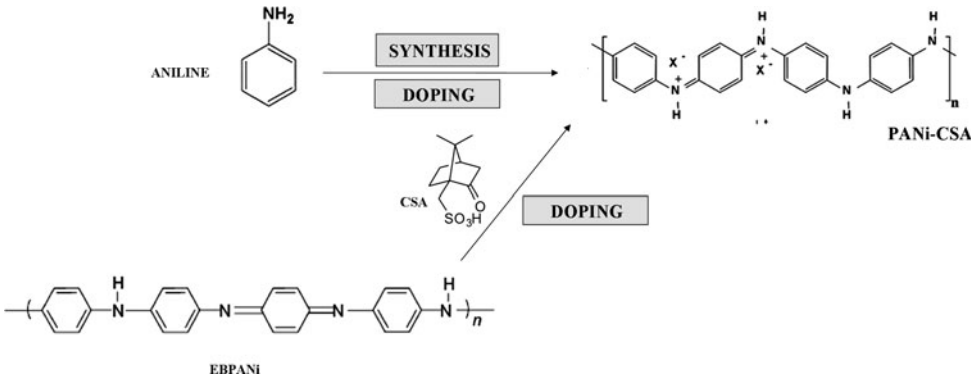

Fig. 1 Scheme of sPANi short fibres synthesis of EBPANi doping

blends, EB and CSA (50/50 wt/wt) were dissolved in HFP (6×10^{-3} M) by magnetic stirring at room temperature in agreement with previous works [13]. After 2 h, PCL was added to the previous solution and dissolved by magnetic stirring up to form the blend.

In both cases, the mixture was placed in circular Petri dish, 6 mm as diameter, up to reach a film thickness of 0.6 mm, properly calculated by micrometric calibre. Finally, the mould was placed under hood under forced flow overnight to assure the complete evaporation of solvent.

2.3.2 *Infrared spectroscopy*

The effect of PANi short fibres on PCL chemical structure was evaluated by FTIR-ATR spectrometer (Nexus, Thermo Nicolet, model 60SX). Infrared absorption spectra were recorded in the 4000–500 cm^{-1} range, at 4 cm^{-1} resolution averaging 144 scans.

2.3.3 *Conductivity measurements*

Bulk electrical conductivity of PCL–sPANi and PCL–EBPANi films has been evaluated by means of two probes DC electrical resistance measurements by using a Signatone 1160 probe station connected to a picometer/voltage source meter (National Instrument) in ambient condition.

A custom conductivity cell was designed and realized in polytetrafluoroethylene (PTFE) in order to provide a simple fixture for loading a film and performing two-points-probe conductivity tests. The cell has an internal circular electrode with a diameter of 10 mm and guard ring placed at a distance of 0.5 cm. (Fig. 4a). The guard electrode prevents surface current errors.

2.4 sPANi/PCL electrospun membranes

2.4.1 *Preparation*

PCL pellets were dissolved in sPANi/$CHCl_3$ solution in as previously described. A PCL/PANi ratio of (99/01) wt/wt was considered for the electrospinning process. An home-made electrospinning apparatus equipped with a high-voltage supply (HV Gamma Voltage, USA) with a maximum working value of 30 kV has been used in this study. Polymer solutions were added into a 5 ml syringe attached to a hypodermic needle used as a nozzle (18 Ga). An aluminium plate was placed at a working distance of 120 mm from the capillary tip. The flow rate of the polymer solution was controlled by a precision syringe pump (KD Scientific, USA) in order to maintain a steady flow at the capillary exit. In particular, voltage of 10 kV and flow rate of 0.5 ml/h were selected as optimal process parameter. Moreover, all the experiments were carried out room temperature and controlled relative humidity. Before each experiment, the collector was covered with aluminum foil and then removed after fibre deposition. All non-woven fiber mats were dried at room temperature for about 6 h in order to completely remove any solvent residue prior the characterization. PCL electrospun membranes from PCL/$CHCl_3$ (20% wt/wt) were prepared as control.

