Cell Proliferative and Antimicrobial Properties of Cactus Mucilage Composite Nanofibers through Forcespinning®

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Abstract

The development of natural nano-scale composed materials has potential for improved wound healing applications. Through the fabrication of Forcespinning® (FS) nanofibers composed of cactus mucilage (CM) combined with chitosan (CH)/pullulan (PL) (CM/CH/PL) biodegradable and biocompatible polymers, a homogenous nanofiber structure and a porous three-dimensional surface area was generated. By resembling the extracellular matrix (ECM), nanofiber materials incorporating bioactive components, may allow the improvement of cell adhesion and proliferation. In this study, the development of CM/CH/PL nanofibers with a fiber diameter average less than 406±127 nm and CH/PL were produced and examined for their ability to sustain adhesion and proliferation of 3T3 embryonic fibroblasts. After a 6 day incubation period CM/CH/PL nanofibers displayed robust 3T3 cell proliferation, while also preventing growth of *Escherichia coli* after 24 hrs. Combining compatible, biodegradable polymers with natural bioactive compounds may provide an enhanced environment for cell growth, with potential wound dressing applications.

Introduction

Nanofiber materials provides a porous three-dimensional environment resembling the extracellular matrix (ECM), with potential biocompatibility towards promoting cell growth within wound environments (1). Current wound healing complications include tissue damage and inflammatory response, which lead to the production of cytokines, free oxidative radicals, and other inflammatory signaling molecules associated with prevalent conditions including diabetes mellitus, cardiovascular disease, and aging (3). Cactus, *Opuntia* spp., mucilage (CM) extracts may contain phenolic and flavonoid compounds with beneficial anti-inflammatory and antibacterial properties (2). In developing FS CM composite nanofibers, CA was used as a crosslinking agent combined with CH as biodegradable and PL due to a fiber mechanical property, as previously shown in ternary composite nanofibers with supporting cell growth capabilities (4). With the properties of CM composite nanofibers, the possibility of cell adhesion and proliferation can be seen compared to CH/PL. In this study, an antibacterial analysis, 3T3 cell adhesion and proliferation is compared with CH/PL and CM composite nanofibers for possible wound healing applications.

Results/Discussion

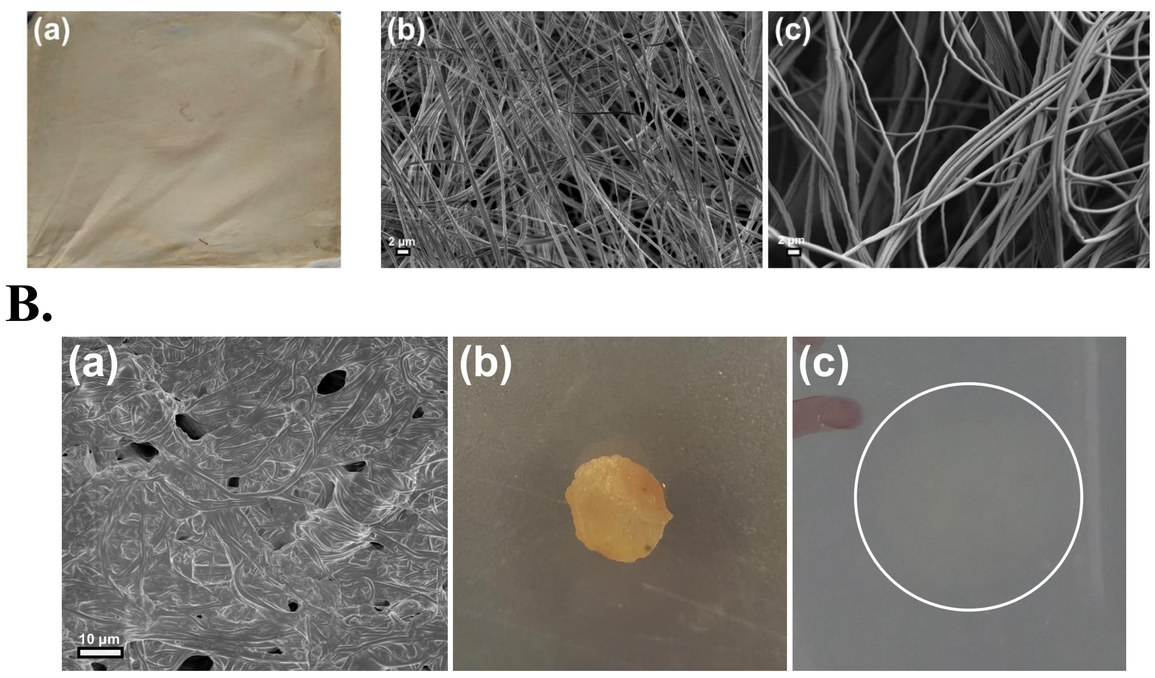

Figure 1 SEM and antibacterial characterization: A. FS CM/CH/PL nanofiber mats (a) are displayed, while the morphology for CH/PL-260±82 nm (b) and C100-406±127 nm (c) nanofibers is shown. Scale bar = 2 µm. (2000X Mag.). **B**. Membrane morphology (1000X Mag.) (a) and images from an antibacterial analysis of DN100 (b) compared to N extract (white circle) (c) are shown against *E. coli*. Scale bar = 10 µm.

