Supporting information

Chitosan/Cellulose-based Porous Nanofilm Delivering C-phycocyanin: A Novel Platform for the Production of Cost-effective Cultured meat

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Experimental section

Preparation of polysaccharide film-based platform

The polysaccharide-based multilayer film was fabricated via LbL assembly and subsequently modified by crosslinking. In LbL assembly, CHI (Chitosan, medium Mw, degree of deacetylation = 75~85%, Sigma-Aldrich) and CMC (Carboxymethyl cellulose sodium salt, Mw ≈ 250,000, Sigma-Aldrich) were selected as a positively charged and a negatively charged polysaccharide, respectively. We first prepared a CMC sodium salt aqueous solution and a CHI aqueous solution at a concentration of 1mg/mL. The pH of both solutions was adjusted to 4 using 1M HCl and NaOH. Silicon wafers, Slide glass, and OHP films were used as substrates for the preparation and analysis of the platforms. We immersed the oxygen plasma-treated substrates in the CHI solution for 10 minutes (min) and then washed the substrate twice with deionized (DI) water to form a stable positively charged layer on the surface of the substrates. Subsequently, the positively charged substrate was immersed in a negatively charged CMC solution for 10 min, followed by washing in the same manner. In this process, a single bilayer (BL) film was formed on the surface of the substrate by electrostatic interaction between the CHI and CMC. This cross-deposition was repeated *n* times to yield a (CHI/CMC) film composed of *n* BLs.

Following the LbL assembly, a twice-crosslinking reaction was introduced to obtain a porous inner-structure of the films. 1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide hydrochloride (EDC, Mw ≈ 191.71, Daejung)/N-hydroxysulfosuccinimide (NHS, Mw ≈ 115.09, Sigma-Aldrich) chemistry was used for the first crosslinking. The substrates coated with the (CHI/CMC) film were placed in a 0.05 M solution of 2-(N-Morpholino) ethane sulfonic acid hydrate (MES buffer, Mw ≈ 195.2, Sigma-Aldrich), supplemented with 0.1 M EDC and 2.5

mM NHS for 20 min, and subsequently soaked in both phosphate-buffered saline (1X PBS Gibco® Life Technologies) and DI water to wash any unreacted residue. For the second crosslinking, the primary crosslinked substrates were incubated in a 2.5% glutaraldehyde solution (Mw ≈ 25,000, Sigma-Aldrich) for 30 min, followed by washing with DI water thoroughly. Non-crosslinked film and crosslinked film are referred to as (CHI/CMC) film and X-linked (CHI/CMC) film, respectively.

Incorporation and protection of C-PC in the platform

A C-PC solution was prepared at a concentration of 0.5 mg/mL using 1X PBS as a solvent. X-linked (CHI/CMC) film-coated substrates were incubated in the C-PC solution for 12 hours (h) under a light-blocked or darkened environment at room temperature, allowing for sufficient incorporation of C-PC into the films. While the films were drying, agarose was dissolved in DI water at a concentration of 0.1 w/v%. The agarose solution was applied to the dried films at 25 μL per cm^2 in order to form the protective capping layer for C-PC. We hardened the prepared films at 4 °C. CPC-incorporated films without the capping layer and CPC-incorporated films with the capping layer are referred to as uncapped (CHI/CMC)/CPC films and capped (CHI/CMC)/CPC films, respectively.