2.4.2 *Morphology and image analysis*

Preliminary, the fibre mesh of PCL and PCL/PANi electrospun membranes was observed by optical microscopy (Olympus BX51P). A qualitative evaluation of fibre morphology of the electrospun PCL membranes was performed by field emission scanning electron microscopy, (FE-SEM—QUANTA200, FEI the Netherlands) after sputter-coating with gold–palladium. Samples were preliminary kept under hood in order to remove residual solvent traces, then, directly located on metal stubs to preserve the fibre morphology. The accelerating voltage was fixed at 5 kV by imposing a low vacuum condition (10^{-2} torr) into the microscope chamber. Moreover, on selected SEM images, fibre size distribution were kept by freeware image analysis software (NIH Image J 1.37) and the average diameter was determined by measuring ca. 15 representative fibres for three different images.

2.5 Biological validation

2.5.1 Cardiogenic differentiation

Human mesenchymal stem cells line (hMSC) obtained from LONZA were cultured in 75 cm^2 cell culture flask in Eagle's alpha minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum, antibiotic solution (streptomycin 100 μg/ml and penicillin 100 U/ml, Sigma Chem. Co) and 2 mM L-glutamin. When the cells became almost confluent, they were released by treating with 0.25% trypsin–EDTA solution for 3 min at 37°C and resuspended in α-MEM and seeded in six-well plates at a concentration of 50,000 cells per well. Twenty-four hours after seeding, the cells were washed with PBS twice. Then the cells were incubated for 72 h in serum-free media containing 10 ng/ml FGF, 10 ng/ml EGF with 10 μM 5-azacytidine. After this period of time the medium was change with fresh complete medium to prevent cell death due to prolonged exposure to 5-azacytidine and the cells were consider cardiomyocyte-like cells.

2.5.2 Cell survival evaluation

The effect of sample on cardiomyocyte-like cells survival was investigated by using MTT colorimetric assay. Prior to cell seeding, the different substrates were placed into 24-well culture plate and sterilized by immersion in 70% of ethanol (v/v) with antibiotic solution (streptomycin 100 μg/ml and penicillin 100 U/ml) for 15 min and then were equilibrated with prewarmed α-MEM medium for 2 h. After sterilization; 2×10^4 cells were seeded onto PCL and PCL/PANi nanofibers scaffolds. Samples were maintained in a humidified chamber at 37°C for 1, 3 and 5 days, respectively and, then, cultured without the support of any external electrical stimulation. At the end of culture time, the scaffolds were washed carefully with PBS and incubated with fresh culture medium containing 0.5 mg/ml of MTT for 4 h at 37°C in the dark. The supernatant was then removed carefully and dimethyl sulfoxide (DMSO) was added to each well. The plate was shaken to dissolve the purple formazan crystal and the optical density value was recorded using a micro-plate reader (Perkin-Elmer, Boston, MA) at the wavelength of 570 nm. To calibrate the cell survival rate, blank and control groups (tissue culture polystyrene plates) were set. In the blank group, only culture media was added into the well, while in the control group cardiomyocyte-like cells and culture media were added. The blank and control groups were treated with the same procedures and incubated for the same time as those of the experimental groups. The measured of optical density values of the blank group, control group and experimental group were coded as ODbla, ODcon, and ODexp, respectively, and the cell survival rate was calculated by equation: Survival Rate = (ODexp − ODbla)/(ODcon − ODbla). During the experiment, the culture medium was changed every two days with fresh media and all viability experiments were conducted in triplicate.

2.5.3 Immunostaining

Cardiomyocyte-like cells cultures after 5 days onto PCL and PANi/PCL scaffolds were fixed with 4% paraformaldehyde and permeabilized with PBS containing 0.1% Triton X-100. Cells were incubated with antibody at 4°C overnight for examination of expression of sarcomeric α-actinin in PBS containing 2 mg/ml of bovine serum albumin (BSA). Then were washed with ice-cold PBS for 10 min at room temperature and incubated for 1 h at 4°C with secondary antibodies conjugated with FITC (3 mg/ml, Sigma Chemical, St. Louis, MO), diluted 1:50 in PBS. After this, were rinsed with PBS plus 0.01% Triton X-100 and immunostaining were visualized by confocal laser scanning microscopy (LSM510; CarlZeiss).

