CHARACTERIZATION OF AN IN VITRO SKIN EXPANSION MODEL BASED ON ELECTROSPUN MEMBRANES

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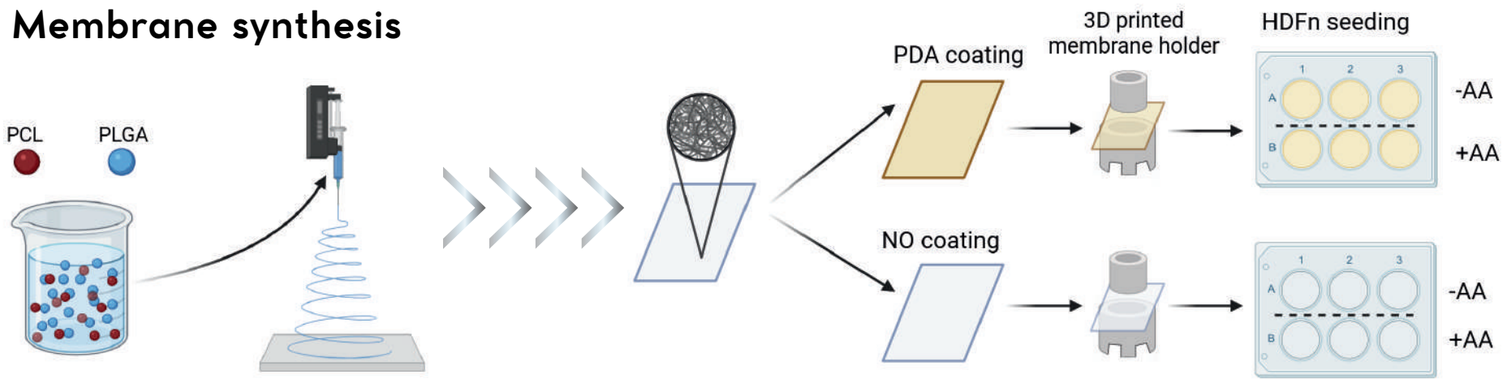
Introduction

The main objective of this internship was to develop a biomimetic Polylactic-co-glycolic acid/Polycaprolactone (PLGA/PCL) electrospun membrane seeded with cells that mimics the skin properties in order to subsequently test in vitro a 4D-printed shape-shifting device for skin expansion. The hypothesis of this work was that coating the membranes with polydopamine (PDA) would increase their hydrophilicity and thus cell attachment, and that increasing the concentration of ascorbic acid (AA) in the cell medium would stimulate the collagen production and favor the cell growth and attachment.

Materials and methods

A 10%(w/v) mix between PLGA and PCL in a 70:30 proportion was dissolved in hexafluoropropanol (HFP) and then extruded in an Electrospinning machine to synthesize the membranes. Some of these membranes were coated with PDA. Neonatal human dermal fibroblasts (HDFn) were seeded in the membranes, and 2 concentrations of AA were tested in their medium: regular concentration of 50uM (-AA), and a higher one of 100uM (+AA). After 4 days results were analyzed using immunofluorescent stanining in the confocal microscope and in the optical microscope.

Membrane synthesis



SEM characterization

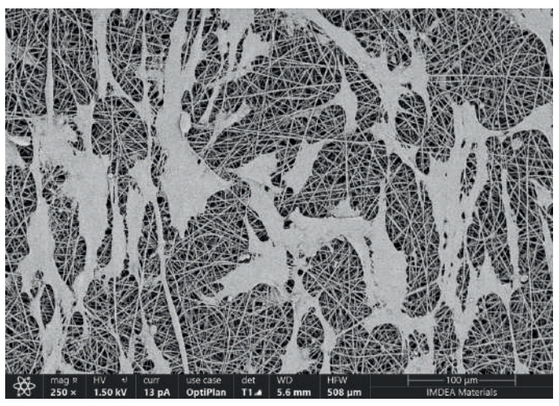


Figure 1: Characterization using scanning electron microscopy (SEM) of an electrospun membrane seeded with HDFn cells

