TECHNISCHE UNIVERSITÄT DRESDEN

Centre for Translational Bone, Joint and Soft Tissue Research

# Influence of fiber orientation in MEW substrates on the growth of keratinocytes and fibroblasts

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## Abstract

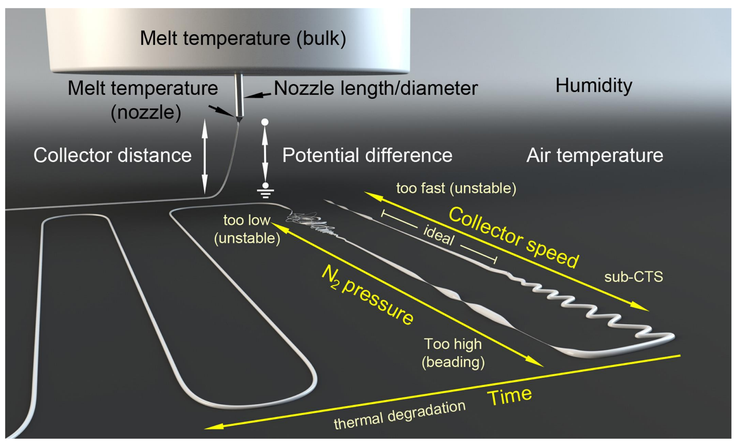
Melt Electro Writing (MEW) is capable of printing patterned scaffolds with high shape fidelity and fiber dimension of single to tens of micrometers. Therewith, the fibers are in the range of cellular dimensions and cells can recognize the whole fiber.

It was already shown that human mesenchymal stem cells (hMSCs) could be oriented with an alternating pattern of thick and thin PCL fibers.[2)]

Perspectives for using oriented cell growth may for example be tympanic membrane (TM) or cruciate ligament replacements.
Regarding the TM, an optimal synthetic replacement needs to mimic the vibrational and mechanical as well as the cell settling properties.

For that purpose, the prominent cell types of keratinocytes and fibroblasts were examined regarding their morphological behavior in dependency on scaffold patterns.

## Method for printing ultrafine fibers from polycaprolactone (PCL)

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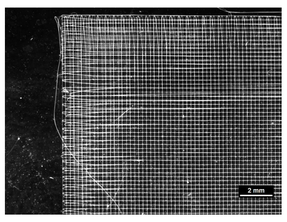
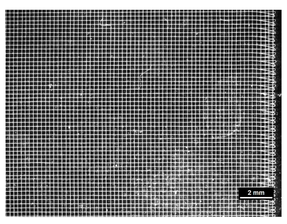
A commercially available printer from GeSim with a MEW module was used, to produce non-woven meshes with uniform fiber diameters and large surface area to volume ratio.

As shown in the picture, a variety of parameters influence the MEW printing process. Speed and pressure are the most prominent parameters to influence the fiber shape.

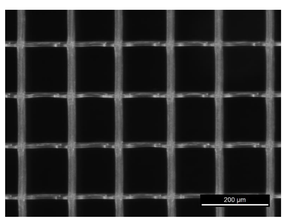
But to obtain high shape fidelity scaffolds, all parameters have to be fairly adjusted.



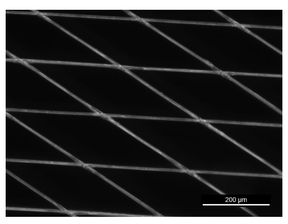
× Low shape fidelity



✓ High shape fidelity

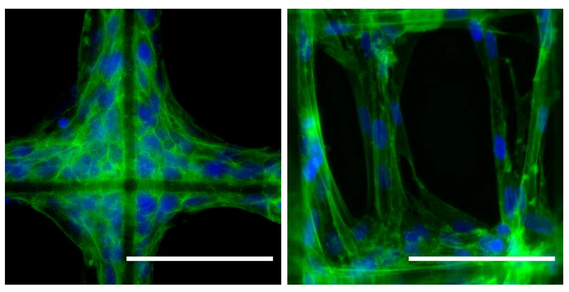


✓ Alternating thick, thin fiber



✓ 30° angle between fibers

## Utilized cell types



*Fluorescence staining with Phalloidin/DAPI for Actin fibers (green) and cell nucleus (blue). Scale bar = 100 µm*