2.6 Statistical analysis

Differences among scaffolds were examined by Student's *t* test and data were reported as mean ± standard deviation. All the results were considered at significant level $P < 0.05$.

3 Results and discussion

Currently, the use of conducting polymers are progressively exploring in scaffold design to electrically stimulate cells, so regulating specific cellular activities and, ultimately, the process of regeneration of damaged tissues. In particular, many studies of electroactive tissues (i.e., muscle, myocardium) which respond to electrical impulses, demonstrates the success of variously doped CPs to entrap/release biological molecules as therapeutic proteins and drugs [18–20] or to transfer charge from a biochemical reaction [21]. Polyaniline is initially nonconductive, but may be transformed to the conductive state by increasing the degree of protonation through treatment with an acidic dopant to convert imine groups of the polymer to iminium. Thus, doping of the polyaniline emeraldine base results in a conductive emeraldine salt [22]. The proper selection of doping strategies, needful to induce the polymer conductivity, has been exploited to non-covalently modify CPs with bioactive molecules in order to improve the biological response, as well as to introduce new functionalities oriented to specific applications (nerve, bone, muscle, and cardiac cells).

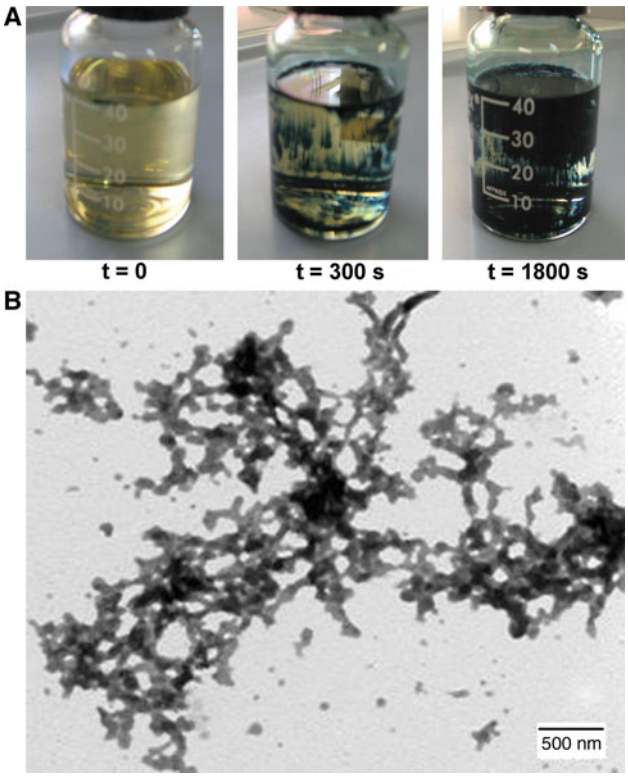

Fig. 2 sPANi synthesis **a** formation of PANi short fibres in water solution with APS initiator, **b** TEM image of sPANi short fibres

In this work, ultrafine sPANi short fibres were prepared by a rapidly-mixed reaction of 2 h duration (Fig. 2a). This involved the fast mixing of the initiator solution (ammonium peroxydisulfate in CSA buffered water) into the monomer solution (aniline in CSA buffered water). As the polymerization begins, the initiator molecules induce the formation of short fibres by rapidly polymerizing aniline monomers in their neighbouring regions. If the initiator molecules are evenly distributed, then they should be consumed during the formation of single fibres. The fibrous PANi morphology is observed by TEM images (Fig. 2b). The diameter of the PANi nanofibers was estimated to be of the order of 50–100 nm, while the length of the fibers was estimated to vary from hundreds of nanometres to several micrometers. Pressed pellets of PANi showed an electrical conductivity of $\sim$0.05 S/cm in air.

