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Influence of poly(*N*-isopropylacrylamide)-CNT-polyaniline three-dimensional electrospun microfabric scaffolds on cell growth and viability

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ABSTRACT

This study investigates the effect on: 1) the bulk surface; and 2) the three-dimensional non-woven microfabric scaffolds of poly(*N*-isopropylacrylamide)-CNT-polyaniline on growth and viability of mice fibroblast cells L929. The poly(*N*-isopropylacrylamide)-CNT-polyaniline was prepared using coupling chemistry and electrospinning was then used for the fabrication of responsive, nonwoven microfabric scaffolds. The electrospun microfabrics were assembled in regular three-dimensional scaffolds with OD: 400-500 μm ; L: 6-20 cm. Mice fibroblast cells L929 were seeded on the both poly(*N*-isopropylacrylamide)-CNT-polyaniline bulk surface as well as non-woven microfabric scaffolds. Excellent cell proliferation and viability was observed on poly(*N*-isopropylacrylamide)-CNT-polyaniline non-woven microfabric matrices in compare to poly(*N*-isopropylacrylamide)-CNT-polyaniline bulk and commercially available Matrigel™ even with a range of cell lines up to 168 h. Temperature dependent cells detachment behaviour was observed on the poly(*N*-isopropylacrylamide)-CNT-polyaniline scaffolds by varying incubation at below lower critical solution temperature (LCST) of poly(*N*-isopropylacrylamide). The results suggest that poly(*N*-isopropylacrylamide)-CNT-polyaniline non-woven microfabrics could be used as a smart matrices for applications in tissue engineering.

KEYWORDS: *smart tissue engineering scaffolds, conducting polymers, carbon nanotubes, responsive-polymers, biocompatible conducting matrices.*

INTRODUCTION

Conducting polymers are proven and widely applied materials with electronic and ionic conductivity.^{1,2} A range of new biomedical applications are currently being considered using conducting polymers including development of artificial muscles,³ controlled drug release,^{4,5} neural recording⁶ and stimulation of nerve regeneration.⁷ Moreover, electrically active tissues such as brain, heart and skeletal muscle offer opportunities to couple them with electronic devices and computers to create therapeutic body-machine interfaces.⁸ These possibilities make them an important class of materials for bioelectronics. Of various conducting polymer, polyaniline is a well-known, versatile conducting material, which has found particular biological utility due to its ease of availability and/or synthesis via chemical and/or electrochemical methods, environmental stability, inexpensive and classical electrical and electronic properties. Bidez *et al.* reported the biocompatibility and cell adhesion behaviour of polyaniline in both conducting, i.e., emeraldine salt, and non-conducting, i.e., emeraldine base forms.⁹ This work facilitates the possibility of smart conductive scaffolds to be used in a range of biomedical applications.

Carbon nanotubes (CNTs) have also attracted much interest in diverse applications due to their unique structural, mechanical and electronic properties.¹⁰ CNTs are popularly applied in energy storage devices, where they are used as a filler to provide mechanical strength in polymeric nanocomposites, although new investigation of CNTs and CNTs based composites have also found application in the biomedical field.¹¹⁻¹³ Recent studies indicated that materials, with or without pre-treatment to promote cell proliferation, exhibited desirable synergistic effects with cells. Such scaffolds can exhibit a well-defined, biocompatible three-dimensional microstructure, which could provide better access for cells to growth factors for regular cellular activities. Furthermore, temperature change is a widely observed phenomenon in physiological systems. Temperature-sensitive materials have, therefore,

attracted significant attention due to their ability to respond intelligently to temperature changes. It is worth noting that a novel design of temperature-responsive materials, which are capable of responding to external temperature, have been reported in the literature.¹⁴⁻¹⁶ The systems are derived from poly(*N*-isopropylacrylamide) and exhibit a lower critical solution temperature (LCST) ranging from 32 to 34 °C. These poly(*N*-isopropylacrylamide)-based materials normally show efficient bioactivity at an ambient temperature, when the transition status of hydrophilicity is present. After their phase transitions to hydrophobicity upon changes of external temperature, the polymeric matrices become insoluble to water, thereby causing a dramatic decrease in the diffusion of substrates. Based on this mechanism, poly(*N*-isopropylacrylamide)-based matrices could act as controllable temperature-responsive bio-switches for biomedical and biotechnology applications.¹⁷⁻²⁰

Taking advantage of the excellent mechanical strength, cost-effectiveness, ease of preparation and high surface-to-volume ratio of these three-dimensional microfabric scaffolds, and utilising novel stimuli-responsive functional materials with temperature self-control ability could be highly useful for the advancement of tissue engineering technology. In this report, we have fabricated electrospun microfabrics of a covalently attached poly(*N*-isopropylacrylamide)-CNT-polyaniline. The advantages of microfabric scaffolds include excellent cells growth and viability with cells detachment behaviour by lowering incubation temperature from 37 to 20 °C. Cell viability and proliferation were studied using mice fibroblast cells L929 and also cross checked with a range of other cell lines as preliminary tests for the potential application of poly(*N*-isopropylacrylamide)-CNT-polyaniline three-dimensional non-woven microfabrics as temperature responsive scaffolds in the tissue regenerations.

