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Letter Article

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Author(s): Yuji Tanikawa, Akiko Obata, Kenji Nagata, Toshihiro Kasuga and Toshihisa Mizuno*

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Abstract

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Objective: In this study, we characterized the effectiveness of core-shell fibermats that possess cross-linked gelatin (CLG) as the shell layer of constituent nanofibers, as a protein-releasing cell-incubation scaffold.

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Results: Although the single-layered poly(AM/DAAM)/ADH nanofiber fibermats did not adhere to the mammalian cells, the core-shell fibermat with the CLG shell layer exhibited good adherence and subsequent proliferation. A sustained release of the preloaded FITC-Lys over 24 days without any burst release was observed, and the cumulative amount of released protein reached over 65% after 24 days. Upon loading bFGF in our fibermats, we succeeded in promoting cell proliferation, and highlighting its potential for use in therapeutic applications.

Conclusion: We successfully confirmed that core-shell fibermats with a CLG shell layer around the constituent nanofibers, were effective as protein-releasing cell-incubation scaffolds.

Keywords: Fibermat, co-axial electrospinning, protein-encapsulation, gelatin, scaffold, growth factor, CLG shell layer.

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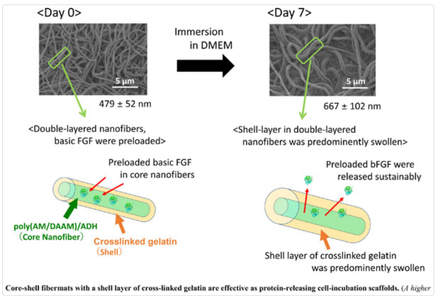
Graphical Abstract



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LETTER

Scaffolds Designing from Protein-loadable Coaxial Electrospun Fibermats of poly(acrylamide)-*Co*-poly(diacetone acrylamide) and Gelatin

Yuji Tanikawa[1], Akiko Obata[1], Kenji Nagata[1], Toshihiro Kasuga[1] and Toshihisa Mizuno[1,2,*]

[1]*Department of Life Science and Applied Chemistry, Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-Cho, Showa-Ku, Nagoya, Aichi 466-8555, Japan;* [2] *Department of Nanopharmaceutical Sciences, Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-Cho Showa-Ku, Nagoya, Aichi 466-8555, Japan*

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Keywords: Fibermat, co-axial electrospinning, protein-encapsulation, gelatin, scaffold, growth factor, CLG shell layer.

1. INTRODUCTION

Often, treatment with several cytokines is necessary to promote the proliferation and differentiation of some cell lines and stem cells, and the maturation of neuronal cell lines [1]. During the development of fertilized eggs, differences in the local concentration of activin A are known to determine the fate of cell differentiation into different germ layers [2]. For *in vitro* differentiation or maturation of such cells on cell-culture dishes or 3D scaffolds, sequential treatment with several cytokines (optimized concentration, duration, and order) through a simple exchange of culture media is thought to recapitulate the events occurring *in vivo* [3]. Through co-culture with endothelial cells or co-differentiation with mesodermal progenitors in the presence of several cytokines and growth factors, the preparation of various types of human organoids has been successfully achieved [4]. In contrast, for a similar application to somatic cells in the body for *in situ* regenerative therapy, installing scaffolds is believed to be effective [5], as it enables the recruitment of somatic stem cells and releases cytokines at the target site. In this context, the design of drug-releasing scaffolds has been extensively studied by many researchers, using various materials made of organic [6], inorganic [7], hybrid polymers [8], inorganic ceramics [9], metals [10], *etc*. Effective

* Address correspondence to this author at the Department of Life Science and Applied Chemistry, Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-Cho, Showa-Ku, Nagoya, Aichi466-8555, Japan; Tel/Fax: +81-52-735-5237; E-mail: toshitcm@nitech.ac.jp

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low molecular weight (MW) drugs that can promote cell proliferation [11], differentiation [12], and direct re-programming in mature somatic cells [13] have recently gained considerable attention; however, proteinaceous cytokines such as growth factors [14], bone morphogenetic proteins [15], and tumor growth factor-β [16] are still crucial for this purpose. Therefore, novel protein-releasing materials that provide a controlled, but sustained release are urgently required and therefore, require intensive research.

