Physical Properties of Hyaluronan Impacts Epithelial-to-Mesenchymal Transition

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Introduction

Epithelial-to-mesenchymal transition (EMT) is well studied biological process that occurs during embryonic development, wound healing, and cancer metastasis. Type II EMT occurs specifically during wound healing [1]. There are notable differences in the wound healing environment of embryos and adults. The ECM of an embryo contains more hyaluronan (HA) and type III collagen fibers. Adult wound healing ECM is characterized by having more type I collagen fibers which are thicker and more aligned, with lower levels of HA [2]. HA is an ECM component that is known to interact with collagen fibers [3]. While the effect of different collagen fiber topographies on wound healing is well understood, the effect of HA with different topographies remains unknown. To address this gap, we created synthetic matrix analogs that are capable of representing different HA topographies utilizing hyaluronan binding peptide (HABP). We further investigated the role of underlying HA topography on EMT. The results of this study will shed light on the role of HA organization on EMT during embryonic development, wound healing, and cancer metastasis.

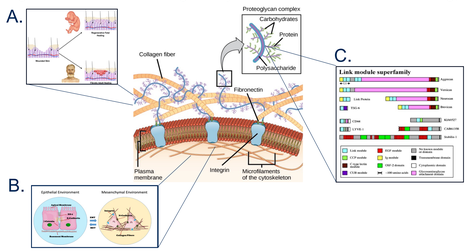


Fig. 1. HA is a unique molecule with diverse functions. (A). Elevated levels of HA is a hallmark of scarless healing in developing embryos. (B). Differences in fiber diameter cause epithelial cells to change their morphology. (C). Native HA is a highly crosslinked molecule and several proteins have a shared LINK molecule sequence to form bonds with HA.

Methods

Making scaffolds and peptide conjugation

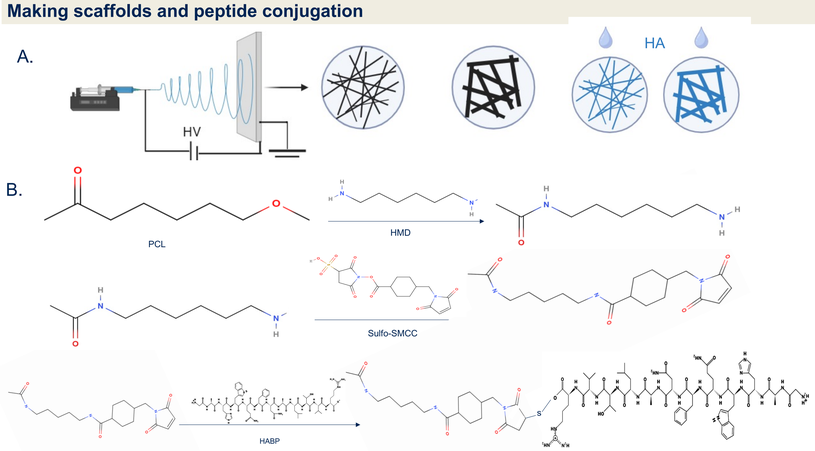


Fig. 2. (A). This figure depicts the electrospinning set-up used (B). This figure shows the chemical reaction that occurs when HABP and Scr HABP are added to PCL scaffolds

Methods

Cell Culture & Cell Seeding onto Scaffolds

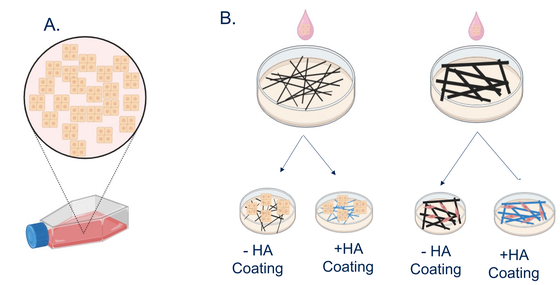


Fig. 2. (A). MCF-7 cells used as a model cell type to reflect EMT. The cells were cultured in T-25 flasks until they were 80% confluent. The cells were then split and used for experiments. (B). One hundred thousand cells were seeded onto scaffolds after UV sterilization. The cells were cultured for 48 hours on scaffolds and then they were used for fluorescence imaging where the nucleus and f-actin were stained. Half of the scaffolds were coated with exogenous HA while the other half were not.