Characterization of the film-based platform

The thickness of the LbL-assembled (CHI/CMC) film was measured using a contact-profilometer (Dektak 150, Veeco Instrument Inc., USA). Qualitative analysis and additional bond formation of the films, before and after crosslinking, were investigated using Fourier transform infrared spectroscopy (FTIR; FT/IR-4700, Jasco, USA). The morphology of the films was observed by AFM, and the images were analyzed using XEI and Gwyddion software. The wettability of the films was analyzed according to morphology by measuring the contact

angle of 4-μL water droplets on each film using a contact-angle goniometer (Smart Drop Standard, Femtobiomed, Korea). The change in morphology of the film due to crosslinking and C-PC incorporation was analyzed by observation field-emission scanning electron microscopy (FE-SEM, JEOS, IT-500HR, USA) cross-section view. To confirm the incorporation of the drug into the films, we investigated the confocal laser scanning microscopy (CLSM; LSM 880, Carl Zeiss) images of the X-linked (CMC/CHI) films before and after incorporation of C-PC. The same laser conditions were irradiated for all samples. The change in the porosity of the film due to C-PC incorporation was analyzed using a porosimeter (PM33GT, Quantachrome, USA).

Analysis of release profile of C-PC

Uncapped (CHI/CMC)/CPC film or capped (CHI/CMC)/CPC film-coated substrates, with a size of 1 cm × 1 cm, were immersed in 1 mL of 1X PBS buffer to induce the release of C-PC. At designated time points, we transferred 1 mL of the 1X PBS buffer containing released C-PC to microtubes. We then replenished the mixture with fresh buffer in order to monitor the continuous release of C-PC. The amount of C-PC (λ_{ex} 609 nm, λ_{em} 643 nm) released was calculated by quantifying the fluorescence of the solution in the microtube using photoluminescence (PL, FP-8300, JASCO).

Additional data

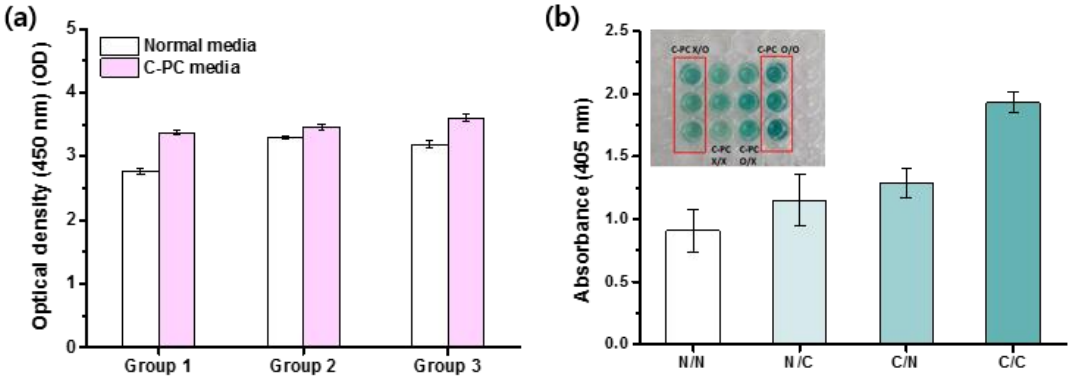


Figure S1. The effect of C-PC on myoblast proliferation. (a) A result of CCK-8 assay for each group (n = 3). "C-PC media" in Group 1, 2, and 3 represent the media containing 100 μg/mL C-PC (treated once), 14.3 μg/mL C-PC (treated every 24 h) and 25 μg/mL C-PC (treated every 48 h), respectively. (b) A result of brdU assay for each group according to additional treatment of C-PC (n = 3).