Results and discussion

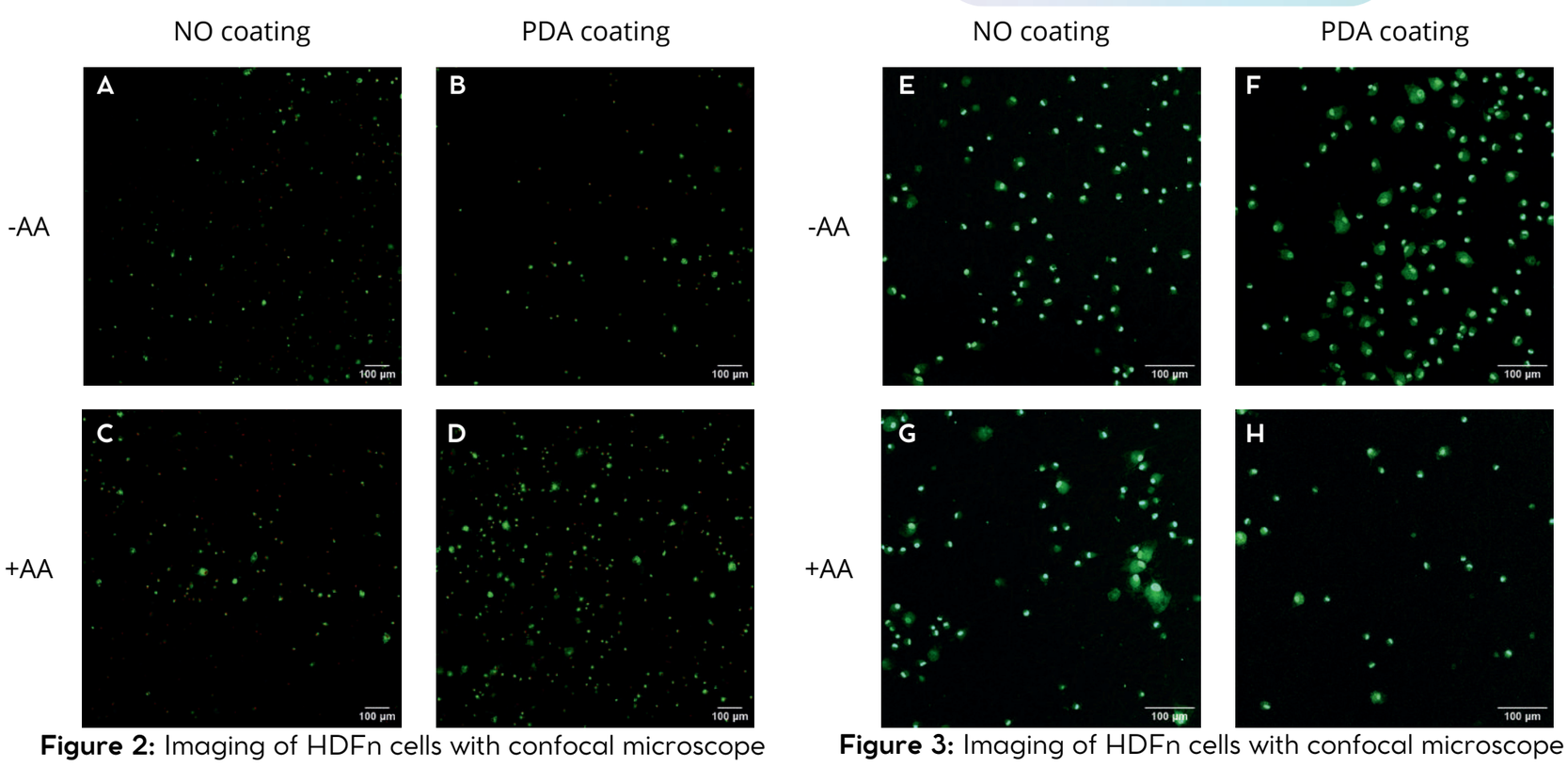


Figure 2: Imaging of HDFn cells with confocal microscope staining live (green) and dead (red) cells. **(A)** Membrane without coating and without additional AA **(B)** Membrane with PDA coating and without additional AA **(C)** Membrane without coating and with additional AA **(D)** Membrane with PDA coating and with additional AA

Figure 3: Imaging of HDFn cells with confocal microscope using DAPI (cyan) for staining of nuclei and Phalloidin (green) for staining of the cytoskeleton. **(E)** Membrane without coating and without additional AA **(F)** Membrane with PDA coating and without additional AA **(G)** Membrane without coating and with additional AA **(H)** Membrane with PDA coating and with additional AA