Electrospun fibermats are assemblies of nano- or microfibers, and because of their similarity to the fiber-assembled structure of extracellular matrixes, fibermat-based cell-incubation scaffolds have been extensively studied [17]. For use as a medical implant scaffold in regenerative therapy, an effective design of the protein-releasing fibermats is expected. To meet the requirement of insolubility in culture medium for a certain period, water-insoluble hydrophobic polymers such as poly (lactic acid) (PLA), poly (ε-caprolactone) (PCL), and polyurethane (PU) are generally chosen as the base materials of fibermats [18-20]. To endow protein-releasing properties, two methods have been frequently examined. One involves the adsorption of proteinaceous humoral factors onto the surface of fibermats *via* non-covalent bonding [21]. To retain biological activity, immobilization *via* non--covalent bonding such as electrostatic interactions is effective. However, due to low binding, most adsorbed proteins might undergo burst release; therefore, this method is practical, but intrinsically not suitable for controlled release. Another method is the use of fibermats prepared using the emulsion method [22]. These fibermats consist of microfibers of hydrophobic polymers such as poly (lactic acid-*co*-glycolic acid) (PLGA), where protein molecules are dispersed within the microfibers as an emulsion of protein molecules in an aqueous buffer with their stabilizers (polysaccharides, *etc*.). Since protein molecules are held in the hydrophobic microfibers, in order to achieve sufficient release, partial or substantial decomposition or solubilization of the hydrophobic polymers is necessary [23]. However, this method includes the possibility of losing the essential function of cell adhesion because of the destruction of fibermats.

Recently, we studied a method to construct protein-loadable fibermats, in which protein molecules were encapsulated within nanofibers [24-26]. Based on the *in-situ* crosslinking during electrospinning (SCES) method using post-crosslinkable hydrophilic polymers and the corresponding crosslinkers [25], we successfully constructed protein-encapsulated fibermats without denaturing the proteins. Owing to the hydrophilic nature of the nanofibers used in this method, the fiber-stacked nanostructures lacked mechanical strength and stability for practical applications. However, by wrapping the nanofibers with other hydrophobic polymers, *i.e*., by modifying the core-shell fibermats, we could overcome these drawbacks [26]. Because of its low hydration, using PCL as the shell layer allows enzymes to be held within the core nanofibers without leakage. However, other materials for the shell layer can be chosen and the protein-releasing fibermats could be designed based on the core-shell fibermat platform. In this study, we used cross-linked gelatin (CLG) [27] as a new shell material for core-shell fibermats and characterized their efficacy as protein-releasing scaffolds. For the base material of the core nanofibers, we used the cross-linked copolymer of poly(acrylamide)-*co*-poly(poly(diacetone acrylamide) (poly(AM/DAAM)) and adipic acid dihydrazide (ADH), poly(AM/DAAM)/ADH Fig. (**1**). In our previous study, we demonstrated the effectiveness of this material in protein-loadable fibermats [25, 26]. The nanostructure, protein-releasing properties, and suitability as cell-incubation scaffolds of the core-shell fibermats, consisting of poly(AM/DAAM)/ADH and CLG, were studied using microscopic measurements and cell-based assays.

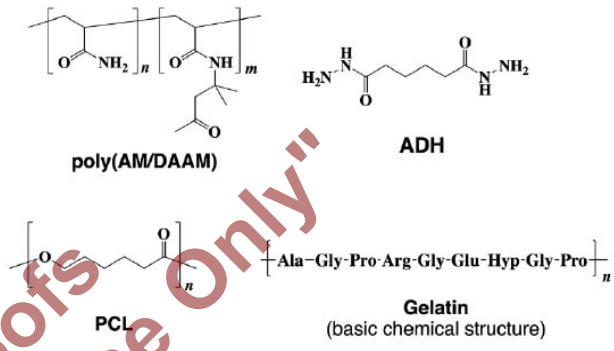

Fig. (1). Chemical structures of poly (AM/DAAM), ADH, PCL, and gelatin.

2. MATERIALS AND METHODS

2.1. Materials

Unless stated otherwise, all chemicals and reagents were commercially obtained and used without further purification. Acrylamide (AM), 2,2,2-trifluoroethanol (TFE), 2,2'-azobis[N-(2-carboxyethyl)-2-methylpropionamidine] tetrahydrate (VA-057), gelatin, and lysozyme from egg white were purchased from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). Diacetone acrylamide (DAAM) and adipic acid dihydrazide (ADH) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Poly(AM/DAAM) featuring AM and DAAM at an 8:2 molar ratio was synthesized as previously described [25]. The M_n and polydispersity index (PDI) of poly(AM/DAAM), determined using gel-permeation chromatography (GPC), were 57000 g/mol and 1.18, respectively. FITC-Lys was synthesized as described in a previous study [28]. NIH3T3 cells were purchased from the JCRB Cell Bank (Japan).