The most significant limitation of using CPs in tissue regeneration is their inherent inability to degrade naturally. In current attempts to overcome this, CPs have been coupled with biodegradable polymers; for example, CPs were blended with degradable materials like polylactide, polyglycolide and their copolymers or ester linkages [10, 23] for different applications in muscle and nerve regeneration.

Here, novel composite substrates comprising polyaniline (sPANi) and poly(caprolactone) (PCL) were developed as biodegradable platforms for cardiac tissue regeneration. In preliminary work, composite films with different PANi concentrations (100/0, 99/1, 95/5 and 90/10 wt/wt) were characterized by ATR spectroscopy. IR spectra are reported in Fig. 3. The spectra of the PCL/PANi composite exhibit strong absorption in the range 4000–500 cm^{-1}, and are similar to that of pure PCL, almost all the main vibrational bands of PCL being present. The vibrational band which may be reasonably assigned to the normal modes of polyaniline is at 3200–3600 cm^{-1}. This band is related to the N–H stretching of an aromatic amine, and is also observed in the doped form. The intensity of this band increases as sPANi content increases, in agreement with the amount used during the composite preparation.

However, the matching of polymer phases with different intrinsic conductivity could compromise the ability of blend to charge transfer. For this reason, the conductivity of PANi/PCL films was measured as a function of the sPANi content. Conductivity is a measure of electrical conduction and thus a measure of the ability of a material to pass a current. Generally, materials with conductivities less than 10^{-8} S/cm are considered insulators, materials with conductivities between 10^{-8} and 10^{3} S/cm are considered semiconductors, and materials with conductivities greater than 10^{3} S/cm are considered conductors.

In order to demonstrate the higher conductivity sPANi short fibres, casted films endowed with synthesized polyaniline (sPANi) short fibres or blended with EBPANi were prepared as reported in the Experimental section. The electrical conductivity of PCL matrix with different PANi nanofiber content (wt%) was tested. A comparative study was also performed, in order to measure the conductivity as a function of the different PANi used, as summarized in Table 1. PCL without incorporated PANi shows minimal conductivity as a dielectric polymer (3×10^{-12} S/cm). The incorporation of PANi significantly increases the conductivity, as a function of PANi content. Moreover, the data reported in Table 1 clearly show that film conductivity increases by up to seven orders of magnitude. It is apparent that, where he level of incorporated sPANi is below 1 wt%, the composite appears as an insulated material. As sPANi content increases, the distance between the fibers decrease until it becomes so small that a tunneling or "hopping" process can occur (Fig. 4). Depending on the conducting filler level and the related distribution inside the polymer matrix, the physical properties of the composite can be finely tuned. At very low filler fractions, the mean distance between the conducting fillers is quite large, and the electrical conductivity is mainly determined by the host matrix. When the filler concentration exceeds a critical value, the filler particles form linkages, resulting in the formation of conductive paths in the whole material: the corresponding filler content is termed the *percolation threshold* [24].