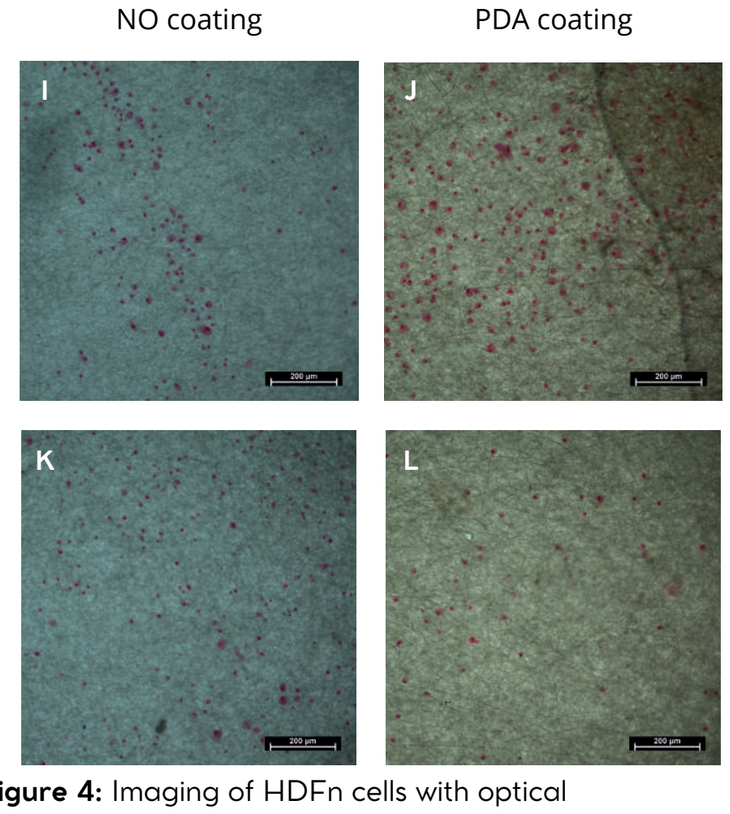


Figure 4: Imaging of HDFn cells with optical microscope staining collagen with Picrosirius red dye. **(I)** Membrane without coating and without additional AA **(J)** Membrane with PDA coating and without additional AA **(K)** Membrane without coating and with additional AA **(L)** Membrane with PDA coating and with additional AA

Conclusions

- Live/Dead results suggest a synergistic effect between PDA coating and higher AA concentration.
- DAPI/Phalloidin results appear inconclusive regarding morphology.
- Picrosirius staining indicates that longer culture periods may be necessary to observe substantial collagen deposition.

Future directions

- Repeat the experiment to further validate the proposed synergistic relationship between PDA and AA.
- Increase cell culture duration to better assess long-term collagen synthesis and ECM production induced by AA and how it influences cell growth and morphology
- Perform co-culture experiments with human dermal fibroblasts (HDFn) and neonatal human epidermal keratinocytes (HEKn) to evaluate the combined effects of PDA and AA on cell viability and growth.

References

Pelaez Morales, M. (2025). Desarrollo y caracterizacion de membranas hibridas de PLGA/PCL obtenidas por electrospinning para el analisis de expansion in-vitro de dispositivos biomedicos con memoria de forma https://hdl.handle.net/20.500.12880/15365

Ángela Castro María. (2026) Desarrollo de biomateriales avanzados para plataformas in vitro de pulmón y piel, UC3M Programa de Doctorado en Ciencia y Tecnología Biomédica.

Description of the center/group

IMDEA materials institute is an international research center focused on advanced materials and their applications in different fields. Dr. Jennifer Patterson leads the biomaterials and regenerative medicine group, participating in various European projects such as BIOMET4D. This particular project aims to develop 4D printed shape-morphing medical devices for skin expansion and for craniosynostosis using biometals.

Significance regarding biomaterials

In this tissue engineered model, the electrospun membrane acts as a biocompatible scaffold, which is then seeded with HDFn cells. Using factors such as AA and PDA, tissue reconstruction is enhanced in order to achieve this in vitro skin model for posterior testing of the medical devices.

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