Results

Material Characterization

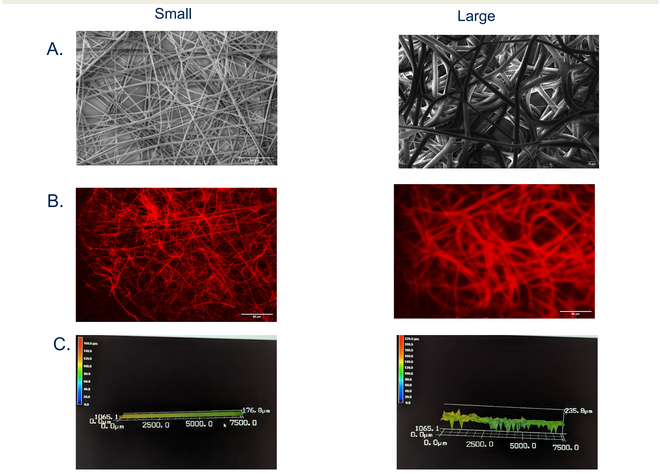
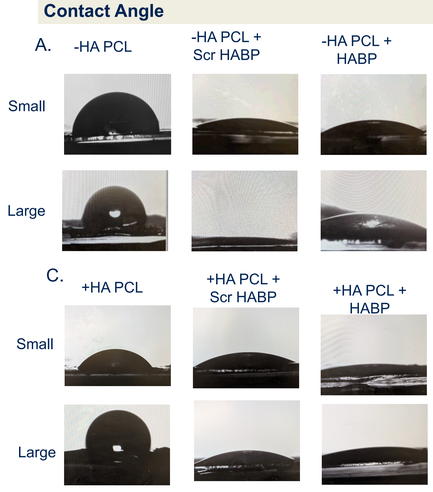


Fig. 2. Material Characterization of PCL electrospun fibers (A). SEM images of small diameter fibers with an average diameter of 0.553577 ± 0.151514µm (left) and large diameter with an average diameter of 4.420268 ± 1.324585µm (right). (B). Auto fluorescence of small diameter (left) and large diameter (right) fibers. (C). Profilometry images of small diameter fiber mats (left) and large diameter fiber mats (right). Small diameter fibers have a height of 176.8µm and large diameter fibers have a height of 235.8µm.

Results

Contact Angle



B.

	-HA PCL	-HA PCL + Scr HABP	-HA PCL + HABP
Small	75.78 ± 24.78°	8.74 ± 15.14° **	8.72 ± 15.10° **
Large	89.90 ± 0.20°	0 ± 0° **	15.04 ± 26.05° **

D.

	+HA PCL	+HA PCL + Scr HABP	+HA PCL + HABP
Small	76.98 ± 22.62°	16.47 ± 14.28° **	0 ± 0° **
Large	78.16 ± 13.03°	12.24 ± 21.19° **	8.75 ± 15.16° **

Fig. 3.(A) Water contact angle results for scaffolds without HA coating with (B) average contact angle ± standard deviation. (C) Water contact angle with HA coating with (D) average contact angle ± standard deviation. (** $p < 0.005$)

Results

Cell Shape

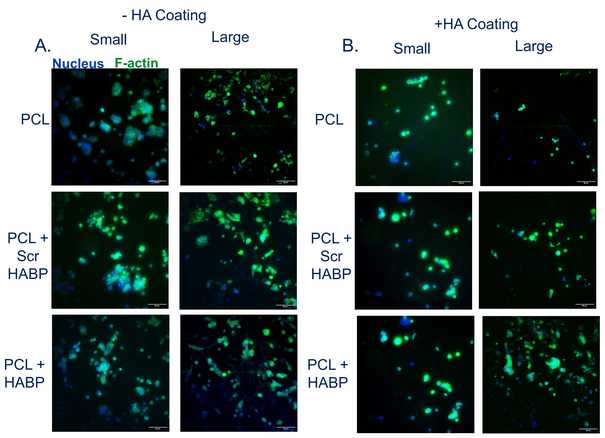


Fig. 4. Fluorescence imaging of MCF-7s cultured on scaffolds for 48hr. (A). MCF-7s cultured on large and small diameter PCL fibers without exogenous HA coating. (B). MCF-7s cultured on large and small diameter PCL fibers with exogenous HA coating.

Conclusions and Ongoing Work

- Mesenchymal morphology on large diameter fibers
- The addition of the peptides cause the scaffolds to be more hydrophilic
- The large diameter fibers have a rougher surface than small diameter fibers
- The peptide-treated scaffolds does not cause cell death to seeded cells, but further optimization needs to be done
- In the future western blots will be done to determine how EMT markers change in the presence of HABP
- Further optimization of large diameter fibers will be done
- The affinity of HA to HABP will be measured using atomic force microscopy (AFM)
- More trials using MCF7 cells will be done
- This study will be repeated with dermal keratinocytes
- Aligned elecrospun PCL fibers of two different diameters will be made to study the different between the effects of aligned HA fibers vs random HA fibers on EMT

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References

Blake, B., & Ozdemir, T. (2023). Developing Fibrous Biomaterials to Modulate Epithelial to Mesenchymal Transition. *Cells Tissues Organs*. https://doi.org/10.1159/000530712

Degen, K. E., & Gourdie, R. G. (2012). Embryonic wound healing: a primer for engineering novel therapies for tissue repair. *Birth Defects Res C Embryo Today*, *96*(3), 258-270. https://doi.org/10.1002/bdrc.21019

Frenkel, J. S. (2014). The role of hyaluronan in wound healing. *Int Wound J*, *11*(2), 159-163. https://doi.org/10.1111/j.1742-481X.2012.01057.x

Ravikrishnan, A., Ozdemir, T., Bah, M., Baskerville, K. A., Shah, S. I., Rajasekaran, A. K., & Jia, X. (2016). Regulation of Epithelial-to-Mesenchymal Transition Using Biomimetic Fibrous Scaffolds. *ACS Appl Mater Interfaces*, *8*(28), 17915-17926. https://doi.org/10.1021/acsami.6b05646