2.2. Construction of Core-Shell Fibermats with PCL or Gelatin Shells and Post-Crosslinking with EDC.

According to the similar protocol in the previous study [26], we prepared the core-shell fibermats, consisting of the core nanofiber of poly(AM/DAAM)/ADH and the shell unit of PCL or CLG. The solution of poly(AM/DAAM) (0.5 g in 2.5 mL of 100 mM phosphate buffer, pH 8) and ADH (0.5

molar equivalent with respect to DAAM in poly(AM/-DAAM)) was prepared for the precursor solution of the core nanofiber. When encapsulating FITC-Lys, 1 wt% with respect to the mass of the polymer was added to the solution. To encapsulate bFGF, 25 μg bFGF was added to the solution. Concomitantly, 8 wt% PCL or gelatin solution in TFE was prepared and used for the precursor solution of the shell unit of core-shell nanofibers. With linear extrusion velocity of 0.2 mL/h for the poly(AM/DAAM)/ADH solution and 0.8 mL/h for the PCL or gelatin solution under high voltage (18 kV), core-shell fibermats were electrospun (SD-02, MECC Co. Ltd, Japan). For crosslinking of the core-shell fibermat with a gelatin shell, the fibermats were cut into a round shape (φ 6.4 mm) and placed at the bottom of a 96-well plate. These round fibermats were immersed in 100 μL of EDC solution (200 mM) in EtOH and incubated for 24 h at 25°C. After several washes in PBS (1 mL × 5), the fibermats were used for experimentation. Fibermat preparation and crosslinking processes were both performed at room temperature. UV sterilization was applied before cell experiments.

2.3. Scanning Electron Microscopy (SEM).

SEM observation of fibermats was performed according to the previous procedure [25, 26]. Samples were coated with amorphous osmium through plasma chemical vapor deposition by using a JEE-420T vacuum evaporator (JEOL, Japan). The mean diameters of the fibermat nanofibers and their standard deviations were evaluated from the SEM images of 30 nanofibers, using the software, Image J. In order to check reproducibility of fiber diameter and fiber-stacked nanostructure of each fibermat, we also did SEM observations of the other batch samples.

2.4. Transmission Electron Microscopy (TEM).

TEM observation of fibermats was performed according to the previous procedure [25, 26]. Samples for TEM observation were prepared by directly collecting the spun core-shell nanofibers from the coaxial spinneret onto a TEM grid (Formvar Carbon Film on Copper 100 mesh (50), Okenshoji Co., Ltd, Japan). To obtain adequate image contrast between core and shell portions, sodium phosphotungstate was mixed into the core precursor solution of poly(AM/DAAM) and ADH at a final concentration of 0.001% (w/v). The mean core diameters and shell thicknesses of the fibermat nanofibers and their standard deviations were evaluated from the TEM images of 30 nanofibers, using the software, Image J. In order to check reproducibility of the mean core diameters and shell thicknesses; we also did TEM observations for the other batch samples.

2.5. Attenuated Total-Reflectance Fourier-Transform Infrared (ATR-FTIR) Spectroscopy.

ATR-FTIR spectra were acquired as previously [25, 26]. ATR-FTIR spectra were acquired using an FT-IR-4000 spectrometer (JASCO, Japan) equipped with an ATR PRO450-S unit (JASCO, Japan). The FID spectra scanned 200 times at ambient temperature were accumulated and Fourier-transformed to obtain FTIR spectra at 4 cm^{-1} resolution. In order to check the reproducibility of ATR-FTIR spectra of the fibermats, we also did ATR-FTIR observations for the other batch samples.

