

Supplementary Information

Chitosan Imparts Better Biological Properties for Poly(ϵ -caprolactone) Electrospun Membranes than Dexamethasone

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Cell culture (before seeding)

Adipose-derived mesenchymal stem cells (ADSCs) are cultured at 5% CO₂ atmosphere in a 175 cm² surface area of polystyrene vented tissue-culture flasks at 37 °C. The cell growth media comprises Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum and 1% penicillin / streptomycin. The cells were seeded onto samples at a concentration of 5,000 cells *per* well in 48-well plates and cultured at 37 °C in 5% CO₂.

Adhesion and proliferation assay

The ADSC cell response on the membrane surfaces was investigated after 1 and 7 days of culture in growth media. Cell adhesion and proliferation tests were evaluated by staining the cells with 5-chloromethyl fluorescein diacetate (CMFDA; Life Technologies), rhodamine phalloidin, and 4',6-diamidino-2-phenylindole (DAPI; Invitrogen) to visualize cytoplasm, cytoskeleton, and nucleus, respectively, using a fluorescence microscope (Zeiss). Before staining, unadhered cells were aspirated and the substrates were gently rinsed (twice) with phosphate-buffered saline (PBS) before being transferred to a new 48-well plate. The samples were incubated (37 °C in 5% CO₂) with a 10 $\mu\text{mol L}^{-1}$ solution of CMFDA in PBS for 45 min. Following incubation, the solution was aspirated and the substrates incubated in growth media at 37 °C under 5% CO₂ for 30 min. The media was aspirated and the

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substrates rinsed (once) with PBS before transport to a new 48-well plate, where the cells were fixed in an aqueous 3.7% v/v formaldehyde solution for 15 min (25 °C). The fixative was aspirated and the substrates rinsed (thrice in PBS for 5 min *per* rinse) before addition to a new 48-well plate. The cells were permeabilized in an aqueous 1.0% v/v Triton X solution for 3.0 min (25 °C). This media was aspirated and the substrates rinsed (thrice), transferred to a new 48-well plate and incubated in an aqueous 5.0 $\mu\text{L mL}^{-1}$ rhodamine-phalloidin solution for 25 min at 25 °C. Then, an aqueous 1.0 $\mu\text{L mL}^{-1}$ DAPI solution was added to each 48-well plate and incubated for 5.0 min. The solution was aspirated, and the substrates rinsed with PBS (twice), stored in a PBS buffer and placed in a light-resistant container at 20 °C. Analysis of the fluorescence was performed with ImageJ software. Cell count numbers were statistically analyzed using Tukey's test at a 5% significance level.

Cell morphology

SEM was used to compare the attachment and morphology of cells on the scaffolds after cell culture assay. After proliferation and adhesion assays (1 and 7 days), the adhered cells to the samples were fixed in an aqueous 3.0% v/v glutaraldehyde, 0.10 mol L⁻¹ sodium cacodylate, and 0.10 mol L⁻¹ sucrose solution for 45 min (25 °C). Then, the samples were soaked for 10 min in a buffer solution based on 0.10 mol L⁻¹ sodium cacodylate and 0.10 mol L⁻¹ sucrose. Sample surfaces and cells were processed in sequential ethanol dehydration for 10 min each, following dehydration in hexamethyldisilazane (10 min) and stored in a desiccator until SEM imaging. SEM images were determined from the same conditions reported in the sub-section "Characterization".

Results (dexamethasone release)

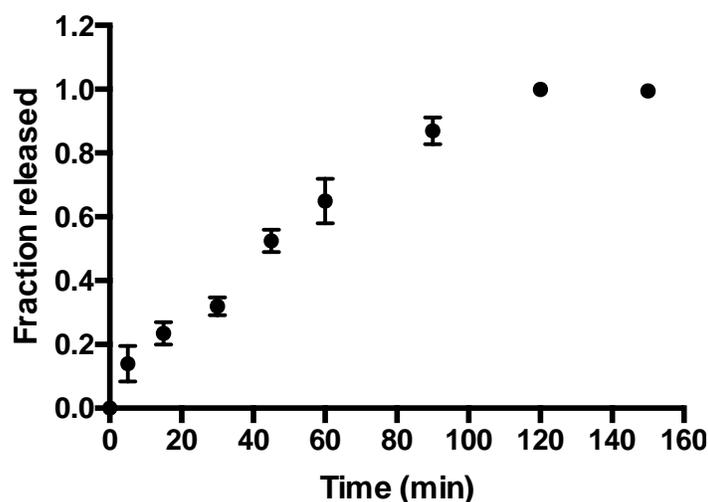


Figure S1. Release curve of dexamethasone obtained in terms of fraction released, which is obtained from poly(ϵ -caprolactone) loaded with dexamethasone. The release study is evaluated in PBS (pH 7.4) at 37 °C (n = 2).