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Coating Nanofiber Scaffolds with Beta Cell Membrane to Promote Cell Proliferation and Function

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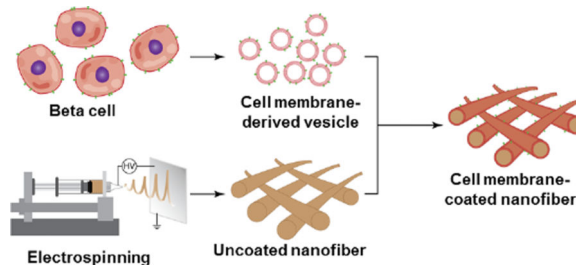
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Abstract

Cell membrane cloaking technique has emerged as an intriguing strategy in nanomaterial functionalization. By coating synthetic nanostructures with natural cell membranes, it bestows the nanostructures with unique cell surface antigens and functions. Previous efforts have been primarily focused on the development of cell membrane-coated spherical nanoparticles and their uses thereof. Herein, we attempt to extend the cell membrane cloaking technique to nanofibers, a class of functional nanomaterials that are drastically different from nanoparticles in terms of dimensional and mechanophysical characteristics. Using pancreatic beta cell as a model cell line, we demonstrate successful preparation of cell membrane-coated nanofibers and validate that the modified nanofibers possess an antigenic exterior closely resembling that of the source beta cells. When such nanofiber scaffolds are used to culture beta cells, both cell proliferation rate and function are significantly enhanced. Specifically, the glucose-dependent insulin secretion from the cells are increased by near five-fold as compared to the same beta cells cultured in regular, unmodified nanofiber scaffolds. Overall, coating cell membranes onto nanofibers would add another dimension of flexibility and controllability in harnessing cell membrane functions and would open new opportunities for innovative applications.

Graphical abstract



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We demonstrate successful coating of polymeric nanofibers with pancreatic beta cell membrane and validate that the modified nanofibers possess an antigenic exterior closely resembling that of the source beta cells. When such nanofiber scaffolds are used to culture beta cells, both cell proliferation rate and function are significantly enhanced.

1. Introduction

The key components for tissue engineering and cell delivery consist of cells, scaffolds, and growth-stimulating factors.^{1, 2} Among this triad, scaffolds provide a solid matrix for cell attachment and proliferation, while supplying physicochemical and/or bioactive cues to the residing cells.^{3, 4} The interactions between cells and scaffolds are of particular importance, which determine the survival and function of the cells.^{5, 6} Therefore, scaffolds, typically made of polymeric biomaterials such as polymeric nanofibers, are often modified with cell surface receptors to stimulate intracellular signaling, alter protein expression, and modulate cell function.⁷⁻¹⁰ Common strategies to implement such surface modifications are chemical conjugation and physical absorption.¹¹⁻¹⁴ A variety of soluble receptors including intercellular adhesion molecules 1 (ICAM-1),¹⁵ E-cadherin,¹⁶ and Ephrin^{17, 18} have been conjugated onto synthetic scaffolds to enhance cell survival and function. To alleviate potential risk of protein denaturation by these chemical and physical processes, synthetic lipid bilayers have been successfully coated onto nanofiber scaffolds.^{19, 20} The lipid membranes not only shield the nanofibers from external environment but also provide a biomolecule-friendly medium to anchor cell surface receptors and preserve their integrity and functionality. While these bottom-up functionalization approaches are competent to present individual receptors or receptor combinations to cells seeded in a scaffold, they are generally inadequate to replicate the complex cell surface properties and functions critical for cell-cell interaction and cell proliferation in a scaffold.^{21, 22} Herein, we demonstrate a new and robust strategy in nanofiber scaffold modification to promote cell proliferation and function by coating polymeric nanofibers with the functional utility of cell membranes derived directly from natural cells.

The use of cell membranes to cloak synthetic nanoparticles through a top-down fabrication method has emerged as a promising technique for nanomaterial surface functionalization.^{23, 24} Such cloaking technique bestows nanoparticles with complex cell surface properties and functions that are otherwise difficult to replicate. Currently, a variety of cell membrane-coated nanoparticle systems have been developed with unique features and functions, which involve different cell types (e.g., red blood cell, platelet, leukocyte, cancer cell and bacterium) and different synthetic nanoparticles (e.g., polymeric nanoparticle, gold nanoparticle, and silica nanoparticle)²⁵⁻²⁸. These biomimetic nanoparticles have demonstrated a wide range of biomedical applications including drug delivery, photodynamic therapy, detoxification, and vaccination.²⁹⁻³² However, it remains untested whether the cell membrane cloaking technique can be generalized from spherical nanoparticles to spidery nanofibers, which exhibit aspect ratios drastically different from nanoparticles. Hence, this work represents the first attempt to investigate the 'coatability' of polymeric nanofibers with natural cell membranes.

We choose pancreatic beta cell as a model cell line to conduct the study because these cells rely on direct cell–cell interaction to maintain their survival and function,^{33, 34} and such characteristics have been explored to design scaffolds aimed to promote beta cell function.^{18, 35} We hypothesize that beta cell membrane-coated nanofiber scaffolds would possess an antigenic exterior closely resembling that of the source cells, thereby recapitulating the characteristics of intercellular interaction among beta cells found in the pancreas. As illustrated in Figure 1A, cell membrane-derived vesicles are collected from beta cells and then coated onto polymeric nanofibers. In the study, we demonstrate successful preparation of cell membrane-coated nanofibers (CM-fibers) and validate that the CM-fibers can enhance cell survival and promote cell function when they are used as scaffolds to culture beta cells.