2.6. Release Behavior of Preloaded FITC-Lys from the Core-Shell Fibermats.

As in the case of the core-shell fibermats with the CLG shells, the FITC-Lys loaded fibermats (4 mg) with gelatin shells were immersed in 1 mL EDC solution (200 mM) in EtOH and incubated for 24 h at room temperature to crosslink the gelatin shell. After washing in PBS (1 mL × 5), these fibermats were immersed in DMEM (1.5 mL) and the amount of FITC-Lys released for 1–24 days was quantified from the fluorescence intensities at 521 nm (excitation wavelength, 495 nm) of the DMEM medium using a fluorescence microplate reader. For the core-shell fibermats with a PCL shell, the FITC-Lys loaded fibermats with PCL shell were immersed in DMEM (1.5 mL) and the amount of FITC-Lys released for 1–24 days was quantified from the fluorescence intensities at 521 nm (excitation wavelength, 495 nm) of the DMEM medium using a fluorescence microplate reader. At all-time points, when observing the fluorescence intensity of DMEM, the media was replaced with fresh DMEM (1.5 mL).

2.7. Cell-Incubation in the Fibermats with or without Preloaded bFGF.

NIH3T3 cells were routinely cultured in DMEM (Wako Pure Chemical Ind. Ltd., Japan) supplemented with 10% FBS (Wako Pure Chemical Ind. Ltd., Japan) and 1% antibiotic solution (including 10,000 units penicillin and 10 mg streptomycin, Wako Pure Chemical Ind. Ltd., Japan) at 37°C in a humidified atmosphere containing 5% CO_2. Cells (3×10^4 cells) were seeded on the fibermats with or without preloaded bFGF, which were cut into a round shape (φ 6.4 mm) and placed at the bottom of a 96-well plate, and incubated for 1, 3, 5, and 7 days. Alteration of cell numbers growing on the fibermats was measured using a Cell Counting Kit-8 (CCK-8, Dojindo, and Kumamoto, Japan) according to the manufacturer's instructions. To avoid miscounting the cells in the fibermats, each fibermat was moved to a new 96-well plate before the addition of the CCK-8 solution.

2.8. Confocal Laser Scanning Microscopy (CLSM) of Cell Morphologies.

The morphology of NIH3T3 cells, incubated on the fibermats after 1 or 3 days, were imaged using a confocal laser scanning microscope (LSM880 microscope (Zeiss, German). The sample at each time point (1 or 3 days) was fixed with 4% paraformaldehyde, treated with 0.1% Triton solution and then,1% BSA solution. After staining the actin filaments and nuclei with Phalloidin-iFluor 488 Conjugate (Cayman Chemical Co. Ltd., USA) and DAPI, included in Vectashield (Vector Laboratories, USA), respectively, each fibermat sample was sandwiched between two cover slips (25 × 60 mm) and imaged under a CLSM.

2.9. Statistical Analysis

All biological experiment data were presented as the mean standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significance as a post hoc test ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Preparation and Characterization of Core-Shell Fibermats Containing a Shell Layer of Crosslinked Gelatin

In our previous study, based on the SCEP method, we successfully constructed protein-loadable fibermats with single-layered nanofibers, using the crosslinked copolymers poly(AM/DAAM) and ADH as a base material [25]. Further, to support the weak structure of the poly(AM/DAAM)/ADH fibermat in terms of mechanical strength and long-term stability of the fiber-stacked nanostructure in an aqueous environment, we modified it by wrapping the poly(AM/DAAM)/ADH nanofibers with a hydrophobic PCL shell [26]. PCL is a cell-adhesive polymer, and thus, the core-shell fibermats with a PCL shell layer could be used as a protein-releasing cell-incubation scaffold. However, due to the low hydration of PCL, sufficient protein release was not expected. Thus, we prepared a core-shell fibermat with a crosslinked gelatin (CLG) shell layer and characterized its protein-releasing ability, capability for cell adhesion, and inducing proliferation. Gelatin is a well-known cell adhesive material, but crosslinking is necessary to make it insoluble in an aqueous environment [27]. Therefore, after co-axial electrospinning to prepare the core-shell fibermat with a gelatin shell, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) was applied to the core-shell fibermats (details in *Experimental Section*). As a reference, we also prepared core-shell fibermats with PCL shells.

