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First conference paper presented

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Project coordinator name	Prof. Richard M Hall
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Report prepared by	Prof. Richard M Hall Judith Schneider Andre Souza Plath

Dissemination Level of Report

PU	Public	x
PP	Restricted to other program participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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Version	Date	Comment	Modifications made by
D7.4.1	19-10-23	First draft	Created/Edited Richard M Hall
D7.4.1	19-10-23	First draft sent to Andre Souza Plath, Judith Schneider and Scientists in charge.	
D7.4	**23-10-203**	**Submitted**	Judith Schneider

Introduction

The dissemination and exploitation of the new knowledge and innovation developed not just in BioTrib, but within Horizon Europe activity as a whole, is critically important to the economic and social well-being of the community. It is particularly important for the health sector where the public expects investments to lead to tangible benefits both in the short and long-term. As such the BioTrib European Training Network is tasked with producing outputs that are of benefit to academia, industry and the wider community as well as for the clinical sector in this activity.

The Network has a stratified approach to disseminating the new knowledge and innovation which is set out in the table below.

Stakeholders and Dissemination Channels.

Stakeholder[1]	Information type	Vehicle	Involvement/Activity Level
Programme partners	Overview mgmt. information	Project website, supervisory board [ALL ESRs]	Regular from beginning of project.
Supervisory Board	Detailed Science and Technology Information	Supervisory Board Meetings and Briefings. [ALL ESRs]	Engaged from beginning. Kick off meeting then meets every 2 months.
General Public incl. patients	General inform. builds trust, creates interest	Website, workshops, Impact summaries [ALL ESRs]	Engaged from beginning. Science fairs panels. ESR Use of Twitter and/or Blogs
Clinical Opinion Leaders	S&T information	Symposia, Conferences & Publications. [ALL ESRs]	From year 3 participation in conferences and other clinical meetings. Supervisory Board
Regulation. and Standards	Detailed S&T evidence	Symposia, Conferences [ALL ESRs]	Year 3 & 4 - Clinicians, Technical Personnel, Regulators
Academic and Scientific Commun.	Detailed S&T evidence	Conferences, Journal publications [ALL ESRs]	Conferences from 2nd year of ESR and publications from 3rd year of ESR. 1 output/ESR/year from 2nd year

The ESRs have thus far produced a range of dissemination activities focused at different stakeholders particularly LinkedIn and other social media channels.

First Conference publication

The first conference publication was delivered by Andre Souza Plath from ETH Zurich. The details of the conference publication can be found here:

Plath, A.M.S., Greutert, H., Alfarano, S.R., Abbott, D., Mezzenga, R., Mougel, V. and Ferguson, S.J.	Poly (e-caprolactone) process parameter optimized nanofibres promote the growth of 3T3 human cells	32nd Conference of the European Society for Biomaterials	04.09 – 08.09.2022, Bordeaux, France

Andre on attending the conference noted that he gained considerable positive experience from presenting his work and was of the opinion that:

> '*Participating at ESB2022 in Bordeaux allowed me to extend my network and meet former colleagues. This experience also inspired future work directions and increased my curiosity in relevant biopolymer topics (e.g., non-fouling materials, cell-on-chip). The discussions and feedback I received there helped me crafting the first peer-review manuscript that was published later in June 2023'.*

The conference abstract can be found in Appendix 1 and the poster presentation in Appendix II. Further research in the area led to this abstract being enhanced and submitted for publication, This submission has now been published and has the following doi:

https://doi.org/10.3390/bioengineering10070771

[1] Abbreviation: S&T – science and technology.

Appendix I – Abstract submitted to and presented at the ESB conference.

Poly (ε-caprolactone) process parameter optimized nanofibers promote the growth of 3T3 human cells

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Keywords: Nanofibers, scaffolds, wound-healing.