2. Materials and methods

2.1 MIN6 cell culture and membrane derivation

A mouse pancreatic beta cell line, MIN6, was obtained from AddexBio Technologies (San Diego, CA) and cultured in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) supplemented with 10% v/v fetal bovine serum (FBS, Hyclone) and 1% v/v penicillin-streptomycin (Invitrogen). Cell membrane was harvested by following a previously published protocol²⁵. Specifically, MIN6 cells were grown in T-175 culture flasks to full confluency and detached by scrapping the culture flask surfaces. Washed cells were suspended in a hypotonic lysing buffer containing 20 mM Tris-HCl (pH = 7.5), 10 mM KCl, 2 mM MgCl₂, and 1 EDTA-free mini protease inhibitor tablet (Pierce), and then disrupted using a dounce homogenizer with a tight-fitting pestle. The cell suspension was subjected to 20 passes and then centrifuged at 20,000 ×g for 20 min, after which the pellet was discarded. The collected supernatant was centrifuged again at 100,000 ×g for 45 min, and the pellet was collected and used as purified MIN6 cell membrane for subsequent experiments.

2.2 Synthesis of polycaprolactone nanofibers

Polycaprolactone (PCL, $M_w = 70\sim 90$ KDa, Sigma-Aldrich) and poly-D-lysine ($M_w = 30\sim 70$ KDa, Sigma-Aldrich) were dissolved in a formic acid-acetone mixture solution (7:3 v/v) with a final polymer concentration of 10 % (w/v) and 1% (w/v), respectively. The polymer solution was loaded into a 10 mL syringe with a gauge 23 needle, which was then placed onto a microinjection pump (New Era Pump Systems Inc). To inject the polymer solution, a 20 kV high voltage (Gamma High Voltage Research) was applied to the needle and the flow rate of the solution was 0.5 mL/h. To collect the nanofibers, a glass cover slip (0.15 mm in thickness, VWR) was cut into 4 × 4 mm square pieces and taped onto a large piece of aluminum foil, which served as a collector and was connected to the ground. The distance between the needle and the aluminum foil collector was 10 cm.

2.3 Coating nanofibers with MIN6 cell membrane

The collected MIN6 cell membrane was sonicated with an FS30D bath sonicator (Fisher Scientific) at a frequency of 42 kHz and a power of 100 W. The sonication lasted 3 min to form beta cell membrane-derived vesicles (denoted β C vesicles). Immediately following the sonication, the nanofibers cast on the glass cover slip were immersed into the β C vesicle

suspension and kept for 30 min at room temperature. Then the solution was discarded and the nanofibers were rinsed with deionized water. After drying in air, the morphology and the thickness of the cell membrane-coated nanofibers (denoted CM-fibers) were examined by scanning electron microscopy (SEM). Nanofiber diameter and size distribution were obtained by measuring diameters of 100 CM-fibers randomly selected from five independent samples.

2.4 β C vesicle-nanofiber fusion study

To study and verify the fusion between β C vesicles and the nanofibers, β C vesicles were fluorescently labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate (DiD; excitation/emission = 644/665 nm; Life Technologies) into the cell membrane and calcein-AM (excitation/emission = 495/516 nm; eBioscience) into the inner aqueous compartment of the vesicles, respectively. Specifically, DiD was first mixed with a trivial amount of egg PC in chloroform (approximately DiD:egg PC = 1:9, molar ratio), and then dried by flowing nitrogen gas over the sample. To the dried film, calcein-AM solution and MIN6 cell membrane were added. The mixture was sonicated with an FS30D bath sonicator (Fisher Scientific) at a frequency of 42 kHz and a power of 100 W for 3 min to form fluorescently labelled β C vesicles. The fusogenic β C vesicles were then incubated with the nanofibers at room temperature for 30 min. The resulting CM-fibers were rinsed and imaged with an EVOS® inverted fluorescence microscope (Thermo Fisher Scientific). The fluorescence intensity was quantified with a microplate reader (BioTek Instruments). To prepare non-fusogenic β C vesicles as a control group, poly(lactic-co-glycolic acid) (PLGA) polymeric cores with a diameter of ~ 100 nm were prepared using 0.67 dL/g of carboxy-terminated 50:50 PLGA (LACTEL Absorbable Polymers) through a nanoprecipitation process.²³ For fluorescent labeling of the polymeric cores, Alexa 488 (excitation/emission = 495/519 nm; Life Technology) was covalently conjugated to about 5% of the PLGA polymers that form the cores. Finally, the DiD-labeled β C vesicles were coated onto the Alexa 488-labeled polymeric cores following a previously published protocol³⁰. The nanoparticle-supported cell membrane would lose its fusion activity^{24, 29} and thus can serve as a non-fusogenic control of the fusogenic β C vesicles.