Scanning electron microscopic measurements of the poly(AM/DAAM)/ADH-gelatin fibermat, confirmed the formation of nanostructures composed of stacked homogeneous nanofibers with an average diameter of 484 ± 70 nm, similar to that of poly(AM/DAAM)/ADH-PCL (666 ± 80 nm) Fig. (**2**). Furthermore, the existence of double-layered structures, consisting of core poly(AM/DAAM)/ADH nanofibers and the gelatin shell was successfully confirmed using transmission electron microscopy (TEM) measurements. The average thickness of the gelatin layer was estimated to be 76 ± 20 nm. To make the gelatin shells water-insoluble, post-crosslinking treatment with EDC was carried out. The fiber-

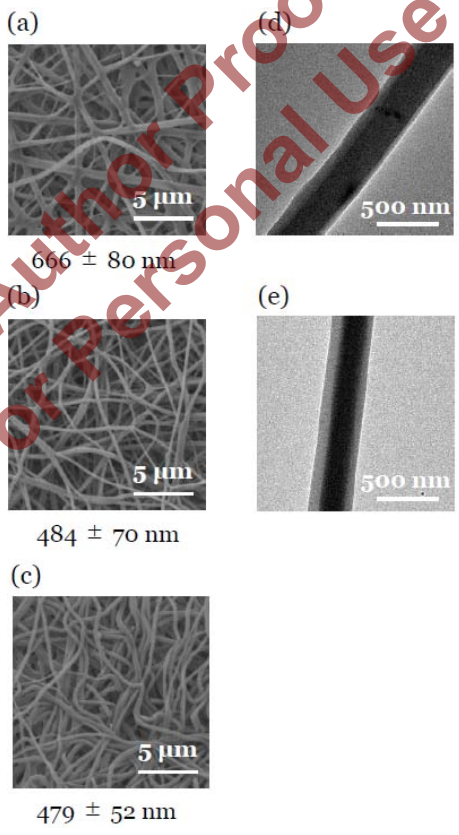

Fig. (2). Scanning electron microscopic (SEM, X5000 magnification) images of the core-shell fibermat with PCL shell (**a**), those with gelatin shell before (**b**) and after (**c**) crosslinking with EDC, and transmission electron microscopic (TEM, X4000 magnification) images of the core-shell fibermat with PCL shell (**d**) or gelatin shell (**e**). For TEM, the poly(AM/DAAM) core nanofibers were stained with phosphotungstate (see methods).

stacked nanostructure and their average diameter (479 ± 52 nm) were maintained following crosslinking. To the best of our knowledge, this is the first study to successfully construct the core-shell fibermats with the CLG shell layer as there are no previous reports on this topic. Using attenuated total reflection-infrared (ATR-IR) measurements, we investigated the coexistence of the poly(AM/DAAM)/ADH core nanofiber and the CLG shell. The ATR-IR spectra of the core-shell fibermats and the references (gelatin and poly(AM/DAAM)/ADH fibermats) are summarized in Fig. (**3**). Considering the characteristic IR band of poly(AM/-DAAM)/ADH fibermats (1540, 1615, and 1650 cm^{-1}) [26], additional IR bands at 1525 and 1630 cm^{-1}, typical of the amide groups in gelatin [29], were observed. This indicated the coexistence of poly(AM/DAAM)/ADH and gelatin; in other words, the core-shell fibermats were successfully constructed.

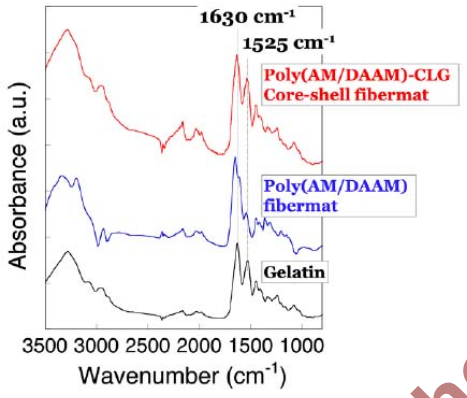

Fig. (3). Attenuated total reflection-infrared (ATR-IR) spectra of the poly(AM/DAAM)/ADH-CLG core-shell fibermat (red line), gelatin (black line), and the poly(AM/DAAM) fibermat (blue line). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.2. Cell Adhesion and Protein Release Properties of the Core-Shell Fibermats.