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INTRODUCTION

Electrospinning is a nanomaterials fabrication technique that produces cost-effective high surface-to-area biomaterials. Electrospun materials are versatile extracellular matrix (ECM) mimicking materials with tunable fiber diameters for a wide range of applications. The ECM is a fibrous natural structural and mechanical support that provides cells with biological, physical, and chemical signaling. In the literature, poly (ε-caprolactone) (PCL) electrospun materials have shown good cell compatibility for human fibroblasts, cartilage, mesenchymal stem cells, and other tissues. The optimization of nanofiber topology and diameter is strongly related to the envisioned applications of nanofibrous mats. In previous works, fiber diameter influenced mechanotransduction and the cell differentiation path. Herein, we sought to obtain with an experimental design uniform, reproducible cytocompatible nanofibers based on a binary system and a fixed content of weight per volume of poly (ε-caprolactone) for a versatile set of applications.

EXPERIMENTAL METHODS

Mats were electrospun using an experimental design varying the acetic acid content, voltage, distance to the collector, and flow rate (Figure 1). The experiments were planned with a 2^4 factorial design using the software JMP Pro 14.0. Surface morphology was assessed with scanning electron microscopy with a voltage of 3kV in the samples previously coated with an 80/20 Platinum-Palladium alloy. Spectroscopy (FTIR-ATR and XPS) studies were conducted on the surface of the electrospun mats to assess chemical alterations after the solubilization and electrospinning of the polymers. FTIR spectroscopy was performed for 16 scans and a resolution of 4 cm^{-1} from 4000 to 600 cm^{-1}. X-ray photoelectron spectroscopy was performed with 200W power and HR spectra were deconvoluted with OriginPro 2021 using Shirley Background to minimize χ^2. Cell viability, proliferation, and morphology were examined at day 5 for 3T3 fibroblasts seeded at 5000 cells/cm^2.

RESULTS AND DISCUSSION

Within the range, most solutions were electrospinnable, but most of them presented jet splitting, conglutination, thus, low reproducibility. The nanofibrous mats had an average fiber diameter within the 124 to 289 nm range. These results are compatible with reported pure PCL electrospun in a Formic/Acetic acid binary solvent by Ekram (2019). Two process parameters also yielded beads-on-string mats. Process parameters have shown increasing acetic acid content and collector distance promote finer nanofibrous structures. Contrary to the initial hypothesis, electrospinning voltage increase did not promote finer nanofibers. We report here microscopy images of sample 11, the most reproducible parameter produced, that was also characterized for its surface chemistry and cell viability. FTIR-ATR and XPS characterizations showed no significant hydrolysis of the ester bonds in PCL after solubilization in the Formic or Formic/Acid solvent system, an important parameter that impacts the mechanical properties of the mats. Finally, the nanofibrous electrospun mats were characterized according to their cytocompatibility. Cell staining of 3T3 fibroblast cells with DAPI and phalloidin showed cell viability, spreading, and proliferation after 5 days. The actin filaments, stained with Alexa Fluor 568, showed a significant spreading of the actin filaments and the clustering of cells.

RUN	DISTANCE (CM)	VOLTAGE (kV)	FEED RATE (μL·H⁻¹)	ACETIC ACID (VOL.%)	FIBER DIAMETER (NM)
1	12	18	750	0	171 ± 17
2	12	12	325	30	171 ± 14
3	12	18	325	0	289 ± 40
4	16	12	750	30	180 ± 10
5	16	18	750	30	*
6	12	12	750	30	124 ± 13
7	12	12	750	0	*
8	16	12	750	0	*
9	16	18	325	30	265 ± 37
10	16	18	750	0	076 ± 12**
11	12	18	325	30	156 ± 13
12	16	12	325	30	173 ± 15
13	16	12	325	0	*
14	16	18	325	0	046 ± 05 **
15	12	18	325	30	182 ± 20
16	12	12	325	0	*

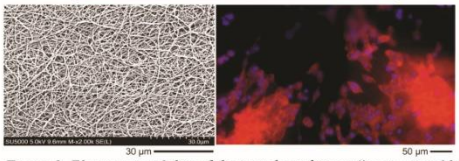

Figure 1. Electrospinnability of the tested conditions (not spinnable, ** beads-on-string), SEM, and inverted microscopy images of 3T3 cells on day 5 for sample 11 (5000× and 20× magnification, respectively.*

CONCLUSION

Distance to the collector and acetic acid content were the most significant parameters affecting uniformity of nanofiber mats. Applications are envisioned for fibroblast compatible materials such as artificial blood vessels and wound dressings. Other tissue engineering applications can be proposed upon the conjugation of other macromolecules to the electrospun surfaces.