2.5 Protein analysis of CM-fibers

For protein characterization using sodium dodecyl sulfate polyacrylamide gel electrophoresis, all samples were prepared at a final protein concentration of 1 mg/mL in lithium dodecyl sulfate (LDS) loading buffer (Invitrogen) as measured by a BCA assay (Pierce). CM-fibers were rinsed in PBS and then disintegrated via sonication using a FS30D bath sonicator at a frequency of 42 kHz and a power of 100 W for 5 min. Samples were heated at 70°C for 10 min and 20 μ L was loaded into each well of a NuPAGE Novex 4–12% Bis-Tris 10-well minigel (Invitrogen) in MOPS running buffer (Invitrogen) in an XCell SureLock Electrophoresis System (Invitrogen) following manufacturer's instructions. Protein staining was accomplished using SimplyBlue (Invitrogen) and destained in water overnight before imaging. For western blot analysis, protein was transferred to Protran nitrocellulose membranes (Whatman) using an XCell II Blot Module (Invitrogen) in NuPAGE transfer buffer (Invitrogen) following manufacturer's instructions. Membrane proteins were probed using antibodies against E-cadherin (147301, Biolegend) and Na⁺/K⁺-

ATPase (A01483, GenScript) along with horseradish peroxidase (HRP)-conjugated anti-mouse IgG (Poly4053, Biolegend). Films were developed using ECL western blotting substrate (Pierce) and developed with the Mini-Medical/90 Developer (ImageWorks).

2.6 MIN6 cell aggregation and proliferation on CM-fibers

To analyze the cell-cell aggregates, CM-fibers on glass cover slips were placed into the wells of 96-well microplates (Corning). To each well, approximately 1×10^4 MIN6 cells were seeded and cultured in DMEM medium containing 15% v/v FBS and 1% v/v penicillin-streptomycin. Uncoated nanofibers (Un-fibers) and bare glass cover slips were used as control groups. On day 1, 4, and 7, samples were treated with calcein-AM and PI (Thermo Fisher Scientific) to label the live and dead cells, respectively. For labeling, the cell culture medium in the plate was removed and then 200 μ L PBS containing 3 mM Calcein-AM and 10 mM PI was added to each well. The plate was incubated under culture conditions for 30 min and imaged with an EVOS® inverted fluorescence microscope (Thermo Fisher Scientific). The size and size distribution of the MIN6 cell aggregates were quantified using ImageJ software. The results shown were representative images of at least independent samples. For MIN6 proliferation study, the cell number was quantified by measuring the fluorescent signal with an excitation wavelength of 485 nm and emission wavelength of 530 nm. All experiments were repeated at least three times.

2.7 Insulin secretion study

The glucose-stimulated insulin secretion from MIN6 cells were measured following a previously reported method³⁵. Briefly, MIN6 cells cultured on CM-fibers, Un-fibers, or bare glass cover slips were pre-incubated in the Krebs Ringer HEPES (KRBH) buffer solution at 37°C for 45 min. Then MIN6 cells were incubated in KRBH buffer containing 2.5 mM glucose for 1 hour and the supernatant was collected. Subsequently, the cells were incubated in KRBH buffer containing 25 mM glucose for another 1 hour and the supernatant was collected. The insulin concentration in the collected supernatants was quantified with mouse Insulin ELISA kit (Merckodia Inc) following manufacturer's instructions. The amount of insulin secreted under 25 mM glucose was normalized to insulin secreted under 2.5 mM glucose, which defines the insulin secretion index used in this study. Statistical analysis was performed with a two-tailed, unpaired Student's t-test.

3. Results and discussion

The fabrication of CM-fibers can be divided into three steps (Figure 1A). The first step was to prepare beta cell membrane-derived vesicles (β C vesicles). Specifically, we used MIN6 mouse pancreatic cells as a model beta cell line to collect cell membrane by emptying intracellular contents through a combination of osmotic lysis, mechanical membrane disruption, and differential centrifugation.²⁵ The purity of the membrane was confirmed by Western blotting analysis, which showed the absence of nuclear and cytosolic markers. The collected MIN6 cell membrane was then sonicated to generate β C vesicles with an average diameter of 120 ± 5.3 nm. The second step was to produce uncoated nanofibers (Un-fibers). Specifically, we chose polycaprolactone (PCL) to prepare nonwoven nanofibers through an established electron-spinning process.^{36–38} In the study, PCL and poly-D-lysine were

dissolved in a formic acid-acetone mixture solution (7:3 v/v) with a final polymer concentration of 10% (w/v) and 1% (w/v), respectively. Then the polymer solution was loaded into a syringe and injected by using a microinjection pump with a 20 kV high voltage applied to the needle. The flow rate of the polymer solution was kept at 0.5 mL/h. The generated nanofibers were collected using glass cover slips mounted on an aluminum foil. In the third step, we coated the polymeric nanofibers with β C vesicles through a fusion process. Freshly prepared β C vesicles were immediately added to the Un-fibers and the two components were allowed to incubate at room temperature for 30 min. After the incubation, the vesicle suspension was removed and the nanofiber sample was washed and dried. When examined with scanning electron microscope (SEM), the CM-fibers showed a smooth outer surface and an overall long fibrous morphology (Figure 1B). Based on the SEM micrographs, CM-fiber diameter distribution was found in the range of 50 – 280 nm (Figure 1 C).