EXPERIMENTAL SECTION

Materials. Amine terminated, poly(*N*-isopropyl acrylamide) (M_n 5,500; Aldrich, Schnelldorf, Germany); carboxyl functionalised multi-walled carbon nanotubes powder (HOOC-MWNTs, >95%, 1-6 %wt. -COOH functionalised, OD: 30-50 nm, L: 10-20 μ m, Nanostructured and Amorphous Materials Inc., Texas, USA), polyaniline (M_w 10,000; Aldrich, Schnelldorf, Germany), *N,N'*-dicyclohexylcarbodiimide (DCC, 99%, Wako, Osaka, Japan), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 99%, Wako, Osaka, Japan) and *N*-hydroxysuccinimide (NHS, 99%, Wako, Osaka, Japan) were used as received from the companies. Foetal calf serum, penicillin, streptomycin and L-glutamine (complete media) were purchased from Sigma (Schnelldorf, Germany); and MatrigelTM, propidium iodide, Calcein-AM and 24-well plates were supplied by B. D. Biosciences (Tokyo, Japan), Dojin (Tokyo, Japan) and B. D. Falcon (Tokyo, Japan), respectively. All supplementary chemicals were used of analytical grade and solutions were prepared with Milli-Q water with resistance of 18.2 M Ω .

Preparation of poly(*N*-isopropylacrylamide)-CNT-polyaniline. Poly(*N*-isopropylacrylamide)-CNT-polyaniline was synthesised in two steps: 1) HOOC-MWNT was covalently coupled with polyaniline using *N,N'*-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) as coupling agents;²¹ and 2) poly(*N*-isopropylacrylamide) was selectively grafted with residual carboxyl groups, i.e., -COOH groups available on the CNT-polyaniline through 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and NHS mediate amide formation.²²

Synthesis of CNT-polyaniline. The CNT-polyaniline was prepared by reacting 100 mg of HOOC-MWNTs with 0.6 mM polyaniline using 0.62 mM of DCC and 0.65 mM of NHS as coupling agents (Fig 1a). First, 100 mg of HOOC-MWNTs was added into 25 mL of anhydrous dimethyl formamide under >20 kHz ultrasonic wave agitation for 2 h. To this mixture, a calculated amount of polyaniline, DCC and NHS were added and then reaction was allowed to continue for 12 h under continuous stirring at room temperature. Unreacted reagents were then removed via dialysis using cellulose dialysis membrane (M_w cut-off 10 kDa) against anhydrous dimethyl formamide for 48 h. CNT-polyaniline was filtered and dried under vacuum. CNT-polyaniline yield (%) = 94.20.

Synthesis of poly(*N*-isopropylacrylamide)-CNT-polyaniline. The amine terminated, poly(*N*-isopropylacrylamide) was covalently coupled with available carboxyl groups of the CNT-polyaniline using EDC and NHS as coupling agent (Fig. 1b). A 200 mg aliquot of CNT-polyaniline was dispersed in 25 mL of dimethyl formamide under ultrasonication. To the resulting mixture, 75 mL aqueous solution containing 1.2 mM amine terminated, poly(*N*-isopropylacrylamide), 1.25 mM EDC and 1.3 mM NHS were added under continuous stirring at room temperature and the reaction was allowed to proceed for 12 h. The resulting poly(*N*-isopropylacrylamide)-CNT-polyaniline was collected by dialysing the reaction solution using cellulose dialysis membrane (M_w cut-off 10 kDa) against Milli-Q water for 48 h. Next, poly(*N*-isopropylacrylamide)-CNT-polyaniline was filtered and dried under vacuum. The Poly(*N*-isopropylacrylamide)-CNT-polyaniline yield was 93.8%.

Fabrication of three-dimensional electrospun microfabric scaffolds. The electrospun microfabric was obtained via a well-established needle-collector electrospinning method as shown in Fig. 1c.²³ A typical electrospinning system with a rotating drum collector (NANON,

MECC Co. Ltd., Japan) was employed to fabricate large nonwoven microfabric scaffolds. An 8 mg/mL solution of poly(*N*-isopropylacrylamide)-CNT-polyaniline was prepared using 1,1,1,3,3,3-hexafluoro-2-propanol and *N,N*-dimethylformamide (8: 2, v/v) solvent mixture. Next, the solution mixture was placed for 12 h under moderate stirring at room temperature. The resulting homogeneous solution was taken up into a syringe (1 mL) fitted with a metallic needle of 22 gauge. The syringe was fixed horizontally on the syringe pump, and an electrode of high voltage power supply was clamped to the metal needle tip. The flow rate, voltage supply, humidity and distance of tip of needle to collector surface (i.e., aluminium foil) were 0.32 mL/h, 15 kV, <15% and 24 cm, respectively. Finally, the microfabric scaffolds (Fig. 1d) were collected on aluminium foil and dried in vacuum desiccators for 48 h at room temperature.

Characterisation. ^1H NMR spectroscopy was used to examine the chemical structure of synthesised samples. ^1H NMR spectra were obtained with a 600 MHz Bruker DRX 600 spectrometer using tetramethylsilane as an internal standard at 20 °C. The samples were prepared by dissolving 10 mg of material in 1 mL of CDCl_3 under stirring for 5 h. The morphology of the bulk and electrospun microfabrics three-dimensional scaffolds based on poly(*N*-isopropylacrylamide)-CNT-polyaniline was observed with a JEOL EDSEM scanning electron microscope (SEM) at accelerating voltage of 20 k. The aluminum foil containing electrospun microfabric was coated with about 5 nm thin layer of platinum using ELIONEX platinum coater prior to measurement.

