NANOFIBROUS PATCHES FOR CARDIAC DRUG DELIVERY

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Abstract

Potential application of nanofibers in drug delivery has attracted a huge attention recently highlighting their properties including increased surface reactivity, high specific surface and improved drug bioavailability. Cardiac regenerative therapy, whereby pro-regenerative cells, drugs or growth factors are administered to myocardium has demonstrated significant potential in post-operative therapy. One of the main conditions applied on drug delivery system materials are cardiac biocompatibility, tolerability, treatment efficacy.

Our objective in this study was to develop a nanofibrous patch with a short degradation time for cardiac drug delivery and evaluate its properties relevant for the application site. Silk fibroin based nanofibres were manufactured from *Bombyx mori* raw cocoons by degum procedure, ionic liquid dissolution and subsequent needle-less electrospinning. The electrospun nanofibres were crosslinked by alcohol dehydration. Stabilized and unstabilized nanofibrous patches were characterized in terms of morphology, chemical composition and degradation kinetics. The main objective was focused on cardiac cytotoxic effect of nanofibres and its evaluation on H9C2 rat myoblastic cell line.

Keywords: Cardiac drug delivery, nanofibres, silk fibroin, biocompatibility, biodegradability

1. INTRODUCTION

Cardiovascular disease, such as ischemic impairment, abnormalities in the morphogenesis, muscle function and cardiac rhythm, is an important cause of morbidity and mortality worldwide. A promising solution to heart disease and postoperative stress reduction is direct cardioprotective drugs delivery into the infarcted myocardium and cardiovascular system. The conventional application of drugs is limited by several hurdles, such as weak effectiveness, poor biodistribution and low selectivity. All of these drawbacks drive researchers forward to further develop and optimize new drug carriers. Targeted drug delivery enhances the specific deposition of drugs in the abnormal foci after administration. [1] Unlike the recent therapeutic approaches to prevent heart failure after myocardial infarction, which are based on systemic injection of proangiogenic peptides and stem cell therapy, novel therapeutic methods based nanoparticle systems show advantage of longer half-life and better stability of biomacromolecules and increased effectiveness of the treatment. [1-3]

Very potent nanomaterials for this heart-targeted drug delivery include liposomes [4], inorganic and biopolymer based nanoparticles [5] and nanofibres [6, 7] able to bind specific ligands, drugs and beta-blockers and/or serve as a potential proangiogenic protein source. Silk fibroin nanoparticles (NPs) and nanofibres (NFs) possess a very high potential in this therapeutic field as non-immunogenic protein based system. [8, 9]

2. MATERIALS AND METHODS

2.1. Materials

Thai silk cocoons of *Bombyx mori* Linn. Silkworms (Nang-Noi Srisakate 1) were obtained from Amphoe Mueang Chan, Si Sa Ket Province, Thailand. Sodium carbonate, formic acid and ethyl alcohol were purchased from Penta, Czech Republic. Myoblastic cell line (H9C2) was obtained from ATCC/LGC Standards, UK. Chemicals used for cell cultivation (DMEM, FBS), metabolic activity evaluation (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)) and staining (3,3'-dihexyloxacarbocyanine iodide (DiOC6(3)) and propidium iodide (PI)) were purchased form Sigma-Aldrich, USA.

2.2. Silk fabrication, electrospinning and stabilization

Bombyx mori raw silk cocoons were degummed with 1% sodium carbonate (100°C, 30 minutes). After the sericin removal, the silk fibre was dissolved in calcium chloride solution, dialyzed and dried. The silk fibroin (SF) spinning solution was prepared by dissolving the obtained product in 98% formic acid at final concentration of 10%. The electrospinning process was performed using wire electrode with applied voltage of 50 kV on NanoSpider NS 1WS500U device (Elmarco Ltd.). Electrospun silk fibroin nanofibres of a basis weight 9.5 g/m^2 were consequently stabilized in ethanol for 1 hour. Part of the nanofibrous sheet remained unstabilized for further analyses.