To confirm the fusion between β C vesicles and Un-fibers, the cell membrane and the inner aqueous compartment of the β C vesicles were labeled with DiD (excitation/emission = 644/665 nm) and calcein-AM (excitation/emission = 495/516 nm), respectively. As shown in Figure 2A, the fluorescence spectrum of these dual-dye labeled β C vesicles display two distinct fluorescence bands characteristic of the signals from the membrane and the inner space. The dual-dye labeled β C vesicles were then incubated with Un-fibers for 30 min, followed by excess washing to remove excess vesicles. Florescent measurement of the resulting CM-fibers showed only red fluorescence signal from the cell membrane while the green fluorescence from the intra-vesicle compartment was largely absent. This observation was further confirmed by examining the fluorescence distribution over the CM-fibers. Figure 2B shows that the near infrared fluorescence emitted from the cell membrane spread evenly on the nanofibers and the fluorescent pattern matched the long fibrous morphology of the nanofibers. In contrast, the intra-vesicle fluorescence signal was not observed over the CM-fibers. Collectively, these results indicate a uniform membrane coating over the fibers, resulting from an effective vesicle-nanofiber fusion process, in which the vesicles wrap around the nanofibers and release their intra-vesicle content. It has been reported that emptied cell membrane-derived vesicles such as red blood cell (RBC) vesicles and platelet vesicles are prone to fuse with solid substrates to reduce their high surface energy^{23,27}. It's expected that the β C vesicle-nanofiber fusion is driven by a similar mechanism, which renders the system energetically favourable.

To further validate the fusion process, we prepared non-fusogenic β C vesicles by pre-coating the vesicles onto PLGA nanoparticle cores, which was labeled with green fluorescent dye Alexa 488 (excitation/emission = 495/519 nm). These vesicles wrapped around PLGA nanoparticle cores and form a stable membrane-core interface, which passivates their fusion ability with nanofibers. The non-fusogenic β C vesicles were then added to the Un-fibers, followed by the same incubation and washing steps as for the fusogenic β C vesicles. Different from the fusogenic β C vesicles, fluorescence signals corresponding to both the membrane and the core were detected and their intensity ratio remained unchanged after incubation with the Un-fibers (Figure 2C). Fluorescent images further demonstrated that both fluorescent signals were observed over the fibers and the fluorescent signals exhibited spotty patterns that co-localized with each other on the fibers (Figure 2D). These results

suggest that the non-fusogenic β C vesicles randomly adsorb onto the Un-fibers rather than fusing with the fibers.

The membrane coating was further verified by examining the surface properties of the CM-fibers. First, we examined the wettability of the CM-fibers by measuring the surface contact angles. Prior to the coating, the static water contact angle of Un-fibers was $69.1 \pm 2.7^\circ$ (Figure 3A). After cell membrane coating the water contact angle of CM-fibers was $30.9 \pm 3.4^\circ$. This significant decrease of contact angle indicates that the CM-fibers become more hydrophilic following the coating process and this change in wettability is attributable to the hydrophilic nature of the coated cell membrane. The membrane coating was also verified with a protein bicinchoninic acid (BCA) assay (Figure 3B). The Un-fibers showed approximately 1 wt% of protein loading yield (defined as the weight ratio of protein content to the nanofibers) due to the addition of poly-D-lysine in the electrospinning process. In contrast, the CM-fibers showed a significantly increased protein loading yield of 2.8 ± 0.5 wt%. This indicates that the maximal membrane protein content coated onto the fibers is about 1.8wt% of the fiber weight. No obvious protein loss was observed when the CM-fibers was stored in 1X PBS at 4°C for 24 hr, implying a stable membrane coating. This result also indicates ‘wrapping’ as opposed to adsorption as the membrane adsorbed onto the fiber via electrostatic interaction tends to detach in a high salt environment. Analysis of the protein content on the CM-fibers was also carried out to confirm successful functionalization of the nanofibers with MIN6 cell membrane antigens. CM-fibers were rinsed to remove uncoated vesicles. Gel electrophoresis followed by protein staining showed that the protein profile of the CM-fibers matched closely with that of β C vesicles (Figure 3C). Western blotting analysis further demonstrated significant enrichment of Na^+/K^+ -ATPase and E-cadherin, two plasma membrane-specific markers, in the CM-fiber formulation as compared to β C vesicles, suggesting the successful translocation of membrane proteins onto the nanofibers (Figure 3D). Following the confirmation of its presence on the CM-fibers, E-cadherin was immunostained with a fluorescence dye and examined with fluorescence microscopy. Figure 3E shows that the protein marker evenly distributed over the long fibrous nanofiber scaffolds. In contrast, Un-fibers treated with the same staining method showed no fluorescent signal, indicating the absence of E-cadherin. Collectively, these results not only verify successful membrane coating on the nanofibers, but also demonstrate effective retention of membrane antigens on the nanofibers through the preparation process.

After having evaluated the preparation and characteristics of CM-fibers, we next tested the effectiveness of the CM-fibers in promoting beta cell proliferation and function. In the study, we cultured MIN6 beta cells on three different substrates including CM-fibers, Un-fibers, and non-coating cover slips. We first tested cell viability using a live/dead assay, where live cells were stained with calcein-AM that emits green fluorescence and dead cells with propidium iodide that emits red fluorescence (Figure 4A). Under our experimental conditions, on day 1, MIN6 cells evenly distributed on all three substrates and no obvious differences in cell viability were observed. On day 4, cells on CM-fibers showed obvious growth and formed small clusters. In contrast, fewer live cells were seen on Un-fibers and even fewer on non-coating cover slips. On day 7, cells on CM-fibers grew into an even higher density and formed large clusters, whereas cells on Un-fibers and cover slips only showed few sporadic, surviving cell clusters.