Cell adherence is an important factor that should be considered when designing scaffolds for adherent mammalian cells. When designing artificial scaffolds that are applicable to regeneration therapy, such as *in vitro* construction of artificial tissues and organs, or implant therapy in which the introduced scaffolds can recruit somatic stem cells and promote tissue regeneration, the choice of cell adhesive materials is indispensable. In general, hydrogels made from synthetic polymers such as polyacrylamide and polyethylene glycols are not likely to adhere to mammalian cells [30]. However, those made from proteins or peptides exhibit exceptionally good adherence. Although the core nanofibers of poly(AM/-DAAM)/ADH were expected to have low cell adhesion, we hypothesized that the addition of a proteinous CLG shell would enhance adherence to mammalian cells. To verify this, we tested cell adhesion and proliferation using NIH3T3 cells as a model. Each fibermat, cut into a round shape (φ 6.4 mm), was placed in the bottom of a 96-well culture dish and then, NIH3T3 cells were seeded. Based on an MTT assay using the water-soluble WST, we estimated the number of live cells on the fibermats at each time point (1, 3, 5, and 7 days) (Fig. **4**). In the case of the poly(AM/DAAM)/ADH fibermat, with only core nanofibers of poly(AM/-DAAM)/ADH, cell numbers increased slightly but saturated within 5 days. However, for the core-shell fibermats with CLG shell, the increase in cell numbers was maintained for 7 days. Using a confocal laser-scanning microscope, we confirmed extended pseudopodia and elongated morphology using the core-shell fibermats with CLG shell (Fig. **5**). The NIH3T3 cells plated on the single-layered poly(AM/-DAAM)/ADH fibermat were round and pseudopodia development was limited even after 3-days of incubation. These results indicate that by modifying the core-shell fibermat with the CLG shell, cell adhesion and proliferation were successfully improved.

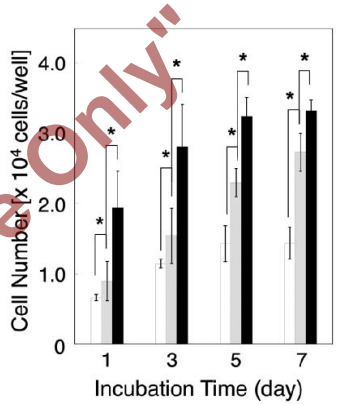

Fig. (4). Cell proliferation of NIH3T3 cells seeded on poly(AM/-DAAM)/ADH fibermats (white bar), core-shell fibermats with PCL shell (zebra-patterned bar) and CLG shell (black bar). 3.0 X 10^5 cells were seeded on each fibermats, set at the well bottom of 96 well plate; *significance difference between pairs of substrates shown ($p < 0.05$). (Mean ± SD; $n = 4$).

We next characterized the protein-releasing properties of the core-shell fibermat with the CLG shell. Protein molecules were preloaded in the core nanofibers inside the core-shell fibermats. In the next section, we evaluate the impact of the pre-encapsulated basic fibroblast growth factor (bFGF) on cell proliferation. Therefore, a FITC-labeled lysozyme (FITC-Lys), in which Lys has a similar cationic property (pI ~ 11.3) [31] to bFGF, was chosen as a dummy protein. The FITC-Lys was synthesized as described in a previous study [28] and was encapsulated by addition to the precursor solution for the poly(AM/DAAM)/ADH core nanofibers in phosphate buffer (pH 8). The fiber-stacked nanostructure of the FITC-Lys loaded core-shell fibermats was confirmed using SEM measurements (data not shown), indicating no adverse impact on their nanostructures. The protein-release experiments were performed by immersing the fibermats in Dulbecco's modified Eagle's medium (DMEM) at 37°C, and the released FITC-Lys at each time point was

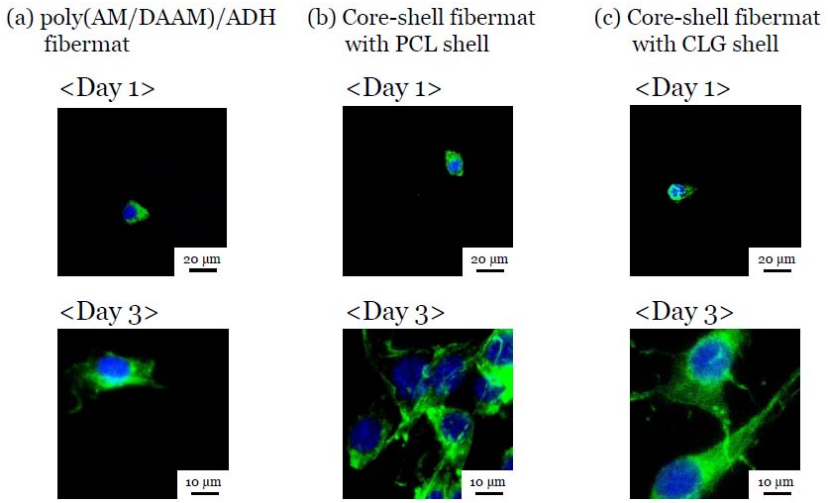

Fig. (5). Morphologies of NIH3T3 cells seeded on poly(AM/DAAM)/ADH fibermats (**a**) core-shell fibermats with PCL shell (**b**) or CLG shell (**c**) after 1 and 3 days of incubation at 5% CO_2 and 37°C. Nuclei were stained with DAPI and actin fibers were stained with Phalloidin-i-Fluor 488 Conjugate. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