Properties of the electrospun nanofibres were evaluated in terms of morphology, chemical structure and degradation kinetics. Morphology prior and after the stabilization process was analyzed using electron microscopy (Vega3 SEM, Tescan Ltd., Czech Republic). Changes in fibre diameter were evaluated by a statistical analysis of the data (n = 100) measured for each sample. Effect of the stabilization procedure on the chemical structure was studied using FT-IR spectrometry (Nicolet iZ10, Thermo Fisher Scientific, USA). The short-term degradation kinetics of the stabilized and unstabilized SF nanofibres in simulated body fluid [10] *in vitro* were documented under high resolution using electron microscopy (UHR FE-SEM ULTRA Plus, Carl Zeiss Ltd., Germany) and complemented by weight loss measurements.

The cardio biocompatibility was evaluated according to the ISO 10993-5: 2009 on H9C2 myoblastic cell line as an appropriate model for cardiac cells. Cells were cultured under standard conditions (37°C, 5% CO2) in DMEM supplemented with 10% FBS. Biocompatibility tests in direct and indirect contact were performed. For both the tests, cells were pre-cultured in vessels prior the exposition. For the test in direct contact, each sample covered one tenth of the pre-cultured cell layer. For the test in indirect contact, the eluates of tested samples in various concentrations (0.5, 1, 2 µg / ml) were prepared for 24 hours prior the exposure to cells. The cell viability was evaluated after 24 hours of exposure via standard mitochondrial oxidoreductases metabolic activity test. Degree of reduction of MTT was determined spectrophotometrically by reading at 570nm (background subtracted at 650nm). Each test was performed in triplicate.

3. RESULTS

3.1. Structure of nanofibres

Analysis of morphology showed fibrous structure with randomly oriented nanofibrous and high porosity, which remained after the dehydration. Slight changes in the structure of the SF nanofibrous sheets after the stabilization procedure were revealed. The mean diameter decreased after the stabilization to (320 ± 87) nm from the original (346 ± 106) nm. This change could appear due to effect of the dehydration and drying leading

to closer rallying of molecular chains. Comparison of the morphologies prior and after the stabilization process is shown in **Figure 1**.

Analysis of the chemical structure performed via FTIR spectroscopy showed absorption peaks at approximately 1625 cm^{-1} (amide I) and 1530 cm^{-1} (amide II), which are typical for silk fibroin structural vibrations and are related to its ß-sheet structure. [11] Moreover, peak related to the -OH group vibrations appeared at 3200 cm^{-1}. Intensity of this peak decreased after the stabilization process leading to partial elimination of hydroxyl groups.

