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Investigation of adherent 3T3 cell line growth on electrospun polyacrylonitrile–polyethylene oxide (PAN-PEO) nanofiber nonwovens with varying material ratios

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Introduction & Aim

- The fourth industrial revolution encompasses fields such as nanotechnology, biotechnology, and new materials among other things [1]
- Tissue engineering overlaps with these three fields when nanofibers are used as tissue, which is promising due to their advantageous high surface-to-volume ratio in terms of tissue engineering [2]
- Biocompatibility plays a decisive role here, which is why the weakly biocompatible polyacrylonitrile (PAN) must be combined with biocompatible polymers such as polyethylene oxide (PEO)
- The goal is to achieve the ideal compromise between adherent cell growth, spinnability, and porosity without the nanofibers dissolving in the cell medium by varying different polymer chain length of PEO in combination with different PAN-PEO ratios

Materials and Methods

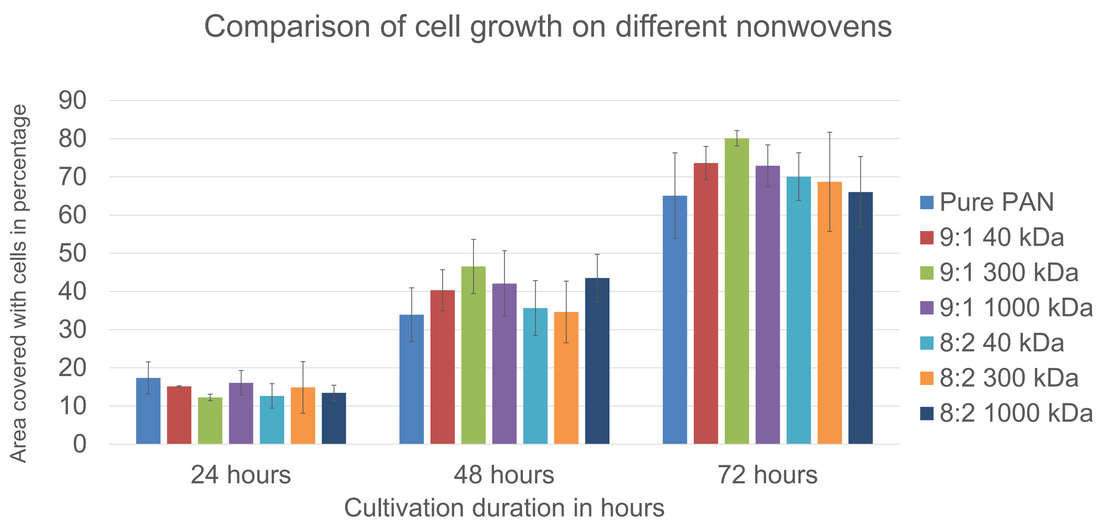
The adherent 3T3 standard cell line was used to assess adhesion. Cultivation was carried out in six-well plates for three days using Gibco Dulbecco's Modified Eagle Medium. Standard glass slides measuring 21 mm x 26 mm were used as carriers for the nanofiber mats, which were attached only at the edges with silicone adhesive to prevent any influence on adhesion. After sterilization, all samples were inoculated with a cell density of $5x10^5$ cells. After a cultivation period of one, two, and three days, the samples were stained with hematoxylin-eosin and then examined optically with a ZEISS Axio Observer microscope. To evaluate the area covered by growth, an area of approximately 14 mm x 14 mm was automatically recorded and analyzed using ImageJ.

Nanofiber mats were prepared with the needleless electrospinning machine “Nanospider Lab” by Elmarco. The following parameters are used for all samples: air humidity 33 %, carriage speed 100 mm/s, substrate speed 0 mm/min, electrode-electrode distance 240 mm, electrode-substrate distance 50 mm, nozzle diameter 0.9 mm, voltage 65 kV, and duration 30 minutes. The varying parameters are shown in the following table.

Sample	Current in mA	Temperature in °C
Pure PAN	0.045	20.2
PAN-PEO 9:1, 40 kDa	0.03	19.9
PAN-PEO 9:1, 300 kDa	0.035	20.3
PAN-PEO 9:1, 1000 kDa	0.033	20.1
PAN-PEO 8:2, 40 kDa	0.024	20.1
PAN-PEO 8:2, 300 kDa	0.013	20.2
PAN-PEO 8:2, 1000 kDa	0.02	19.6

Results and Discussion

- As expected, PAN alone shows the least growth after three days
- In contrast, the combination of PEO with a polymer chain length of 300 kDa and a PAN:PEO ratio of 9:1 shows the most growth after three days
- This is because PEO influences the spinning solution and thus the viscosity and fiber formation
- In this case, too low of viscosity (correlated here with a short polymer chain length of 30 kDa) has a “diluting” effect, while the high length of 1000 kDa prevents the formation of fine and uniform fibers, which mimics the extracellular matrix more precisely
- In addition, an excessively high proportion of PEO prevents the formation of stable nanofibers, which is why the entire network is less like the extracellular matrix and therefore exhibits lesser growth



Literature

[1] Malik, S.; Muhammad, K.; Waheed, Y. Nanotechnology: A Revolution in Modern Industry. Molecules 2023, 28, 661. https://doi.org/10.3390/molecules28020661

[2] Doersam, A., Tsigkou, O., & Jones, C. (2022). A review: textile technologies for single and multi-layer tubular soft tissue engineering. Advanced Materials Technologies, 7(11), 2101720.

Conclusion

- Adding PEO always increased the possible cell growth
- Combination of PAN and 300 kDa PEO with a ratio of 9:1 shows stable and homogenous fibre structures with regular pores and the highest percentage of cell growth
- In future experiments, other biopolymers and additives such as fibronectin will be tested to mimic the extracellular matrix even more closely

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