

**Electrospun extracellular matrix from decellularized Wharton's Jelly for Artificial Skin Grafts**

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**Summary:**

This is a request for the funding of an advanced project focusing on artificial skin grafts. The goal would be to determine a process to produce extracellular matrices that can be fabricated via a process called electrospinning. These matrices would be made from a decellularized matrix from Wharton's Jelly, a material that has showed promising results in the tissue engineering field. If the grant is awarded, the student (Kyle Jerreld) working on the project has spent nearly a year working on tissue engineering related projects and has worked intimately with the processes and materials that will be used. The student's goal is to pursue a PhD in the tissue engineering field, this project would help to further bolster this student's research portfolio and allow them the opportunity to gain invaluable experience that they can apply in future research.

**Introduction:**

The skin is the largest organ in the body and is vital to our survival as it protects us from outside intruders. Many afflictions of the skin that become chronic can pose a legitimate risk to one's survival. For that reason, skin grafts—both autologous and non-autologous—are used to remedy the burns, chronic wounds etc. However, autologous grafts are often less than ideal since they need to be taken from another portion of the patient and in patients with large portions of effected skin, as with many burn victims, there are often few good locations to graft from. That makes non-autologous grafts ideal if not necessary, the problem with non-autologous grafts is that they don't help to regrow skin appendages (sebaceous glands, hair follicles) which are important to healthy skin function [1].

Recent studies have shown the benefits of using decellularized extracellular matrices (dECM) for the fabrication of custom grafts which there are a variety of applications for [2]. A popular source for this dECM is Wharton's Jelly, a constituent of the human umbilical cord [2]. An extracellular matrix (ECM) that is derived from decellularized human tissues have shown greater potential for culturing mesenchymal stem cells (MSC) than artificial matrices [3]. MSC's when cultured in the proper microenvironment which have shown the ability to properly differentiate into skin appendages [4].

Electrospinning is a technique that is commonly used to produce artificial ECM. It has also shown the ability to extrude dECM in conjunction with other artificial polymers such as PLLA [5]. Electrospinning is an ideal fabrication method because it allows for a high level of control over matrix properties which is essential to creating the proper microenvironment for proper MSC differentiation.

**Objective:**

Produce an artificial ECM that can successfully culture MSC's. The optimization of differentiation of MSC's into skin appendage cells is out of scope for this study, simply the ability to culture MSC's successfully on the ECM.

## Methods:

1. **Electrospinning:** Using an electrospinning setup with environment control (temperature and humidity) the dECM from Wharton's Jelly along with a variety of co-polymers (PCL, PLLA, PLGA, or Gelatin).
2. **Analytic Techniques:**
  - a. Scanning electron microscopy (SEM): High resolution electron microscopy will allow for visualization of the morphology of the matrices.
  - b. Uniaxial Mechanical Testing: Allow for characterization of the mechanical properties, namely, stress/strain curve and by proxy elastic modulus.
  - c. Fourier Transform Infrared Spectroscopy (FTIR): Confirmation of the constituent polymers and other native factors that remain of the dECM
  - d. Rhodamine/Phalloidin Staining: Visualization of actin which indicates cell adhesion to the matrix (confocal imaging).
  - e. Live/Dead Cell Staining: Will help determine if the matrices are cytotoxic or have any other properties that cause cell death.
  - f. Cell culture/proliferation assays: Determine whether the MSC's can simply survive or proliferate on the different matrices

## Materials:

A list of published literature that has helped inspire the study and will subsequently help to guide it is included in the references section. Other literature will be used as needed and referenced in the reporting of the findings at the end of the study. The research will primarily be carried out in the Department of Biomedical Engineering research labs in Institute Hall at the Rochester Institute of Technology.