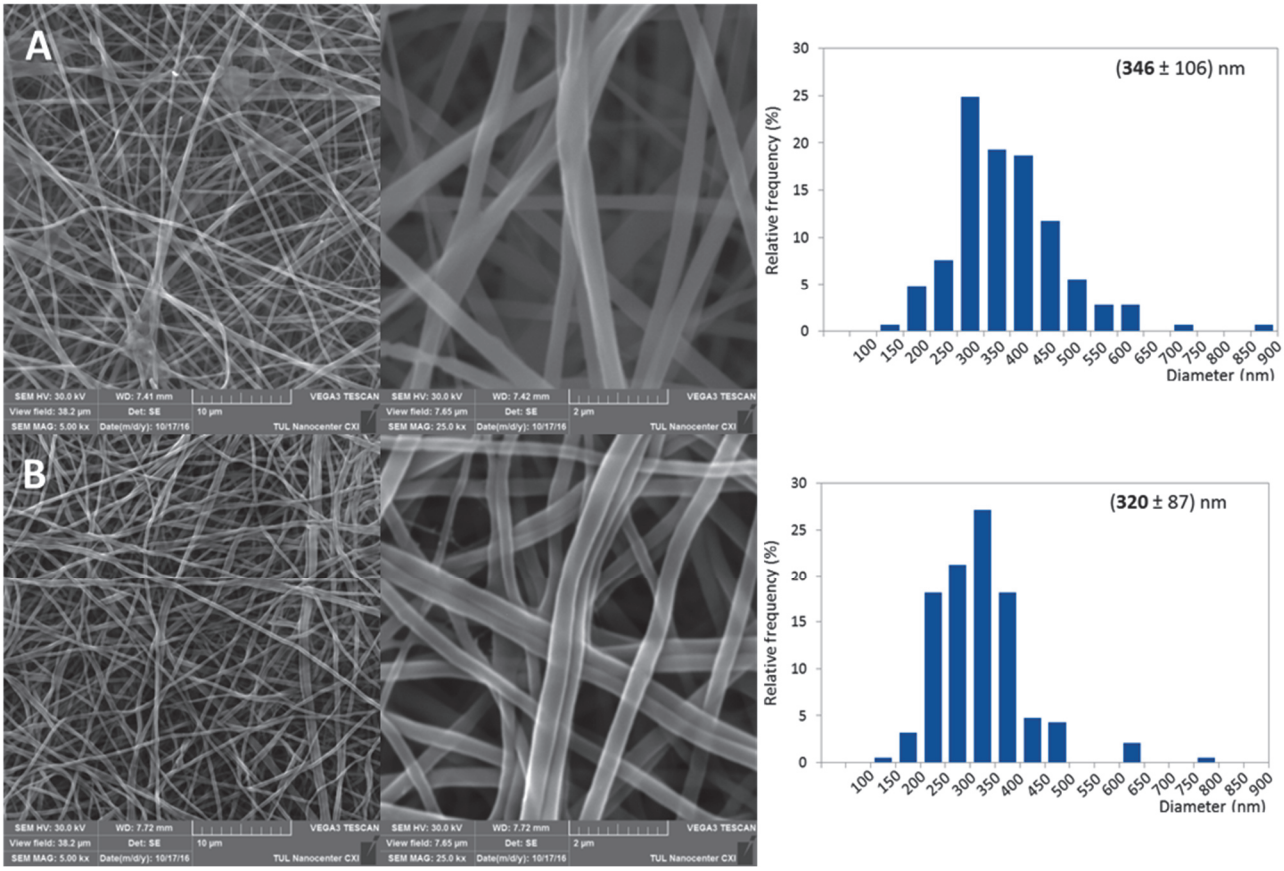

Figure 1 Morphology of SF nanofibres (A) prior the stabilization and (B) after the stabilization. SEM under magnification 5.000 and 25.000 (bar1 = 10 µm, bar2 = 2 µm), histogram of relative frequency of the fiber diameters, mean ± S.D. (n=100)

3.2. Degradation characterization

The *in vitro* degradation analysis under static conditions showed intense differences between unstabilized and stabilized silk fibroin nanofibres. Swelling of the nanofibres was observed in both the cases, but it was confirmed that the performed dehydration stabilization led to decrease of swelling and kinetics of the degradation process was confirmed to be slowed down. Whereas, the unstabilized nanofibres showed weight loss of 92% of the original mass and complete loss of the original porosity and specific surface area, the stabilized nanofibres only increased their diameter slightly due to swelling, but their mass remained almost unchanged (- 3.2 %). The weight loss was recorded after 72 hours of the degradation process. Those results were supported by the SEM observation shown in **Figure 2**.