Cell culture assay. Mice fibroblast cell line L929 was used to examine cell growth and viability on the poly(*N*-isopropylacrylamide)-CNT-polyaniline electrospun microfabric three-dimensional scaffolds and poly(*N*-isopropylacrylamide)-CNT-polyaniline bulk

nanocomposite. The cells were supplied by Riken cell bank, Tsukuba, Japan. In the cell culture, cells were directly placed over the surface of electrospun microfabrics in the medium supplemented with 10% (w/v) foetal calf serum, penicillin/streptomycin and L-glutamine (complete medium) in 24 well tissue culture plate, c.f., the medium was changed every 72 h. The tissue culture plate was incubated in a humidified environment at 37 °C with 5% (v/v) CO₂ supply. The cells (1×10^4 cells/well) were seeded in respective cell culture plates and cells morphology and growth were assessed at 24, 48, 72, 96, 120, 144 and 168 h. For cell viability, assays cell-loaded with electrospun microfabric scaffolds and bulk nanocomposite were washed with phosphate buffer solution (PBS) of pH 7.4 and stained with 1 µL of Calcein-AM and 1.5 µL of propidium iodide (PI) for 30 min at 37 °C. Cell viability was checked at 168 h after cells seeding. The cells detachment behaviour of scaffolds was accomplished by incubating culture plates at 20 °C for 2 h. The control tests were also performed using the commercially available gel Matrigel™ and native media under identical conditions.

All live cells were labelled with Calcein-AM, while dead cells were labelled with PI. Cells were imaged using an Olympus phase-contrast light microscope. To analyse cell growth, the number of cells present within a 0.15 mm² area was determined with three representative images from each sample and each time point. All analyses were completed under identical conditions. When cell clumps were encountered they were counted as one unit, unless separate cells were resolvable. All the results are presented as 5th mean of standard deviation (SD).

RESULTS AND DISCUSSION

Synthesis, characterisation and scaffold fabrication of poly(*N*-isopropylacrylamide)-CNT-polyaniline. Poly(*N*-isopropylacrylamide)-CNT-polyaniline was prepared via coupling

chemistry as shown in Fig 1a-b: 1) HOOC-MWNTs and polyaniline using DCC/NHS; and followed by 2) grafting of amine terminated, poly(*N*-isopropylacrylamide) on the CNT-polyaniline with EDC/NHS. The amine terminated, polyaniline and poly(*N*-isopropylacrylamide) were grafted onto the HOOC-MWNTs one by one through amide bonds between the amine groups of polymers and the carboxylic acid groups of MWNTs. The three-dimensional scaffolds of poly(*N*-isopropylacrylamide)-CNT-polyaniline were fabricated by the technique (Fig 1c-d). Typically, electrospun microfabrics were extruded under an anode spinneret with the electric force to grounded collector. Uniform non-woven microfabric without beads of poly(*N*-isopropylacrylamide)-CNT-polyaniline was assembled in a three-dimensional microstructure using 3 wt% of poly(*N*-isopropylacrylamide)-CNT-polyaniline in 8: 2, v/v solvent system of 1,1,1,3,3,3-hexafluoro-2-propanol and *N,N*-dimethyl formamide at a fixed flow rate of 0.32 mL/h,. The voltage, humidity and distance from needle to collector were 15 kV, <15% and 24 cm, respectively.

The chemical structure of poly(*N*-isopropylacrylamide)-CNT-polyaniline was confirmed by ¹H NMR spectroscopy. The ¹H NMR spectrum of poly(*N*-isopropylacrylamide)-CNT-polyaniline is shown in Fig. 2A. The peaks at 1.1 ppm (a), 1.7 ppm (b), 2.1-2.2 ppm (c), 3.8-3.9 ppm (d) and 6.6-6.8 ppm (e) were due to presence of -CH₃, isopropyl group, >CH-C=O, -COOH, -CH₂- long non-aromatic polymer chain and amide protons, respectively for HCOO-MWNTs coupled with poly(*N*-isopropylacrylamide) chain by amide linkage.²⁴ The aromatic protons were observed at about 6.9 ppm whereas a triplet peak at about 7.5 ppm was observed because of ammonium proton (-N⁺-H).²⁵ ¹H NMR spectrum was confirmed the formation of poly(*N*-isopropylacrylamide)-CNT-polyaniline.

A comparative morphology of bulk and electrospun microfabrics is shown in Fig. 2B. The typical SEM pictures of poly(*N*-isopropylacrylamide)-CNT-polyaniline 1) bulk- shown miniature of fiber networks with polymeric plumps and 2) electrospun microfabrics-

observed three-dimensional assembly of nonwoven microfabrics with OD: 400-500 μm , L: 6-20 cm. The three-dimensional assembled scaffolds of poly(*N*-isopropylacrylamide)-CNT-polyaniline electrospun microfabrics could be provided an uniform interconnectivity with high surface-to-volume ratio for the cells and nutrients. It directs to a higher degree of fibroblast cells growth and viability.