quantified based on FITC fluorescence in the supernatant. As shown in Fig. (**6**), a sustained release of FITC-Lys without any burst was observed over 24 days. The cumulative release in 24 days reached more than 65% of the input amount. As expected, the property was relatively different in the fibermat with the PCL shell, which showed a low release of FITC-Lys. This suggests that the use of CLG as a shell layer for core-shell fibermats, endows not only cell adhesion but also sustained protein-release from the protein-loadable hydrophilic core nanofibers.

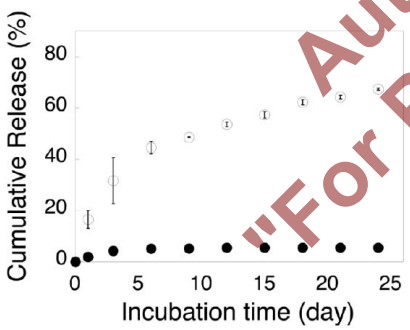

Fig. (6). Comparison of the release profiles (n = 4) of the encapsulated FITC-Lys from the core-shell fibermats with the CLG shell (white circle) and the PCL shell (black circle) when immersed in DMEM for 24 days.

To further investigate the superior protein-release properties of the CLG shell, we next examined the morphological changes in the core-shell fibermats after immersion in DMEM at 37°C for 7 days. The SEM images of the fibermats are shown in Fig. (**7**). For the fibermat with the PCL shell (Fig. **7**), upper, a slight increase in fiber diameter (within 40 nm) was observed after 7 days of incubation. According to a previous study, this increase could be caused by hydration of the core nanofibers with a buffer. In contrast, the core-shell fibermat with the CLG shell showed a significant increase in diameter (over ~200 nm, expansion of average fiber diameter was over 40%) after 7 days of incubation, and this increased linearly over 7 days (Fig. **7**) lower. This observation could be due to the difference in the ease of hydration between PCL and CLG. CLG is a chemically cross-linked protein, and therefore, following hydration, it swells significantly. As a result, the pores between the CLG polymer chains increase and the proteins entangled in the CLG network could be liberated into the DMEM. The key observation here is that it occurred gradually; as a result, the encapsulated FITC-Lys could be released in a sustained manner without any burst release. These data implied that the similarly cationic bFGF could also be released in a similar manner.

3.3. bFGF-Encapsulated Poly(AM/DAAM)/ADH/CLG Core-Shell Fibermat Promotes Cell Proliferation.

For the induction of desirable cell proliferation and differentiation, exposure to humoral growth factors and cytokines under controlled conditions (optimized time period, concentration, and order) is necessary. However, due to digestion by ubiquitously present proteases and/or denaturation in the culture medium, the limited half-life of cytokines can be a serious challenge. For example, upon the addition of bFGF (10 ng/mL) to DMEM, most of its biological activity was lost within 24 h [32]. To circumvent this problem, proteinaceous cytokines can be encapsulated inside scaffolding material and be released when required, especially for the design of implant scaffolds for *in situ* regeneration therapy. Thus, we constructed bFGF-encapsulated fibermats and evaluated their impact on cell proliferation. Since bFGF (pI ~ 9.6) has a cationic property similar to lysozyme [33], it could be similarly released in a sustained manner from the core-shell fibermat. The bFGF-loaded core-shell fibermats