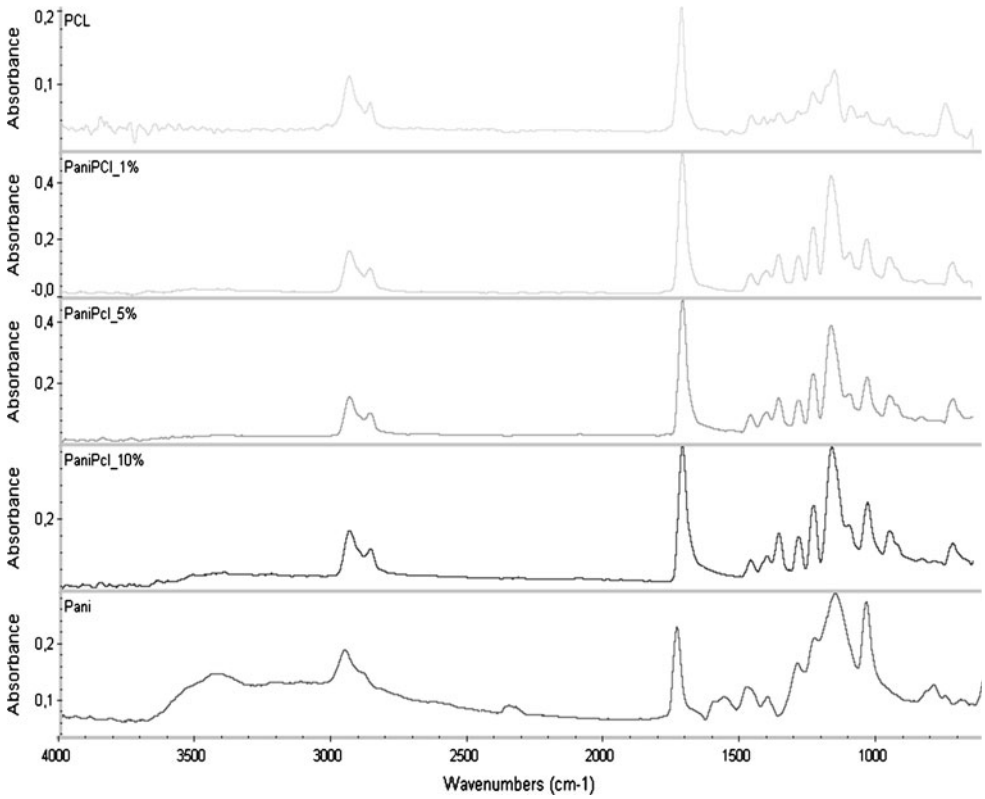

Fig. 3 ATR spectra of PCL/sPANi composites at different sPANi content: from 0 wt% (*upper*) to 100 wt% (*bottom*)

Table 1 Composition, thickness and electrical conductivity of PCL–PANi composite and PCL–EBPANi blend

PCL:PANi or EBPANi %, w/w	PCL–PANi Electrical conductivity S/cm × 10^{-12}	PCL–EBPANi Electrical conductivity S/cm × 10^{-12}
100:0	3 ± 0.5	3 ± 0.1
99:1	85 ± 1.5	6 ± 0.5
95:5	(9 ± 1.3) × 10^4	20 ± 2
95:6	(2 ± 0.7) × 10^6	(3 ± 0.3) × 10^2
90:10	(1 ± 0.2) × 10^7	(7 ± 0.8) × 10^4
80:20	(8 ± 0.5) × 10^7	–

Near this critical value, small changes in the filler amount can induce conductivity modification up to several orders of magnitude. In contrast, at higher filler concentrations, the larger number of conducting paths forms a complete three-dimensional network. Consequently, the resulting conductivity is noticeably increased and much less sensitive to any change in the filler fraction. However, the filler content represents a key aspect in determining the biological response of substrate. To date, it is universally recognized by several studies in literature that polyaniline is not overtly cytotoxic, but did require adhesion promoting modifications to support cell adhesion, survival and functionality. For example, Bidez et al. show that cardiac myocytes grew well onto polyaniline films, and only an initial decrease in proliferation was detected [25]. However, an ideal scaffold for TE should incorporate a wide set of information able to guide the specific tissue regeneration [26–29]. This involves, firstly, the design of proper materials phases (i.e., composite, blends), but also, the control of the architecture on micro/nanometric level (i.e., porosity, roughness) as well as the integration of molecular signals to selectively trigger cell events. One recent fabrication technique for the creation of scaffolds involves the production of nanofibrous meshes via electrospinning [26, 30]. Nanofiber scaffolds produced by electrospinning are considerably smaller than the microfibers produced using conventional extrusion techniques, and are similar in size scale to elements of the native microenvironment thus providing to structurally mimic the extracellular matrix (ECM) [31]. In addition, the process is compatible with a wide number of polymers, both natural (collagens [32], elastin [33] and fibrinogen [34]) and synthetic (poly(glycolic acid) (PGA), poly(lactic acid) (PLA), polycaprolactone (PCL), etc.) [35].