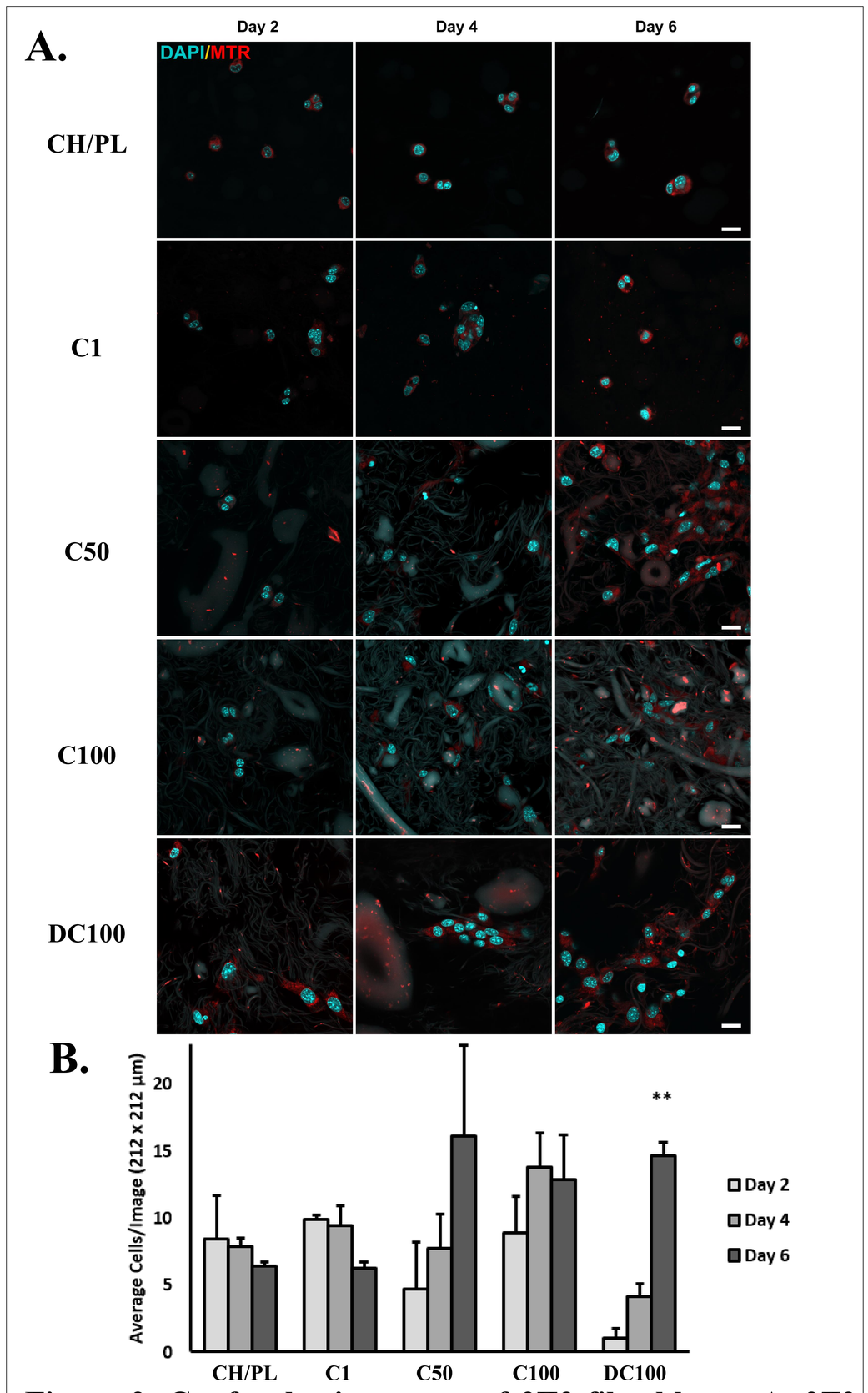

Figure 2. Confocal microscopy of 3T3-fibroblasts: A. 3T3 mouse embryonic fibroblasts were incubated for 6 days. C1/CH/PL (C1), C50/CH/PL (C50), C100/CH/PL (C100), and CM dip-coated C100 (DC100). DAPI (blue) is for cell nucleus and Mito Tracker Red® (MTR) for mitochondria. (60x magnification)(Scale bar = 20 µm). **B**. Quantification of cells/image. n = 3 Expts. An ANOVA/Post-hoc Tukey statistical analysis is shown measuring the standard error of the mean +/- (SEM). (** P - value < 0.01).

FS CM composite nanofibers provide a three-dimensional porous matrix (**Fig. 1A**), while CM dip-coating of DC100 fibers prevents bacterial growth (**Fig. 1B**). CM composite nanofibers significantly support cell adhesion and proliferation (**Fig. 2A,B**), suggesting the possibility of using nanofibers as wound dressings with a decreased infection, and improved wound healing rate. Further, a cell proliferation analysis will be conducted through an extended time course in order to observe cell adhesion in both CH/PL and CM composite nanofibers. A Resazurin test will also be conducted in order to evaluate the cell viability of present adherent cells on CH/PL and CM composite nanofibers.

Acknowledgments/References

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