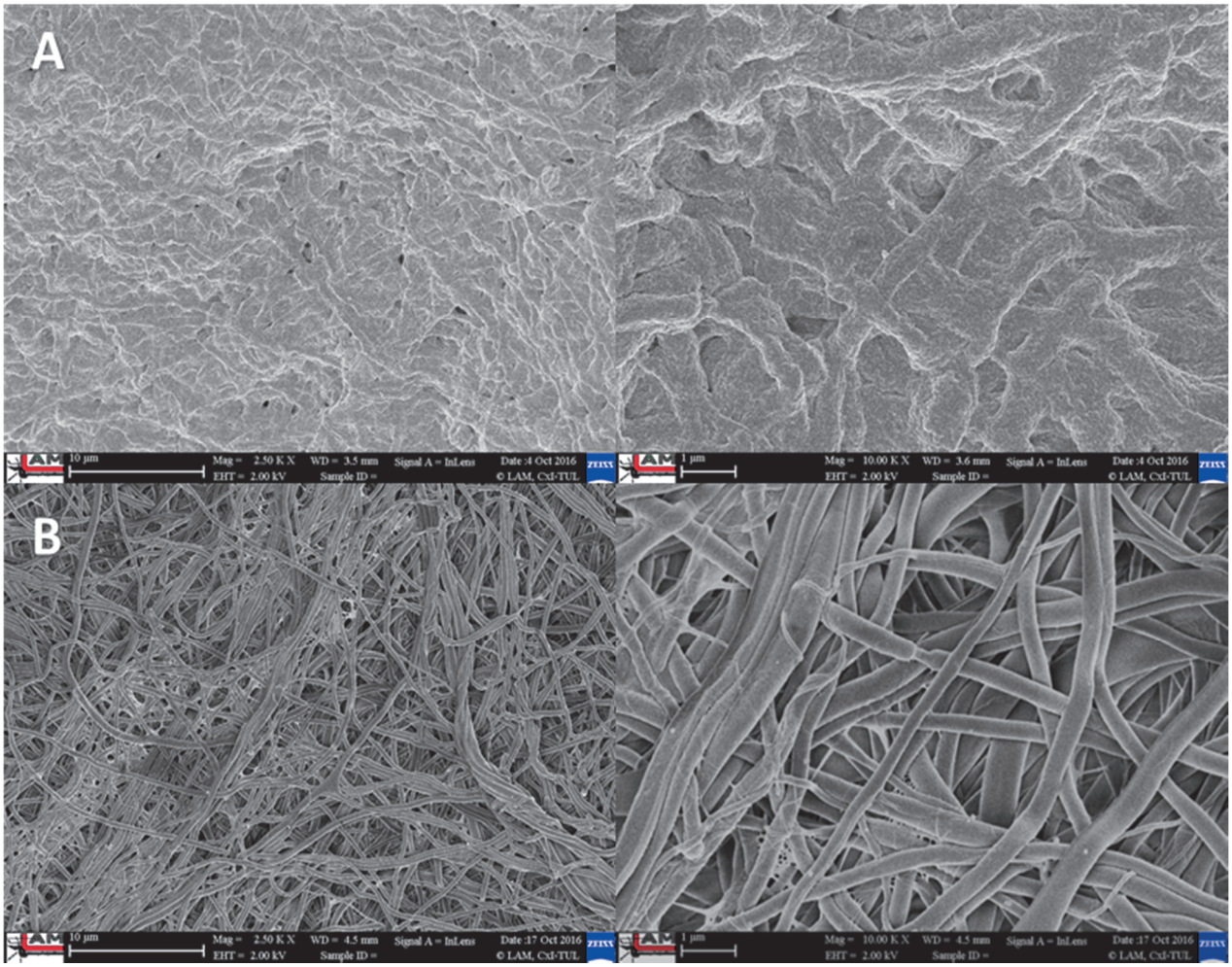

Figure 2 Morphological changes observed after 72 hours of degradation in vitro in SBF. (A) Unstabilized and (B) stabilized Silk fibroin nanofibres. SEM under mag. 2.500 and 10.000 (bar1 = 10 μm, bar2 = 1 μm)

3.3. Biocompatibility evaluation

Biocompatibility of the stabilized and unstabilized SF nanofibres was tested on H9C2 cell line as accurate cardiac tissue model. According to results (shown in **Figure 3**), both types of the samples reached viability of the exposed cells higher than 80 % compared to the cell control (CC) as a blank and can be claimed and cytocompatible to cardiac muscle tissue. In both the tests performed, tested samples were compared to approved biocompatible negative control (NC, RM-C foil by Hatano Research Institute, Japan) and approved cytotoxic positive control (PC, PM-A foil by Hatano Research Institute, Japan) to verify cell sensitivity. In the case of the test in direct contact, for the unstabilized nanofibres the cell viability reached (104.6 ± 3.15) % of CC and (91.2 ± 4.9) % of CC for the stabilized nanofibres (**Figure 3d**).

For the test in indirect contact, viability of cells exposed to the unstabilized SF ranged from (100 ± 3.4) % up to (102.7 ± 2.7) % and increased with the SF concentration. Viability of cells exposed to stabilized SF ranged from (96.2 ± 4.1) % up to (98.6 ± 2.2) % and showed the same trend. Graphical representation of results is in **Figure 3e**. The significant increase of cell viability after interaction with unstabilized silk nanofibres can be attributed to their quick degradation leading to protein release resulting in cell proliferation and increased metabolic activity. Microscopic analysis of H9C2 showed no significant change in cell morphology in terms of shape and size.