Currently, the blending of different polymers (i.e., PCL, gelatin), has been considered a successful strategy to satisfy all the basic requirements of bioactive scaffolds. In particular, this approach enables the development of tailored materials with improved biological response, mechanical and physical/chemical properties which are suitable for long-term applications as for bone [36] and nerve [37] regeneration.

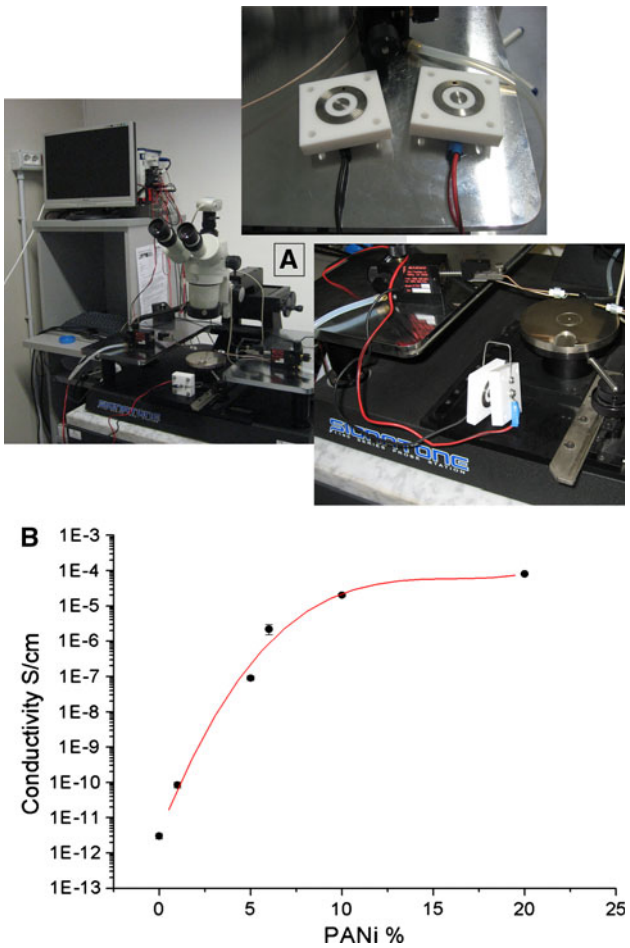

Fig. 4 **a** Conductivity apparatus and measurement cell. **b** Conductivity (scatter) of PCL/sPANi composites films as a function of sPANi wt% and fitting curve (*line*)

Here, the PANi/PCL blending was proposed to create an electroctive bidegradable, and biocompatible scaffold which is potentially capable of supporting the regeneration of the myocardium. Figure 5 shows electrospun membranes obtained from PCL and PCL/sPANi solution, respectively. Optical images of PCL (upper) and PCL/sPANi (bottom) fibers show a random distribution of fibres, homogeneously dispersed onto the collector. Moreover, SEM images at low magnification highlight a network of strictly packed fibers with micrometric scale without the presence of beads. In particular, the influence of sPANi content has been investigated to validate the morphological properties of scaffolds in terms of fibre size and size distribution. As clearly shown in Fig. 5, the presence of short sPANi nanofibres seems to slightly affect the mechanism of fibre stretching during the deposition, so assuring the creation of a fibrous network without defects. This evidence has been confirmed by the image analysis performed on selected SEM images of fibre network (Fig. 6). This shows show a narrow distribution of fibre diameter in the case of PCL substrates with a maximum peak at 7.1 μm. In the case of composite PCL/sPANi substrates, after 2 h of sPANi synthesis, a broader distribution of fibres was detected with a slight reduction of the average fibre size down to 6.4 μm. This is the result of the fine dispersion of sPANi short fibres into the polymer solution prior the electrospinning process, thus limiting the formation of highly conductive clusters able to influence the correct evolution of jet under the electric field forces during the deposition. Despite the higher conductivity of the PANi salts, remarkable process instabilities do not occur during electrospinning process due to the use of a reduced content of sPANi. However, the amount of sPANi may be crucial to the control of the electrospinning process. In particular, we verified that the use of higher amount of sPANi can promote the occurrence of significant instability phenomena which can drastically affect the fibre morphology with the formation of beads along fibres. In perspective, we will provide to leverage the tailored electro-conductive properties by optimizing the entrapment of sPANi short fibres into the