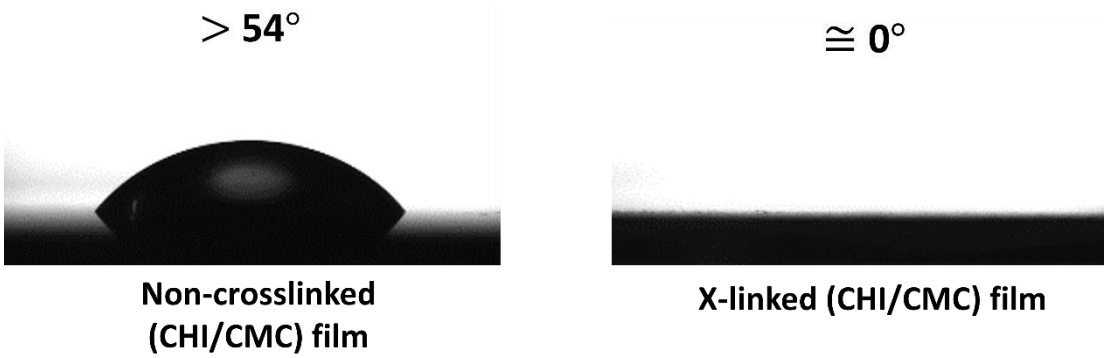
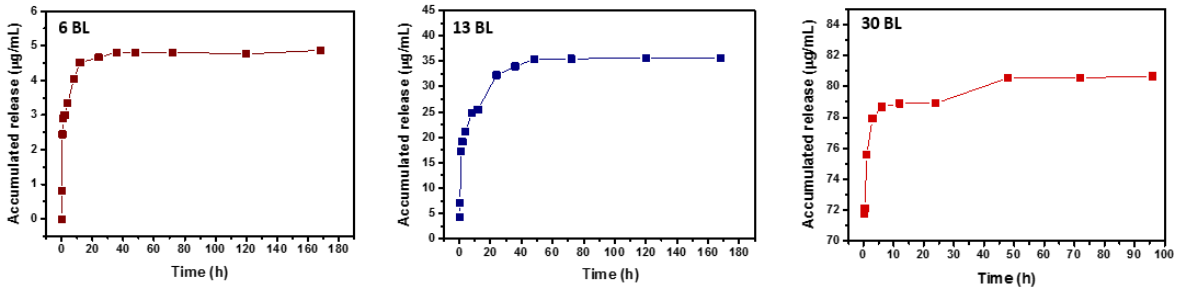


Figure S2. Images of static water contact angle depending on morphology of the films before and after crosslinking.



	Thickness	Amount of released C-PC	Release behavior
6 BL film	27.94 nm	4.87 μg/mL	Saturation after 24h
13 BL film	800 nm	36.57 μg/mL	Saturation after 48h
30 BL film	2.75 μm	81.54 μg/mL	Saturation after 48h

Figure S3. C-PC release profile of X-linked 6, 13 and 30 BL (CHI/CMC) film. The graphs for release behavior (Above) and a table summarizing the total release amount of C-PC depending on a thickness (Under)

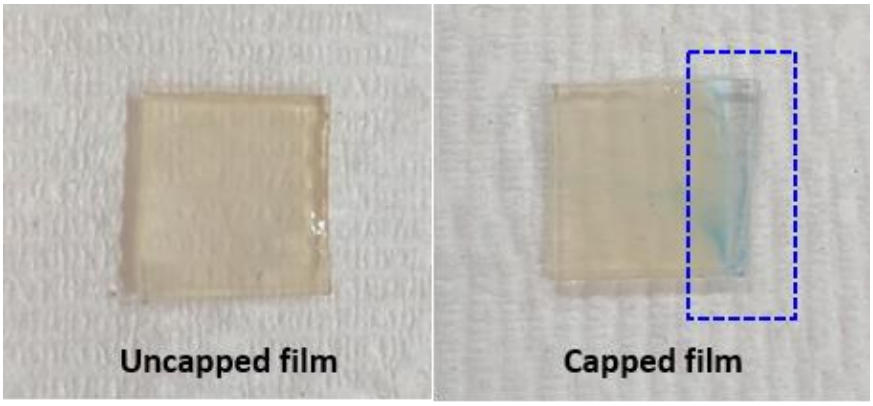


Figure S4. Images of uncapped (CHI/CMC) film and capped (CHI/CMC) film taken out during the release experiment. The part indicated by the blue square represents the blue C-PC remaining on the film.