We further quantified cell proliferation rate based on live/dead assay results (Figure 4B). On day 4 and 7, the number of live cells on CM-fibers increased to 186% and 327%, respectively, as compared to the number on day 1. However, such number dropped to 67% and 18% for cells cultured on Un-fibers and non-coating cover slips, respectively, on day 7. Figure 4C shows the size distribution of MIN6 cell clusters on day 7. The cluster sizes were divided into three categories: $< 2500 \text{ mm}^2$, $2500\text{--}10000 \text{ mm}^2$, and $> 10000 \text{ mm}^2$. For CM-fibers the size distribution of cell clusters in the three size categories were 47%, 26% and 27%, respectively. For both Un-fibers and non-coating cover slips, about 70% of cell clusters were below 2500 mm^2 , while no more than 3% of cell clusters were larger than 10000 mm^2 . Therefore, MIN6 cells cultured on CM-fibers have the highest proliferative rate and tend to form larger cell clusters as compared to the control groups.

Finally, we monitored insulin secretion index for MIN6 cells cultured on the three different substrates. Previous studies have shown that cell–cell contact is critical in maintaining glucose-responsive insulin secretion from MIN6 cell.³⁹ Especially, cells encapsulated at higher cell-packing densities secreted larger amounts of insulin upon glucose stimulation.^{18, 40} In the study, the amount of insulin secreted under 25 mM glucose was normalized to insulin secreted under 2.5 mM glucose, and the ratio is defined as insulin secretion index. We measured such index of cells cultured on the three different substrates on day 1, 4, and 7. As shown in Figure 4D, cells cultured on CM-fibers had an insulin secretion index of 0.97 ± 0.15 , 1.37 ± 0.09 , and 2.76 ± 0.45 on day 1, 4, and 7, respectively. In contrast, the insulin secretion index of MIN6 cells cultured on Un-fibers (0.96 ± 0.17 , 0.96 ± 0.09 , and 0.58 ± 0.18 on day 1, 4, and 7, respectively) and cover slips (0.94 ± 0.12 , 0.72 ± 0.34 , and 0.46 ± 0.15 on day 1, 4, and 7, respectively) decreased with time. These results clearly show that MIN6 cell membrane coating not only promotes the survival of dispersed MIN6 cells in the nanofiber scaffolds, but also enhances the glucose-dependent insulin secretion from the cells.

4. Conclusion

We developed a new and facile approach to functionalizing nanofiber scaffolds to promote cell proliferation and function by coating polymeric nanofibers with functional utility of natural beta cell membrane. Using pancreatic beta cell as a model cell line, we demonstrated the preparation process and validated successful coating of beta cell membrane onto the surface of polymeric nanofibers. The resulting cell membrane-coated nanofibers retained the dimensional and physical properties of uncoated nanofibers while possessing an antigenic exterior closely resembling that of the source beta cell. When seeding beta cells in such modified nanofiber scaffolds, the cell membrane coating provided a natural environment recapitulating the cell-cell interaction among beta cells in the pancreas and thus promoted cell survival and function. Specifically, it significantly enhanced glucose-dependent insulin secretion from the cultured beta cells.

Since the initial report on cell membrane cloaking technique,²³ it has been applied to coat a wide range of synthetic spherical nanoparticles and the resulting biomimetic nanoparticles have demonstrated a versatile therapeutic applications. This work successfully extends the cell membrane cloaking technique from spherical nanoparticles to long nanofibers, which

represents a whole new class of nanomaterials marked with drastic differences in material dimensionality, physicochemical properties, and applications. Future studies will be focused on interfacial interactions between β C membranes and nanofiber substrates including completeness of membrane coverage, membrane sidedness upon coating, and the effects of fiber surface charge and diameter on the membrane coating process. Fundamental studies on these aspects are expected to further advance the understanding of the dynamics between cell membranes and nanoscale substrates and offer valuable information toward rational design and application of cell membrane-coated nanodevices. Overall, cell membrane-coated nanofibers are expected to add additional flexibility and controllability in harnessing cell membrane functions, and open unique opportunities for innovative applications.