Cell culture assay. The feasibility of electrospun microfabrics of the poly(*N*-isopropylacrylamide)-CNT-polyaniline for tissue engineering applications was assessed for potential cells matrices using mice L929 fibroblast cells as a model. Typically, fibroblast cells are very important and play a vital role in wound healing in the body.²⁶ In the cell culture assay, the L929 fibroblast cells were seeded on the i) surface of bulk and ii) electrospun microfabrics of poly(*N*-isopropylacrylamide)-CNT-polyaniline using Matrigel™ and native media as controls. The bulk poly(*N*-isopropylacrylamide)-CNT-polyaniline was directly placed on 24 well tissue culture plate followed by cells seeding on the surface with complete media. Further, the tissue culture plate was incubated in a humidified environment at 37 °C with 5 % CO₂ supply. In similar fashion, cells were also seeded on the three-dimensional scaffolds of electrospun microfabrics, which were directly placed in 24-well tissue culture plate. The results show that poly(*N*-isopropylacrylamide)-CNT-polyaniline (i.e., 0.1 wt%) was biocompatible and cells completely covered materials in the tissue culture plate. Cell growth on the bulk surface and electrospun microfabrics was calculated by counting the number of cells in a 0.15 mm² area. The controls were media and the commercially available gel Matrigel™ containing cells. Fig. 3A shows a bar chart of cell growth in control, Matrigel™, bulk and electrospun microfabric scaffolds with 24 h cells seeding intervals. It can be seen that the electrospun microfabric scaffolds have more than twice the amount of cell growth as compared to controls and bulk at each time point, i.e.,

ranging from 24 to 168 h. This suggests these scaffolds provide a highly compatible micro environment and/or surface for cell culture, attachment and proliferation, even comparison states have lower cells growth with or/and without poly(*N*-isopropylacrylamide)-CNT-polyaniline, 0.1 wt.%. The results support the conclusion that these three-dimensional scaffolds could be a useful material to support good cell growth for tissue engineering^{27, 28}. For any material to be used as a tissue engineering scaffold or in cell culture, a vital condition is a high order of biocompatibility, which means that materials should not poison cells or provide any obstacle to nutrient supply to the cells. The higher cell growth shown on both poly(*N*-isopropylacrylamide)-CNT-polyaniline bulk and electrospun microfabric scaffolds can be attributed due to the balanced hydrophilic functions, conductance and mechanical strength provided by the poly(*N*-isopropylacrylamide), polyaniline and MWNTs, respectively. Fig. 3B show the morphology of the cells on 168 h of cells seeding on i) control; ii) Matrigel™; and poly(*N*-isopropylacrylamide)-CNT-polyaniline iii) bulk and iv) electrospun microfabric scaffolds. The cell morphologies also support the results indicated by the histogram in Fig. 3A. The cells were healthy, had regular growth and were even effectively dividing after 168 h of culture.

Cell viability was checked at 168 h (7 days) of culture. The cells were washed off the poly(*N*-isopropylacrylamide)-CNT-polyaniline bulk and electrospun microfabric scaffolds with PBS solution, pH 7.4. The cells were then stained by calcein-AM and PI dye followed by incubation at 37 °C with 5% CO₂ supply. Fig. 4A indicates the cell viability in the: i) control, ii) Matrigel™ and surface of poly(*N*-isopropylacrylamide)-CNT-polyaniline; iii) bulk and; iv) electrospun microfabrics, viz., green colour represents living cells whereas red colour corresponds to dead cells. On the basis of living and dead cells, the percentage living was calculated. Fig 4B shows the fluorescent micrographs of microblast cells seeded on: i) control; ii) Matrigel™; iii) bulk and; iv) electrospun microfabrics scaffolds of poly(*N*-

isopropylacrylamide)-CNT-polyaniline, 0.1 % wt. for 168 h intervals and stained with Calcein-AM. The observation may be credited to factors such as biocompatibility of matrices, better accessibility of nutrients and gases, homogeneous micro environment, etc. A high degree of green fluorescence in all three cases indicates very good cell activities. Also cells were uniformly spread within the electrospun microfabric scaffold that favours again healthy cell proliferation within the three-dimensional micro environment. A major advantage of these scaffolds is that they do not require any pre-treatment such as pre-coating with adhesive proteins and/or growth factors for cell proliferation, which is usually needed with ionic scaffolds to facilitate homogeneous cells proliferation.²⁹ The fundamental mechanism of improved cell proliferation is still unclear, however the well-defined three-dimensional microstructure, non-toxic non-woven poly(*N*-isopropylacrylamide)-CNT-polyaniline electrospun microfabric scaffolds may provide fibroblast cells with better access to culture media. Although bulk poly(*N*-isopropylacrylamide)-CNT-polyaniline, 0.1 wt.% showed excellent biocompatibility, i.e., 95.38% cells living, electrospun microfabric scaffolds of poly(*N*-isopropylacrylamide)-CNT-polyaniline, 0.1 wt.% had extremely high biocompatibility, i.e., approximately 100% cells living. [In similar set of experiments using other cell types, i.e., A549, NHDF, NHLF, RLE6TN and HEK293, an excellent cell proliferation and viability was further examined on the non-woven microfabric poly\(*N*-isopropylacrylamide\)-CNT-polyaniline matrices up to 168 h \(Fig. 5\). Thus, electrospun microfabric of poly\(*N*-isopropylacrylamide\)-CNT-polyaniline could be potentially used as scaffolds in cell culture.](#)

Moreover, we have also investigated the cell detachment behaviour of the scaffolds by lowering the incubation temperature of culture plates from 37 to 20 °C, i.e., below LCST of poly(*N*-isopropylacrylamide). It was observed that at 20 °C, poly(*N*-isopropylacrylamide)-CNT-polyaniline scaffolds leached the cells, whereas controls resided the cells. It may be due

to the poly(*N*-isopropylacrylamide) chain in the nanocomposites which hydrates and hence cells detach from the surface.³⁰