ACKNOWLEDGMENTS

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 956004. We also thank ScopeM and partner laboratories for the characterizations.

ESB 2022
32TH ANNUAL CONFERENCE OF THE EUROPEAN SOCIETY FOR BIOMATERIALS

Appendix II – Poster presented at the ESB

ETH zürich

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Poly(e-caprolactone) process parameter optimized nanofibers promote the growth of 3T3 fibroblast cells

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Abstract

Poly(e-caprolactone) (PCL) is an aliphatic polyester with good biocompatibility and resorption rates between 2 and 4 years, making it suitable for many tissue engineering purposes. To increase interactions with cells, we aimed to reduce nanofiber diameter. For this, we executed 16 electrospinning experiments varying voltage (kV), distance of spinneret-collector (cm), feed rate (mL·h^{-1}), and formic/acetic acid content. We obtained uniform nanofibrous mats varying from 124 to 289 nm. Beads-on-string mats were produced on the electrospun formic acid system, showing that, despite the higher conductivity, the system could not produce uniform fibers. FTIR-ATR showed a characteristic absorbance peak at 1722cm^{-1} characteristic of the ester bonds. XPS survey showed an approximate 75% to 25% C1s to O1s rate. C1s high-resolution spectra show ester, -C-O, and adventitious carbon bonds at 288.8,286.5, and 285.0 eV respectively. Finally, an optimized condition was used for surface characterizations and cell 3T3 cultures. Fluorescence shows cell clustering after 10 days of culture and confluence on day 14. These results show the potential of nanofibrous mats for wound dressings and cardiac tissue engineering.

Experimental Design

◆ Methods

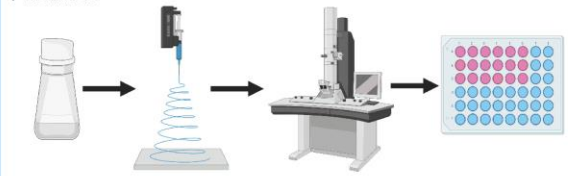

Figure 1. Workflow of the project. (1) Solubilization of PCL (Sigma Aldrich Mw 80kDa in FA or FA/AA (2) electrospinning varying feed rate, collector distance, and voltage (IME EC-CLI) (3) SEM (SU Hitachi 5000), XPS (Thermo Fisher Alpha 110), and FTIR-ATR (Varian Agilent), and (4) 3T3 fibroblast cultures

◆ Nanofiber Optimization (Factorial design)

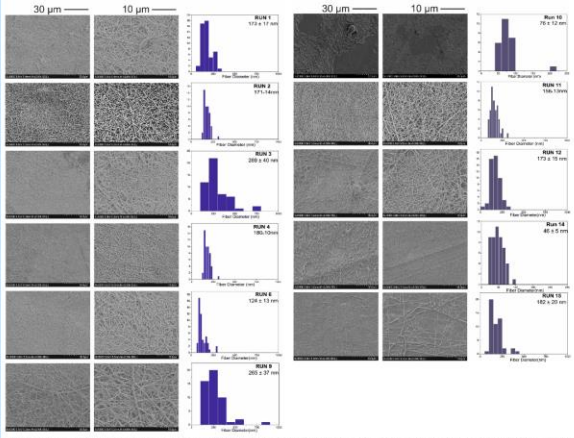

Figure 2: SEM images (2000× and 5000×) obtained from the nanofiber mats coated with Pt-Pd (80:20) of the experimental design varying distance, voltage, feed rate, and acetic acid content. Fiber diameters (n =50 measurements) and 95% CI made calculated with GraphPad Prism 9.0. Sample 11 was the most reproducible.