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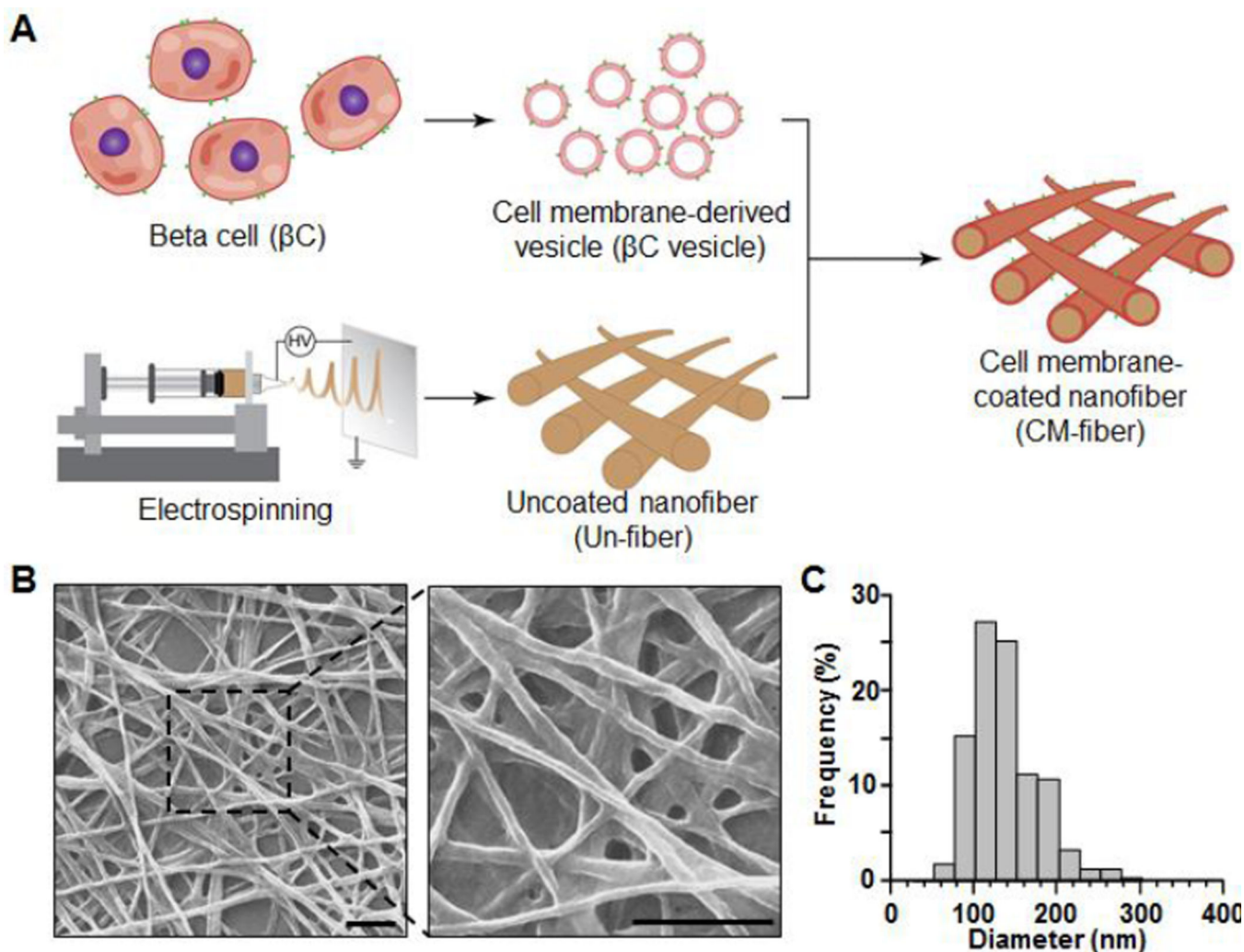


Fig. 1. Preparation and characterization of cell membrane-coated nanofibers (CM-fibers). (A) A schematic illustration showing the preparation of CM-fibers. The process can be divided into three steps: deriving membrane vesicles from beta cells (β C vesicles), fabricating uncoated nanofibers (Un-fibers) via an electrospinning method, and fusing the β C vesicles onto the surface of the Un-fibers. (B) Representative SEM images depicting the fibrous morphology of the resulting CM-fibers (scale bar, 1 μ m). (C) Size and size distribution of the CM-fibers.

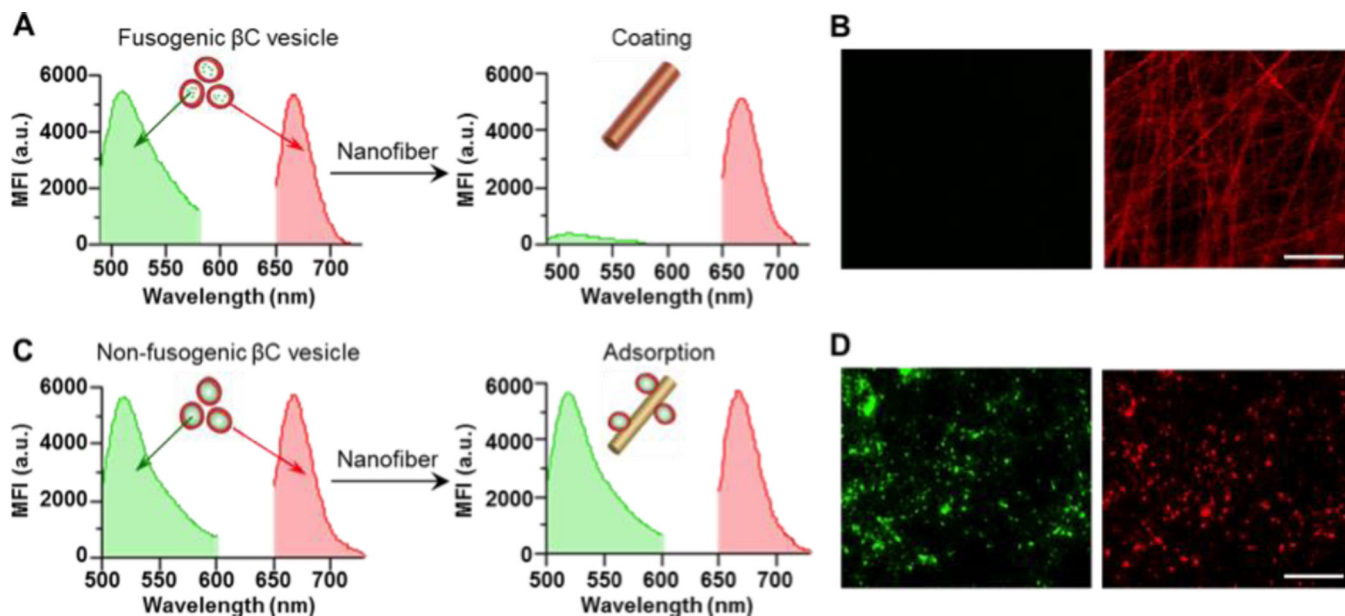


Fig. 2.