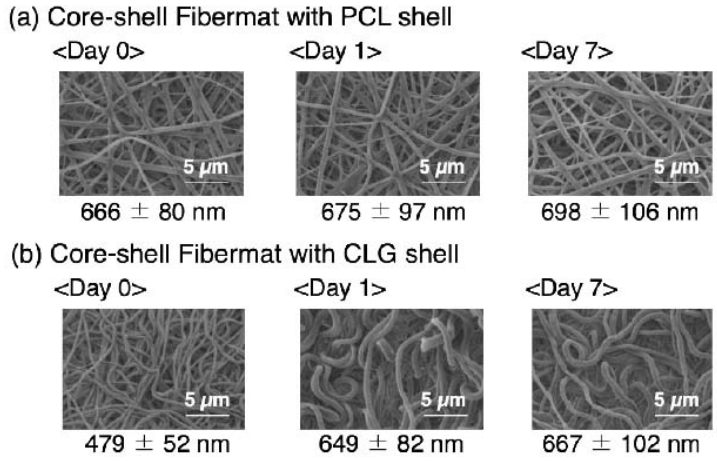

Fig. (7). Morphological changes in the nanofibers of the core-shell fibermats with PCL (**a**) and with CLG (**b**) upon immersion in DMEM with 10% FBS over 7 days, from SEM measurements (X5000 magnification). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

were prepared like the FITC-Lys-encapsulated fibermats. The final amount of bFGF in the fibermat was set to 4.49 ng/cm^2; if 50% of the loaded bFGF in the fibermats (round shape, φ 6.4 mm) was released into the DMEM medium (100 μL), its concentration would be 10 ng/mL. As a reference, a core-shell fibermat without bFGF was also prepared. Live cell numbers in the fibermats at each time point were quantified by the MTT assay using water-soluble WST. As shown in Fig. (**8**), the proliferation of NIH3T3 cells placed in the bFGF-encapsulated fibermat was markedly higher compared to a fibermat without bFGF. Since the lifetime of bFGF (10 ng/mL) in DMEM was less than 24 h [28], this meant that the sustained release of bFGF from the fibermat with the CLG shell could bring about cell proliferation in the incubated cells.

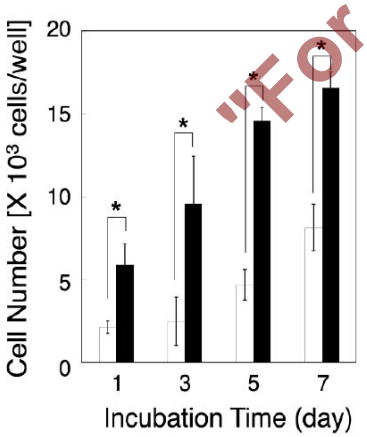

Fig. (8). Comparison of cell proliferation in NIH3T3 cells seeded on poly (AM/DAAM)/ADH-CLG core-shell fibermats with (black bar) and without (white bar) preloaded bFGF over 7 days; *significance difference between pairs of substrates shown ($p < 0.05$). (Mean ± SD; $n = 4$).

CONCLUSION

Intracellular signaling systems participate in the control of individual cell proliferation/differentiation processes and the subsequent development in the well-organized cell assemblies such as tissue and organ. Various proteinous drugs such as hormones and growth factors make pivotal roles in these processes and suitable signal-inputs are performed with the necessary timing, period, and strength (amount). Because in the connective tissues, these proteinous drugs are generally supplied from the neighboring extracellular matrix, the protein-releasable scaffolds have been thought to use as an alternative to the natural extracellular matrix that can promote artificial tissue construction. Electrospun fibermats have fibrous morphology and by choosing suitable base materials they could be used for a cell-incubation scaffold. However, toward application to a protein-releasable scaffold development of the method to construct protein-releasable fibermats is still necessary. In this study, we demonstrated that core-shell fibermats with a CLG shell layer around the constituent nanofibers were effective as protein-releasing cell-incubation scaffolds. Based on the characterization of the release profile of encapsulated lysozyme, sustained-release without any burst release was observed for the core-shell fibermats, consisting of poly(AM/-DAAM)/ADH core nanofibers and a CLG shell. CLG wrapping, in other words, modification of the core-shell fibermats with CLG shells, endowed excellent cell adhesion properties to the poly(AM/DAAM)/ADH core nanofibers. Furthermore, based on the impact of pre-encapsulated bFGF on cell proliferation, the released bFGF effectively promoted cell proliferation. There have been several reports on the use of core-shell fibermats as cell-incubation scaffolds; however, very few detail the importance of choosing the right material for the shell units that can endow efficient release properties. Core-shell fibermats could offer a superior platform for constructing well-designed scaffolds for *in situ* regeneration therapy. For example, by combining the double-layered

nanofibers with different shell materials in one fibermat, a superior scaffold can be designed that can release more than two cytokines with different release profiles. We are in the process of application-focused design of the core-shell fibermats by combining various materials, which would increase its therapeutic potential.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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