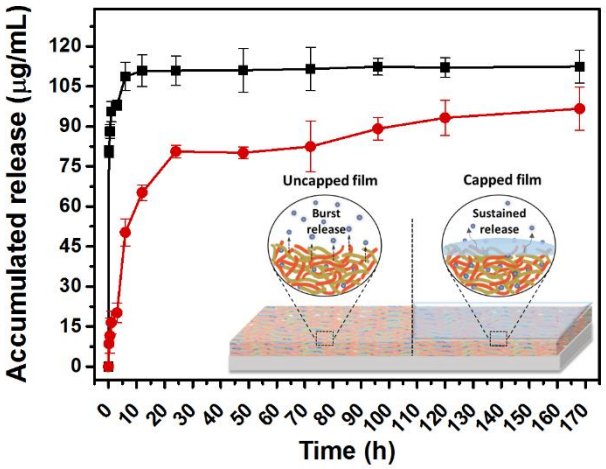


Figure S5. Analysis of total amount of released C-PC from the uncapped- and capped (CHI/CMC)/CPC film (n = 3).

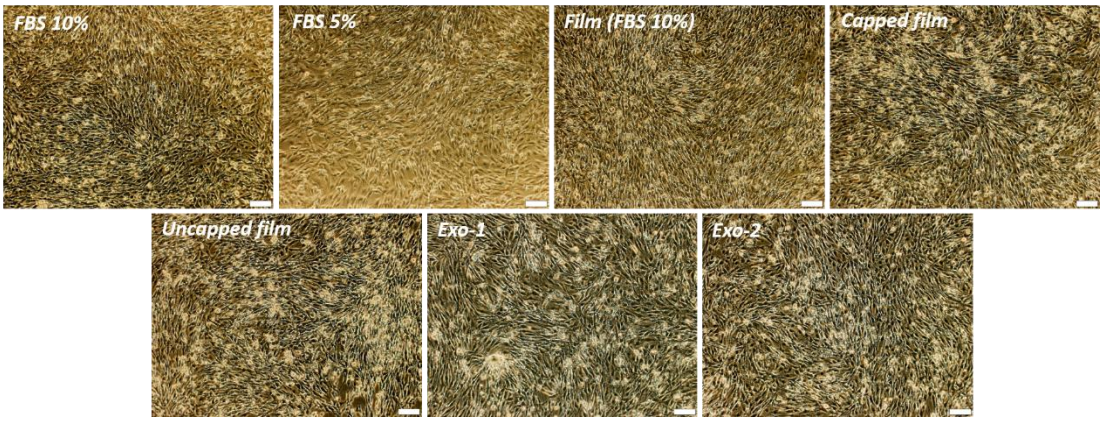


Figure S6. Optical microscope images showing the morphology of C2C12 myoblast cultured for 5 days in the control (FBS 10% and FBS 5%) and experimental ((CHI/CMC) film, Capped film, Uncapped film, Exo-1 and Exo-2) groups. Scale bar: 100 μm

	Initial	FBS 10%	FBS 5%	Uncapped	Capped film	Exo-1	Exo-2
Number of cells ($\times$ 10^4 cells)	0.8	19.63 $\pm$ 1.61	3.2 $\pm$ 2.6	15.85 $\pm$ 2.36	19.68 $\pm$ 5.34	8.44 $\pm$ 0.83	15.72 $\pm$ 2.86
Expansion ratio	1	24.53	3.99	19.81	24.60	10.55	19.63

Table S1. A table with the results for the number of the cells and expansion ratio by each group (n = 3).

	FBS 10% Medium (500 mL)	**FBS 5% Medium (500m mL)**
DMEM, High glucose	\$27.6 / 445 mL	\$29.46 / 475 mL
FBS	\$54.59 / 50 mL	\$ 27.29 / 25 mL
penicillin/streptomycin	\$7.31 / 5 mL	\$7.31 / 5 mL
Total price	**\$89.5**	**\$64.06**

Table S2. Total cost for 500 mL of cell growth medium containing 10% FBS and 5% FBS. 42 units of cell sheets can be prepared using 500 mL of medium.