Confirming the fusion of the β C vesicles onto the nanofibers. (A,B) The β C vesicles were labeled with two distinct fluorescent dyes: DiD (red) in the cell membrane and calcein-AM (green) in the aqueous compartment of the vesicles. (A) Fluorescence emission spectra of the β C vesicles (left panel) and CM-fibers (right panel). (B) Fluorescent images of the CM-fibers in the green channel (left panel) and the red channel (right panel). (C,D) The β C vesicles (labeled with DiD, red) were pre-coated onto PLGA polymeric nanoparticles (labeled with Alexa 488, green) to passivate their fusion ability. (C) Fluorescence emission spectra of the non-fusogenic β C vesicles (left panel) and their mixture with the nanofibers (right panel). (D) Fluorescent images of the nanofibers after incubation with the non-fusogenic β C vesicles in the green channel (left panel) and the red channel (right panel). Scale bars, 25 μ m.

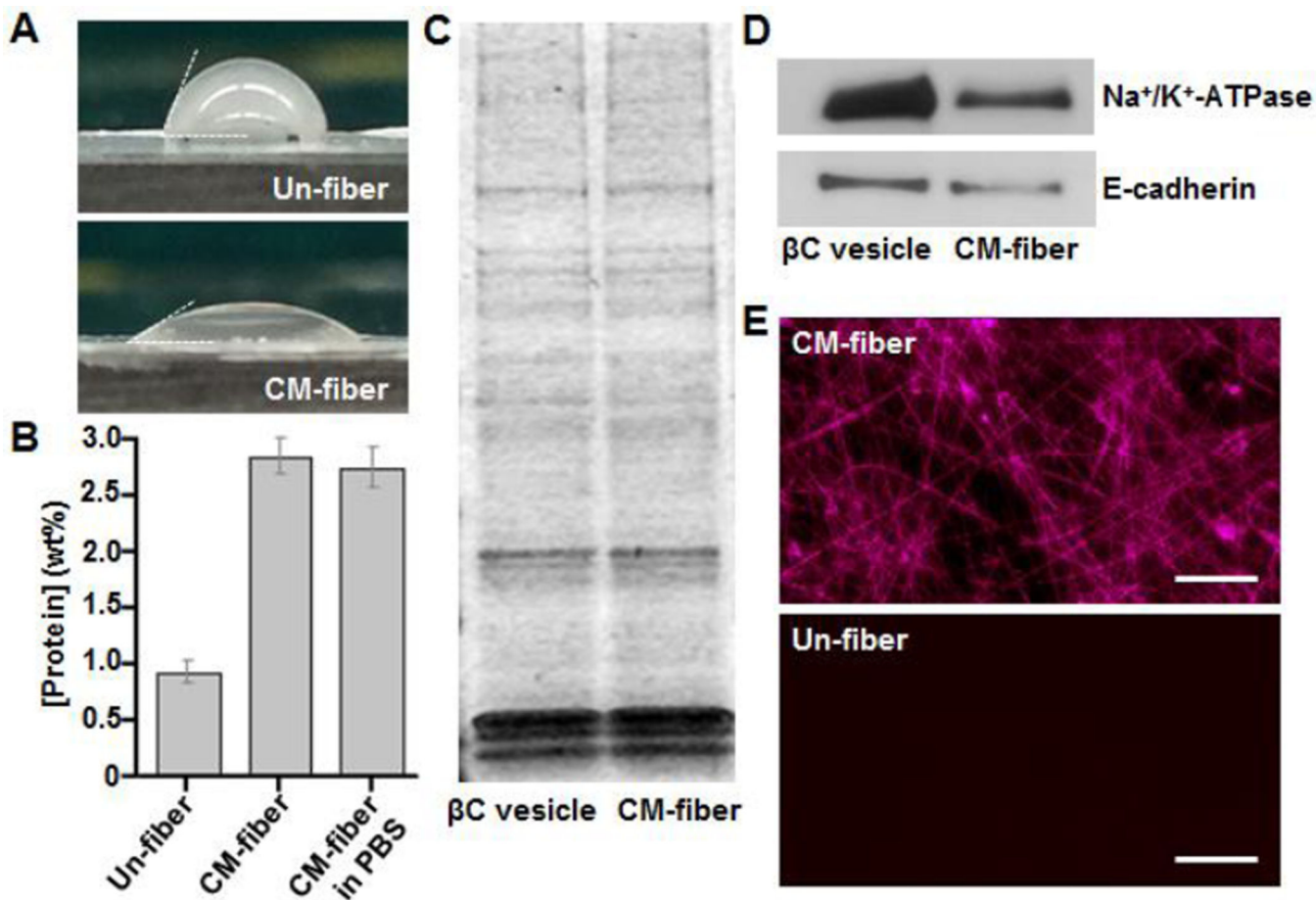