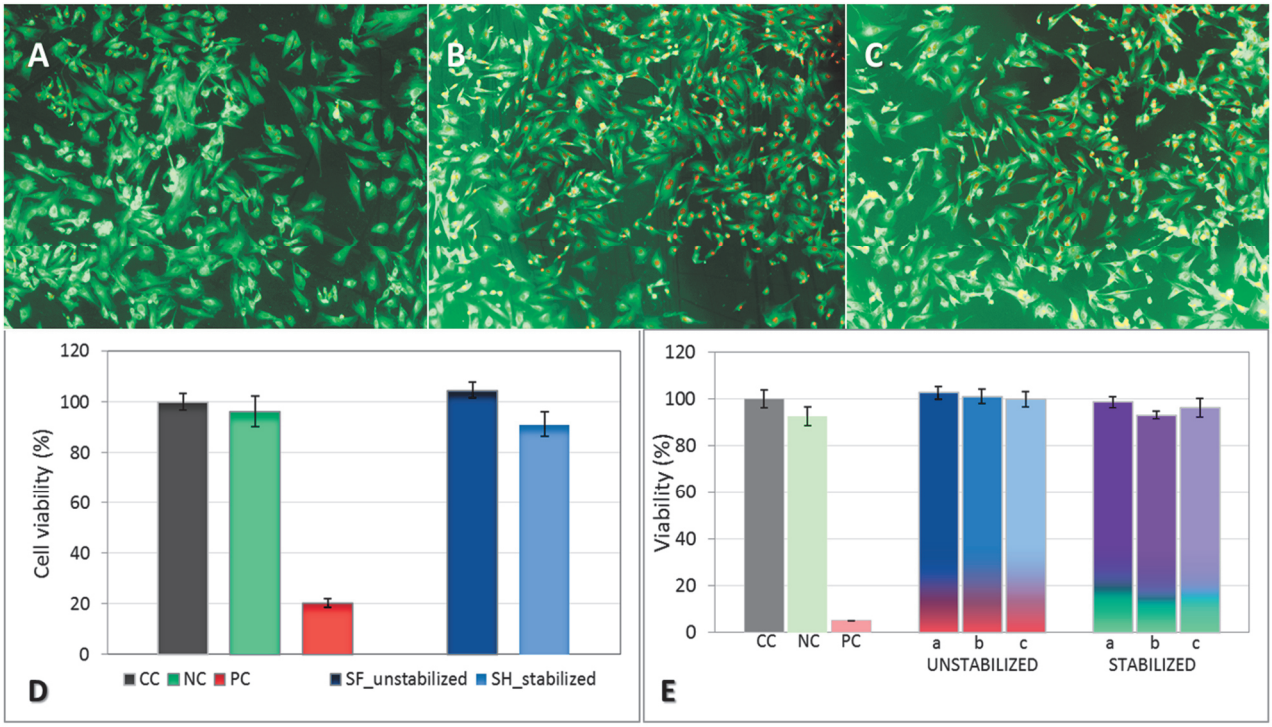

Figure 3 Results of the biocompatibility evaluation. Fluorescent microscopy of the H9C2 cells after exposure to the (A) pure complete medium, (B) unstabilized SF nanofibres eluate and (C) stabilized SF nanofibres eluate. Results of biocompatibility test (D) in direct contact and (E) indirect contact performed in complete culture medium. Concentrations of SF for (E) are a: 2 μg / ml, b: 1 μg / ml and c: 0.5 μg / ml. Abbreviations: (CC) cell control, (NC) negative control, (PC) positive control. For A - C magnification is 100x

4. CONCLUSION

Bombyx mori originated silk fibroin nanofibres were confirmed to have a high potential in heart-targeted drug delivery and the stress therapy as biocompatible matrix for drug releasing system construction. The degradation test *in vitro* showed an important effect of the stabilization procedure on the degradation kinetics and the protein mass release. The degradation period can be modified by the stabilization procedure used and affect the drug releasing period itself. The biocompatibility testing performed on H9C2 myoblast cell line used as a cell model confirmed the stabilized and unstabilized silk fibroin nanofibres as cardiac tissue compatible. No tested silk fibroin NFs concentration resulted in cell viability decrease below the 80% which the ISO EN recognized mandatory biocompatibility limit.

Based on results of the study presented, we believe silk fibroin nanofibres are an interesting candidate for a short-term post-operative drug delivery.

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