RUN	DISTANCE (CM)	VOLTAGE (KV)	FEED RATE (µL.H^{-1})	ACETIC ACID (VOL.%)	FIBER DIAMETER (NM)
1	12	18	750	0	173 ± 17
2	12	12	325	30	171 ± 14
3	12	18	325	0	289 ± 40
4	16	12	750	30	180 ± 10
5	16	18	750	30	*
6	12	12	750	30	124 ± 13
7	12	12	750	0	*
8	16	12	750	0	*
9	16	18	325	30	265 ± 37
10	16	18	750	0	076 ± 12**
11	12	18	325	30	156 ± 13
12	16	12	325	30	173 ± 15
13	16	12	325	0	*
14	16	18	325	0	046 ± 05 **
15	12	18	325	30	182 ± 20
16	12	12	325	0	*

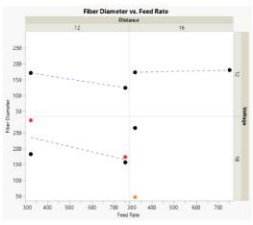

Figure 3: Left-hand side: table with the experimental conditions * not spinnable, ** beads-on-string morphology, and right-hand side: graph with the effect of variables on the main parameters (red dots 100% formic acid and orange dot beads-on-string morphology). Experimental design and graphs made with JMP Pro 14.0.

Characterizations and Cell Cultures

◆ Surface Analysis (FTIR-ATR and XPS)

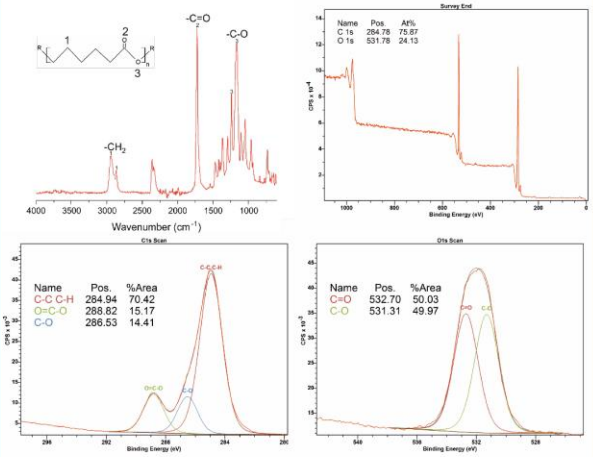

Figure 4 Top-right: FTIR-ATR spectra of the electrospun membrane (n=32 scans)
Top-left: XPS survey of the electrospun membrane (Al source, 100W, 9 scans)
Bottom-left: C1s high-resolution spectra of the electrospun membrane (81 scans)
Bottom-right: O1s high-resolution spectra of the electrospun membrane (81 scans)
*XPS data normalized to adventitious carbon to 285.0 eV with the software CasaXPS

◆ Cell culture (DAPI and f-actin staining)

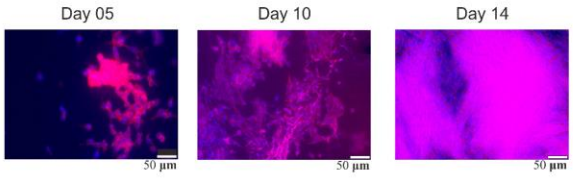

Figure 5: 20× Magnification fluorescence microscopy of the 3T3 fibroblast cells stained on days 5, 10, and 14 seeded on a representative surface (sample 11).

Conclusions

- Fiber diameter was optimized within a process parameter space. Results are comparable to the literature.
- Fibroblast cells had a great affinity for the structures making them suitable for wound dressings or artificial blood vessels.

Acknowledgements

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Grant Agreement N° 956004

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