Fig. 3. Surface characterization of CM-fibers. (A) Static water contact angle measurements of Un-fibers and CM-fibers. (B) The weight percentage of protein content on Un-fibers, CM-fibers, and CM-fibers stored in 1XPBS at 4°C for 24 hr. Error bars represent the standard deviation of three measurements. (C) SDS-PAGE analysis of proteins present on the β C vesicles and the CM-fibers. The samples were run at equal protein content and stained with Coomassie Blue. (D) Western blotting analysis of cell membrane markers, Na⁺/K⁺-ATPase and E-cadherin, present on the β C vesicles and the CM-fibers. (E) Representative fluorescent images of CM-fibers and Un-fibers after immunostaining of E-cadherin (scale bar, 25 μ m).

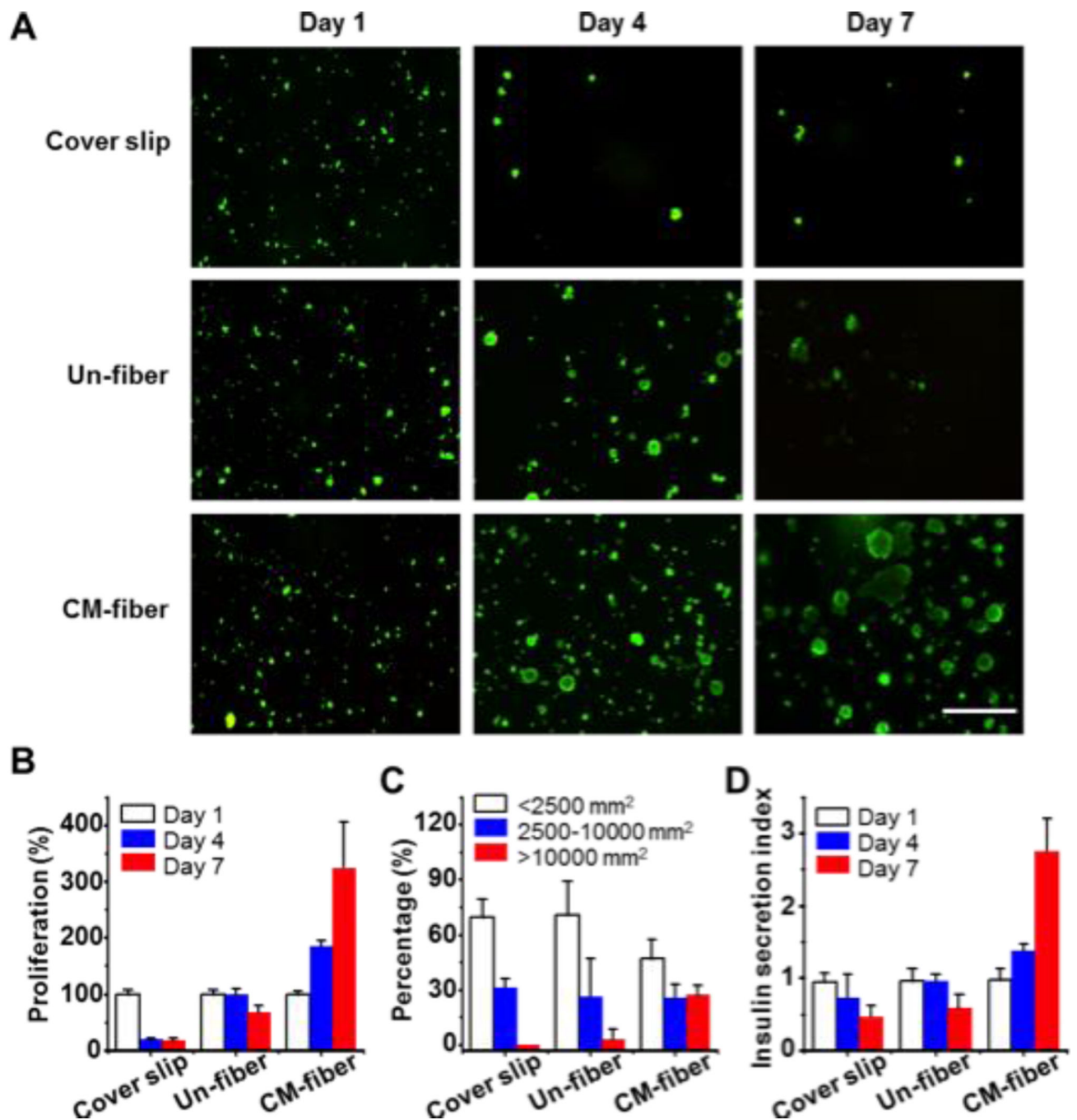


Fig. 4. CM-fibers promoting MIN6 cell proliferation and function. (A) Fluorescent images of MIN6 cells cultured on bare glass cover slip, Un-fibers, or CM-fibers. The cells were stained with calcein-AM/PI assay prior to imaging. (B) The proliferation rate of the cells was quantified on day 1, day 4 and day 7. (C) The size distribution of MIN6 cell clusters on day 7. (D) The insulin secretion index of MIN6 cells cultured on the three different substrates.