Further, the results evident the potential role of temperature-responsive poly(*N*-isopropylacrylamide) in the poly(*N*-isopropylacrylamide)-CNT-polyaniline. At below and above the LCST, as the external temperature increases, i.e., above the LCST hydrogen-bonding interactions become weakened or destroyed, consequently, the hydrophobic interactions among the hydrophobic moieties, i.e., $-\text{CH}(\text{CH}_3)_2$, -B-N-Q-, etc. become strong, which induces the freeing of the entrapped water molecules from the network, while the hydrophilic and ionic moieties, i.e., $-\text{CONH}-$, $-\text{NH}^+$ and $-\text{COO}^-$ may interact with media through hydrogen bonding at below LCST together with polyaniline network upon the HCOO-MWNTs.³¹ However, when the temperature reaches or is above the LCST, the hydrophobic interactions become dominant in the poly(*N*-isopropylacrylamide)-CNT-polyaniline and accordingly cells were happily survived at 37 °C in the scaffolds. Hence, combination of temperature responsive-conducting polymers together with CNTs could be used as an excellent smart matrix with outstanding cell viability and proliferation, as studied with a range of cell lines as a model test for the prospective applications of poly(*N*-isopropylacrylamide)-CNT-polyaniline scaffolds in cell culture.

CONCLUSION

Poly(*N*-isopropylacrylamide)-CNT-polyaniline was synthesised by coupling chemistry using polyaniline, HOOC-MWNT and amine terminated poly(*N*-isopropylacrylamide) as principal components. A non-woven microfabric scaffold of poly(*N*-isopropylacrylamide)-CNT-polyaniline was fabricated by electrospinning. The resulting electrospun microfabrics were endowed with a three-dimensional assembly of non-woven fabrics with OD: 400-500 μm and L: 6-20 cm. Proliferation and viability of a range of cell types were studied to explore the

potential use of poly(*N*-isopropylacrylamide)-CNT-polyaniline scaffolds for tissue engineering applications. The cell growth was compared on both the surface of bulk and on non-woven microfabric scaffolds of poly(*N*-isopropylacrylamide)-CNT-polyaniline. It was observed that the microfabric scaffold provided an excellent surface for cell growth and proliferation as compared to bulk scaffolds and Matrigel™. These findings have led us with future prospective on the spatially ordered tissue architectures using heterotypic cell-cell interactions onto pattern depended temperature-responsive poly(*N*-isopropylacrylamide)-CNT-polyaniline electrospun microfabric surfaces for heterotypic cells co-culture. The progress of these novel approaches will open up new edges for the regenerative medicine.

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Figure Captions

Fig. 1. a) Chemical synthesis of CNT-polyaniline; b) grafting of amine terminated, poly(*N*-isopropylacrylamide) onto the CNT-polyaniline; c) setup for electrospinning and processing of microfabric scaffolds of poly(*N*-isopropylacrylamide)-CNT-polyaniline; and d) schematic illustration of microfabric scaffold.

Fig. 2. A) ^1H NMR spectrum of poly(*N*-isopropylacrylamide)-CNT-polyaniline and B) SEM images of poly(*N*-isopropylacrylamide)-CNT-polyaniline: (i) bulk and (ii) electrospun non-woven microfabrics with inset at higher resolution.

Fig. 3. (A) Cell proliferation assay, the bar chart indicates the viable cell number/ 0.15 mm^2 of control, MatrigelTM, poly(*N*-isopropylacrylamide)-CNT-polyaniline of bulk and electrospun microfabric scaffolds at various time points. Differences were significant; * $p < 0.05$ and ** $p < 0.005$ vs. 1 h, unless otherwise indicated. (B) Cell morphology of neo-fibroblast cells formed in the (i) control, (ii) MatrigelTM, and poly(*N*-isopropylacrylamide)-CNT-polyaniline, 0.1 wt% of (iii) bulk and (iv) three-dimensional electrospun non-woven microfabrics scaffolds after 168 h. Cells were stained with Calcein-AM.

Fig. 4. (A) Living/dead cell assay, the bar chart indicates the present of live cells in the control, MatrigelTM, bulk, and electrospun microfabric scaffolds. (B) Cells images after 168 h of cell culture for showing the viability in the: (i) control; (ii) MatrigelTM; (iii) bulk; and (iv) electrospun microfabric scaffolds after 168 h. Cells were stained with Calcein-AM and PI.

Fig. 5. (A) Cell proliferation assay, the bar chart indicates the viable A549, NHDF, NHLF, RLE6TN and HEK293 cell number/0.15 mm² of poly(*N*-isopropylacrylamide)-CNT-polyaniline of electrospun microfabric scaffolds at various time points. (B) Living/dead cell assay, the bar chart indicates the present of live A549, NHDF, NHLF, RLE6TN and HEK293 cells in the electrospun microfabric scaffolds.