## Schedule:

|         |                                                          |
|---------|----------------------------------------------------------|
| Week 1  | Bulk harvesting and decellularization of Wharton's Jelly |
| Week 2  | Literature review and begin electrospinning matrices     |
| Week 3  | Optimization of co-polymer system                        |
| Week 4  | Optimization of electrospinning parameters               |
| Week 5  | Buffer week for delays in optimization                   |
| Week 6  | Begin chemical analytic techniques                       |
| Week 7  | Live/Dead and Proliferation Assays                       |
| Week 8  | Buffer week for troubleshooting issues with MSC's        |
| Week 9  | Confocal Imaging                                         |
| Week 10 | Aggregation of results and creation of poster            |

**Budget:**

| Materials             | Price       | Quantity     | Total Cost          |
|-----------------------|-------------|--------------|---------------------|
| <b>Polymers</b>       |             |              |                     |
| PCL                   | \$ 500.00   | 1            | \$ 500.00           |
| PLLA                  | \$ 600.00   | 1            | \$ 600.00           |
| PLGA                  | \$ 500.00   | 1            | \$ 500.00           |
| <b>Solvents</b>       |             |              |                     |
| HFIP                  | \$ 800.00   | 1            | \$ 800.00           |
| Ethanol               | \$ 50.00    | 1            | \$ 50.00            |
| Consumables           | \$ 1,000.00 | n/A          | \$ 1,000.00         |
| <b>Staining</b>       |             |              |                     |
| Rhodamine/Phalloidin  | \$ 200.00   | 1            | \$ 200.00           |
| Live/Dead Cell Kit    | \$ 250.00   | 1            | \$ 250.00           |
| <b>RIT Facilities</b> | \$ 2,000.00 | 1            | \$ 2,000.00         |
| <b>Salary</b>         | \$ 6,000.00 |              | \$ 6,000.00         |
|                       |             | <b>Total</b> | <b>\$ 11,900.00</b> |

**Biographical:**

Dr. Karin Wuertz-Kozak and post doctorate fellow Dr. Andrea DePieri have extensive experience in the tissue engineering and biomaterials fields. Dr. Andrea DePieri will be more involved than primary investigator Dr. Karin Wuertz-Kozak. Dr. DePieri has spent his time in his post doctorate fellowship electrospinning scaffolds for research involving skin conditions related to scleroderma. Primary researcher Kyle Jerreld completed an 8-month research co-op in which he was using a method akin to electrospinning called electrospraying and was assisting Dr. DePieri with his electrospinning research. Kyle Jerreld has also been a teaching assistant for the Intro to Biomaterials course at the Rochester Institute of Technology and has taken multiple biochemistry, cell biology, and tissue engineering courses.

**Conclusion:**

This research would provide a jumping off point for the optimal ECM for culturing MSC's with the goal of producing an artificial skin graft that would also have functioning skin appendages. This would be a step towards the development of a product that is much needed in the dermatology field. It would also give primary research experience to a student who would hope to continue this path of research during their graduate studies.

## References:

- [1] Przekora, A. (2020). A concise review on tissue engineered artificial skin grafts for chronic wound treatment: Can we reconstruct functional skin tissue in vitro? *Cells*, 9(7), 1622. <https://doi.org/10.3390/cells9071622>
- [2] Penolazzi, L., Pozzobon, M., Bergamin, L. S., D'Agostino, S., Francescato, R., Bonaccorsi, G., De Bonis, P., Cavallo, M., Lambertini, E., & Piva, R. (2020). Extracellular matrix from decellularized Wharton's jelly improves the behavior of cells from degenerated intervertebral disc. *Frontiers in Bioengineering and Biotechnology*, 8. <https://doi.org/10.3389/fbioe.2020.00262>
- [3] Shakouri-Motlagh, A., O'Connor, A. J., Brennecke, S. P., Kalionis, B., & Heath, D. E. (2017). Native and solubilized decellularized extracellular matrix: A critical assessment of their potential for improving the expansion of mesenchymal stem cells. *Acta Biomaterialia*, 55, 1–12. <https://doi.org/10.1016/j.actbio.2017.04.014>
- [4] Li, H., Fu, X., Ouyang, Y., Cai, C., Wang, J., & Sun, T. (2006). Adult bone-marrow-derived mesenchymal stem cells contribute to wound healing of skin appendages. *Cell and Tissue Research*, 326(3), 725–736. <https://doi.org/10.1007/s00441-006-0270-9>
- [5] Young, B. M., Shankar, K., Allen, B. P., Pouliot, R. A., Schneck, M. B., Mikhael, N. S., & Heise, R. L. (2017). Electrospun decellularized lung matrix scaffold for airway smooth muscle culture. *ACS Biomaterials Science & Engineering*, 3(12), 3480–3492. <https://doi.org/10.1021/acsbiomaterials.7b00384>