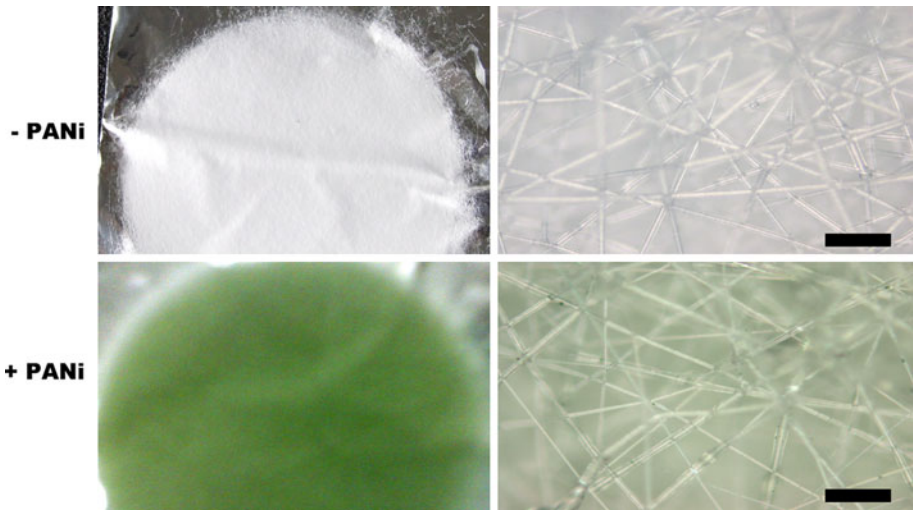

Fig. 5 Electrospun membranes of PCL and PCL/PANi. Optical images (*scale bar* 20 μm)

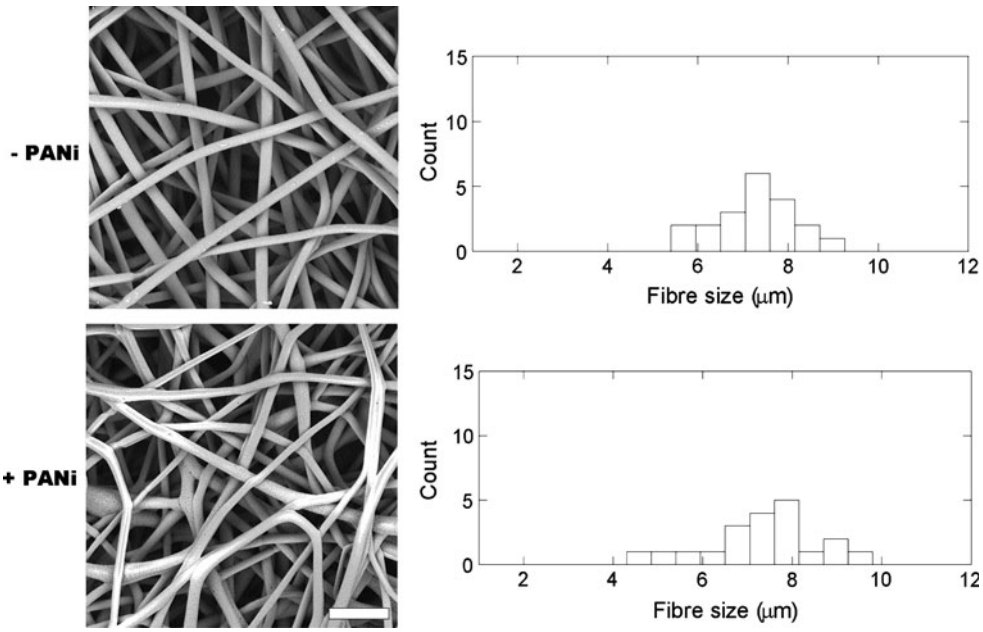

Fig. 6 Evaluation of fibre morphology of PCL and PCL/PANi electrospun membranes for different synthesis times: SEM images and fibre size distribution calculated by image analysis