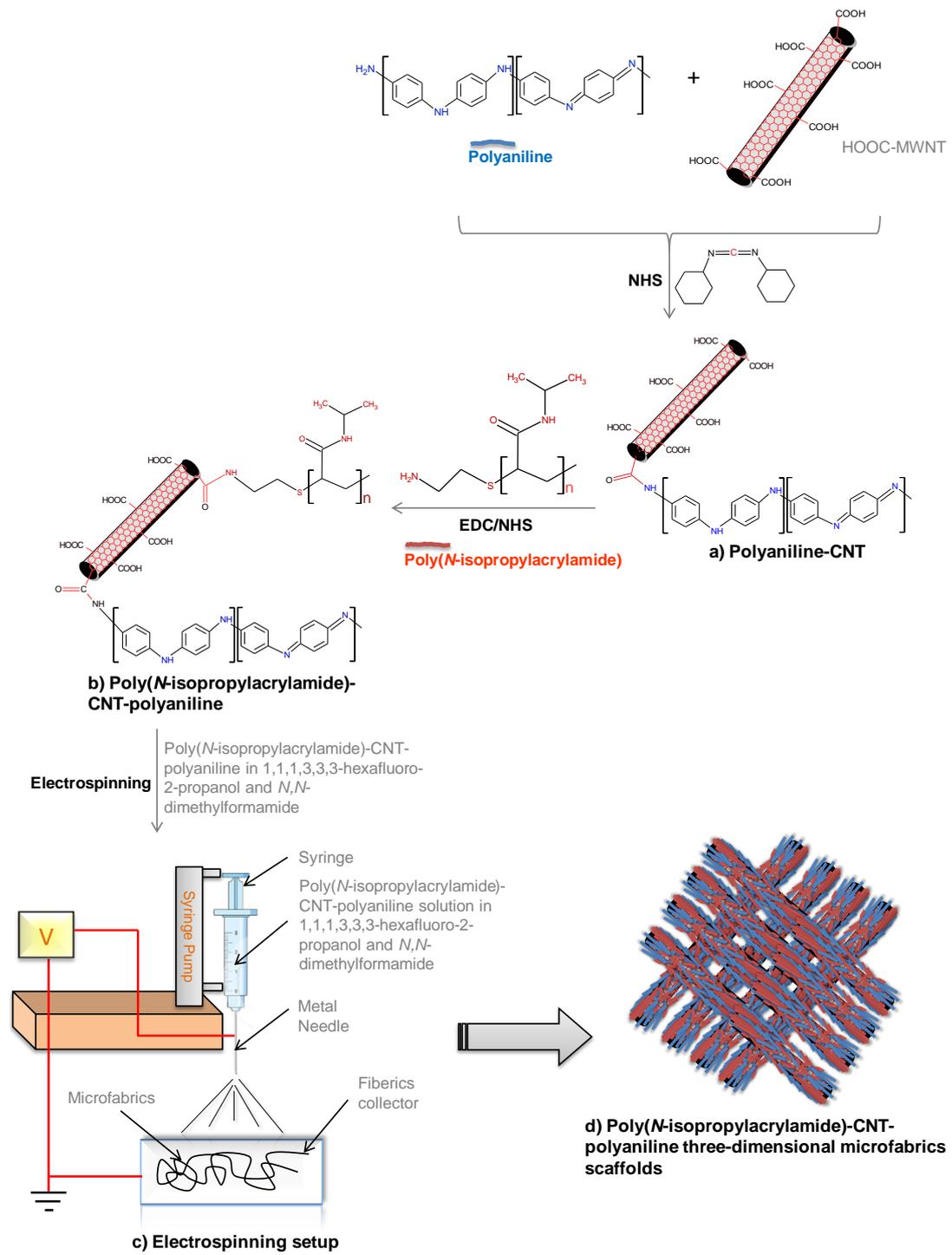


Fig. 1.

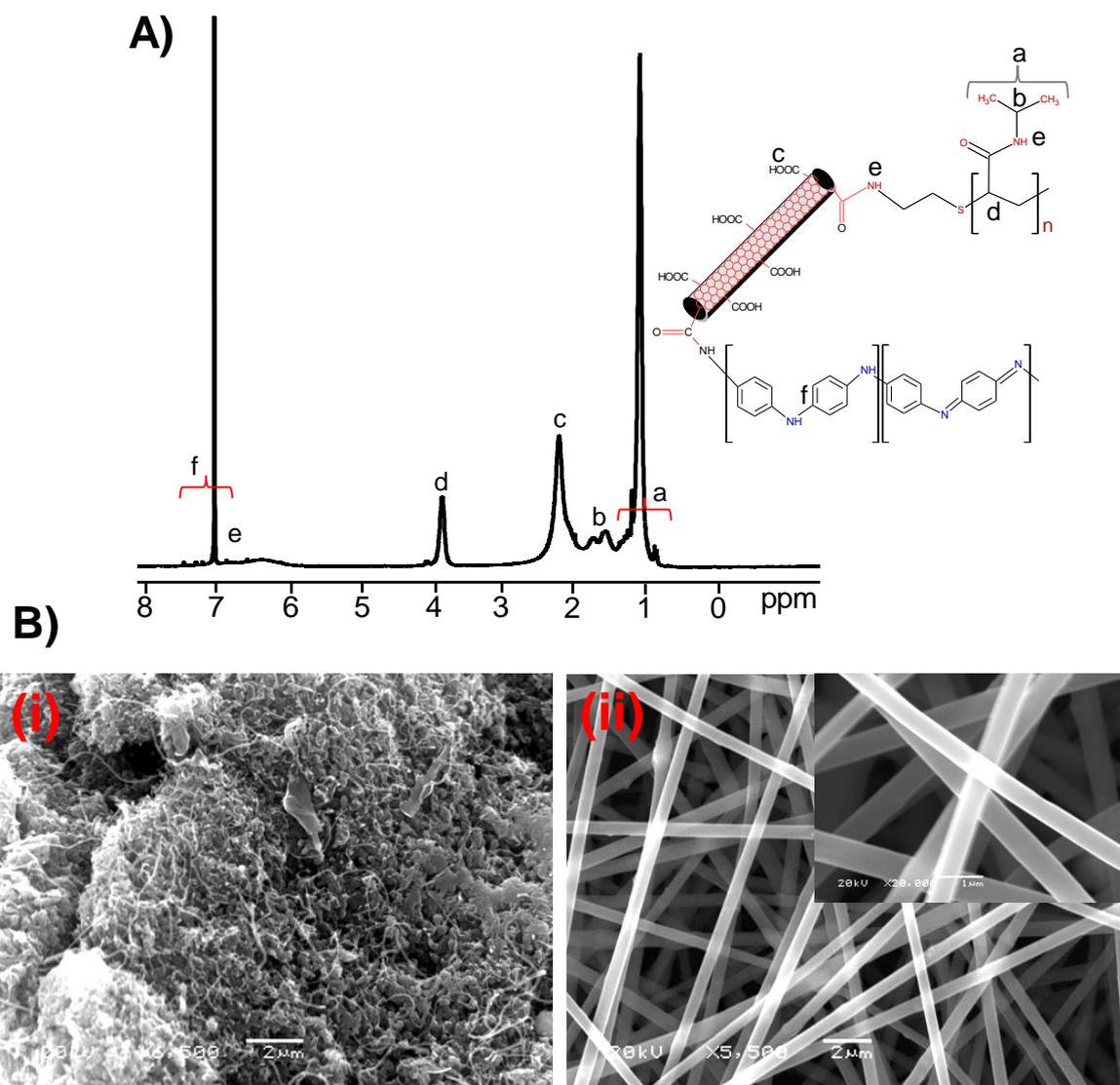


Fig. 2.

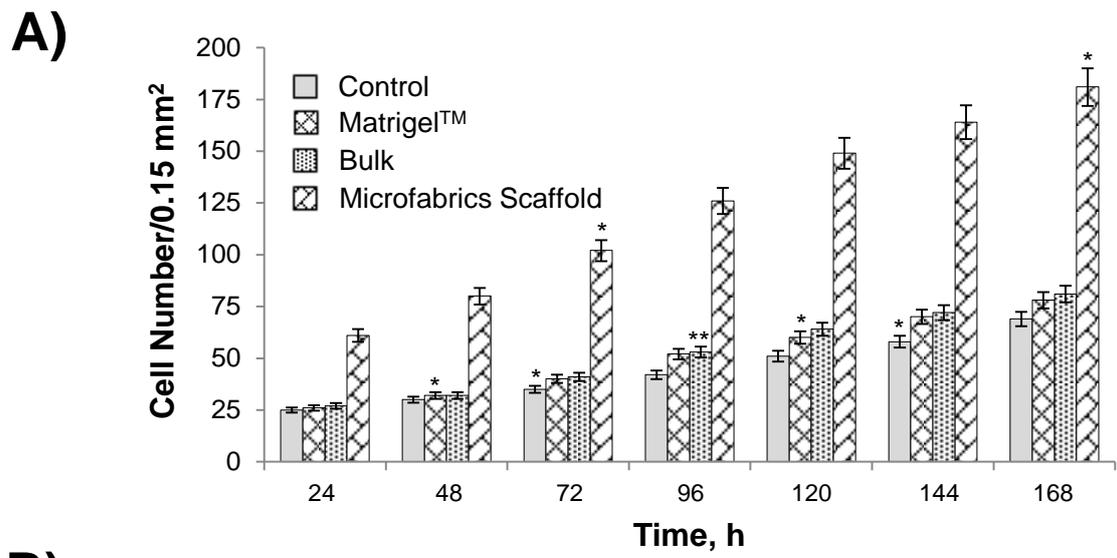


Fig. 3.