electrospun fibres to create electroactive patches able to stimulate extracellularly myocardium cell populations and to accelerate the cardiac muscle regeneration. A preliminary study has been performed to evaluate the attitude of PANi inclusions to promote the differentiation of hMSC into cardiac phenotype. Figure 7a shows the results of cell viability by MTT assays and differentiation in terms of survival rate of cardiomyocyte-like cells in PCL and PANi/PCL nanofibers. After 1 day of cell culture, the survival rate of cardiomyocyte-like cells onto PANi/PCL nanofibers is only related to the adhered cells and only show slight differences as a function of the PANi content. Otherwise, after 3 and 5 days, the survival rate of cardiomyocyte-like cells onto PCL/PANi samples is significantly higher than that on the PCL surface, thus demonstrating the effect of conductive signal of PANi on supporting the cell proliferation. Such findings are well consistent with recent studies on nanostructure PANi films which showed good biocompatibility in terms of adhesion and proliferation [38, 39]. Moreover, according to recent works on cardiomyocites differentiation [40–43], immunoassaying against the expression of sarcomeric α-actinin also confirmed the effect of PANi on the surface interaction of cardiomyocyte-like cells (Fig. 7b) as remarked by a more drastic phenotypic transformation of hMSC to the cardiomyocyte lineage.

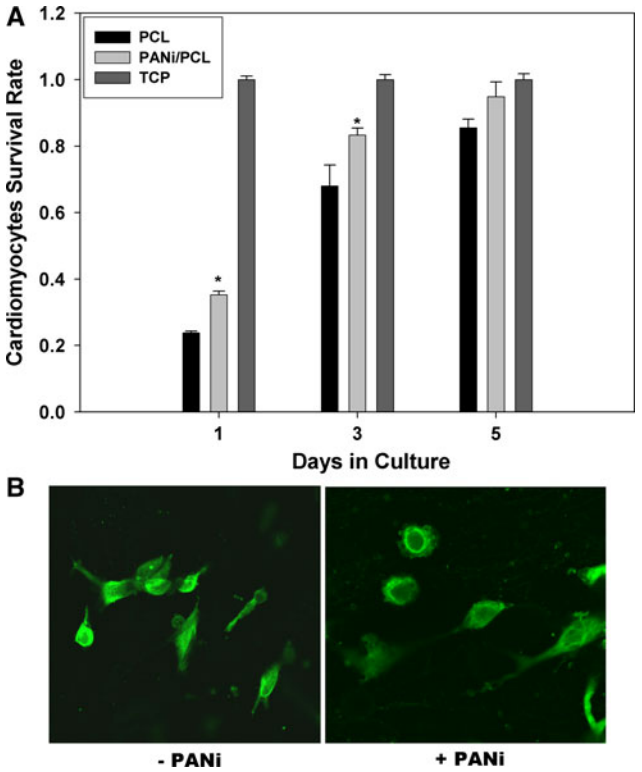

Fig. 7 Cardiogenic differentiation from hMSC onto PCL and PCL/PANi nanofibres **a** evaluation of cell survival rate by MTT assay and **b** immunostaining of sarcomeric α-actinin of cardiomyocites-like cells

4 Conclusion

In this work, we demonstrated that synthesized PANi/PCL films are characterized by appreciable levels of conductivity for a powerful stimulation of cells for cardiac regeneration. The proposed synthesis/doping methods allow to improve the intrinsic conductivity emeraldine base PANi in order to reach equivalent conductive properties by a relevant reduction of the PANi used, and with potential

benefits on the inflammatory response. Moreover, the development of sPANi/PCL electrospun membranes with controlled fibre texture further concurs to create an electrically conductive environment able to stimulate the cell differentiation to cardiomyocites, for a successful use in the myocardium muscle regeneration.

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