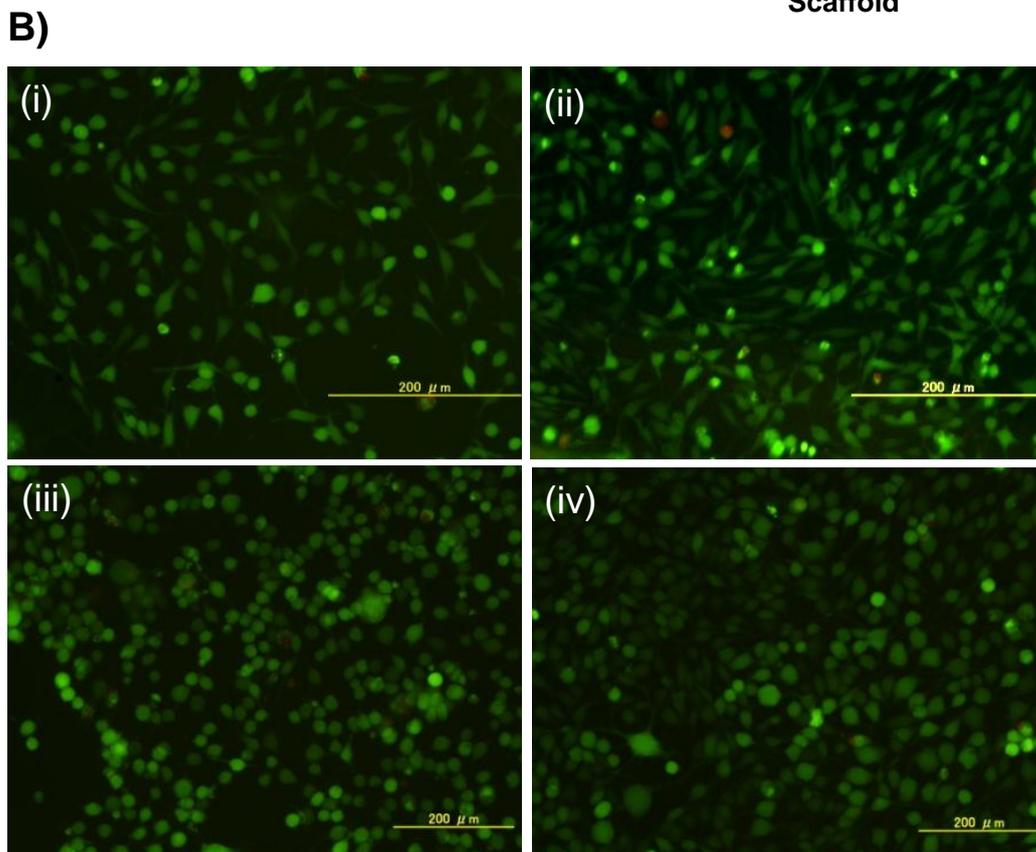
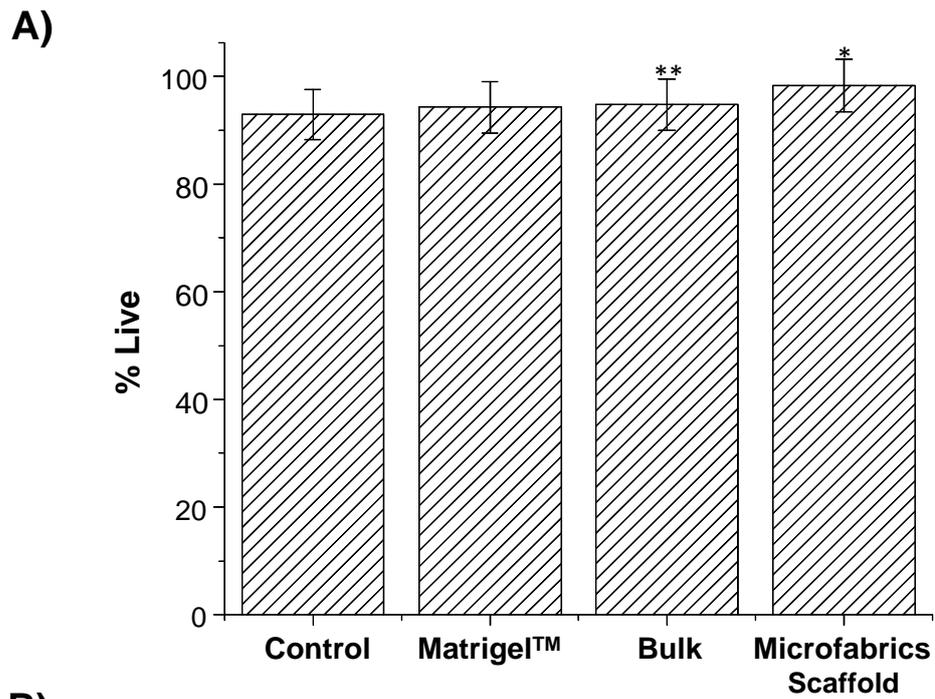


Fig. 4.

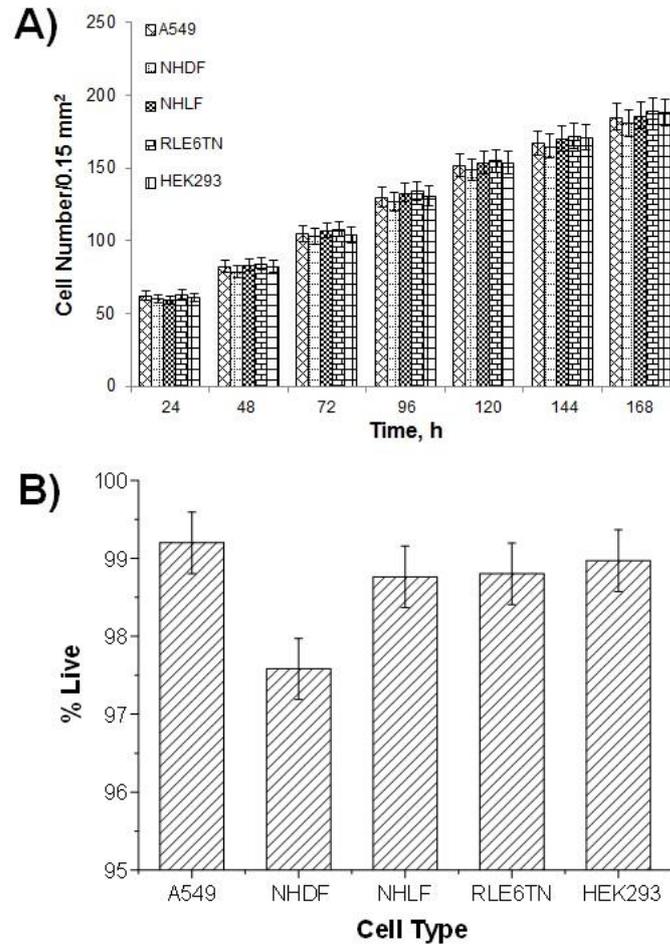


Fig. 5.