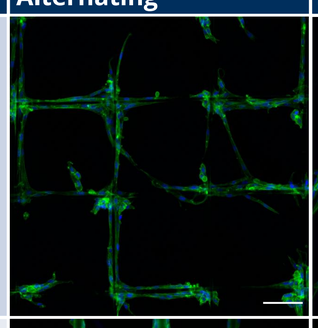
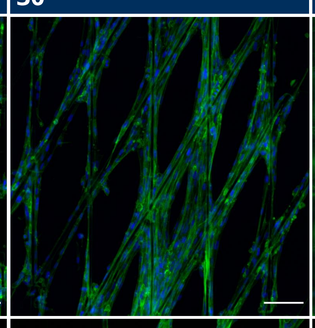
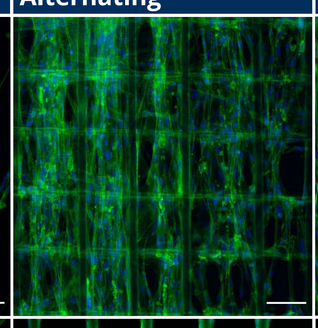
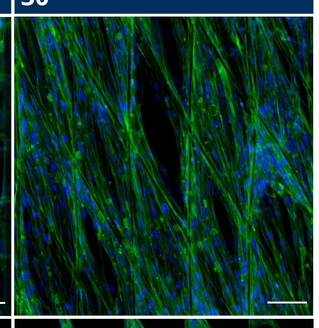
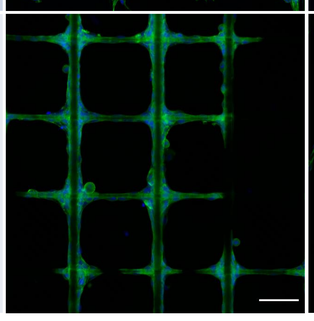
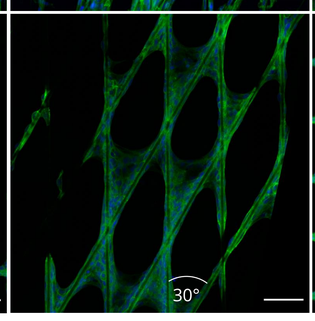
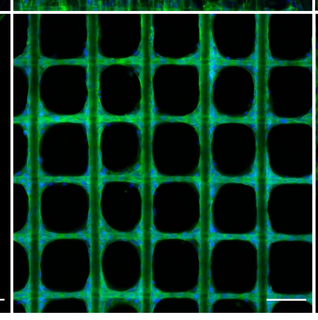
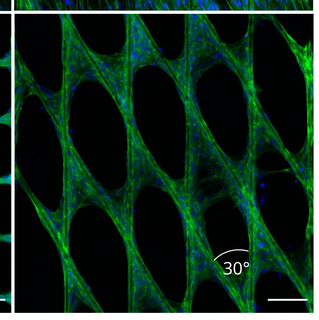
❖ **Keratinocytes**

- Forms protective pathogen barrier
- Main cellular skin component
- Roundish, prefer dense interaction
- Size: ~ 10 - 50 µm

❖ **Fibroblasts**

- Synthesize collagen fibers
- Central role in wound healing
- Elongate and like to adhere to structures
- Size: able to span pores of 250 µm

## Results from cell seeding

| | Day 7 Alternating | Day 7 30° | Day 14 Alternating | Day 14 30° |
|---|---|---|---|---|
| primary fibroblast (NHDF) |  |  |  |  |
| Keratino-cyte cell line (HaCaT) |  |   |  |   |

*Fluorescence staining with Phalloidin/DAPI for Actin fibers (green) and cell nucleus (blue). Scale bar = 100 µm*

The cell morphology behavior of keratinocytes and fibroblasts were tested on scaffolds with multiple different fiber patterns and pore sizes. Below, overgrown scaffolds with alternating thick and thin fibers of ~ 15 µm and ~ 5 µm diameter and with an angle of 30° to the horizontal line are shown.

Fibroblasts are strongly influenced in their cell orientation by the scaffold structure. They span the pores parallel to the thick fibers for the alternating scaffold or orient along the angle bisector for the 30° scaffold.
Keratinocytes tend to stay roundish and grow mostly circular along the pores for the examined scaffold types.

## Conclusion and Outlook

- Investigating cell orientation dependencies on patterned scaffolds for the cell types of keratinocytes and fibroblasts
- Investigate cell orientation for further cell types e.g. ligament cells for cruciate ligament
- Expand scaffold patterns to radial and circular structures as they are present in the TM
- Test different cultivation methods like dynamic cultivation to increase initial cell adhesion

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***References:***

1 *Toby D Brown, Paul D Dalton, and Dietmar W Hutmacher. Direct writing by way of melt electrospinning. Advanced Materials, 23(47):5651–5657, 2011.*

2 *Chaoqi Xie, Qing Gao, Peng Wang, Lei Shao, Huipu Yuan, Jianzhong Fu, Wei Chen,and Yong He. Structure-induced cell growth by 3d printing of heterogeneous scaffoldswith ultrafine fibers.Materials & Design, 181:108092, 2019.2*

3 *Sammy Florczak, Thomas Lorson, Tian Zheng, Miroslav Mrlik, Dietmar WHutmacher, Michael J Higgins, Robert Luxenhofer, and Paul D Dalton. Meltelectrowriting of electroactive poly (vinylidene difluoride) fibers.PolymerInternational, 68(4):735–745, 2019.*

4 *Tomasz Jungst, Iris Pennings, Michael Schmitz, Antoine JWP Rosenberg, J ̈urgenGroll, and Debby Gawlitta. Heterotypic scaffold design orchestrates primary cellorganization and phenotypes in cocultured small diameter vascular grafts.AdvancedFunctional Materials, 29(43):1905987, 2019.*

***Figure:***

**) "Melt electrowriting of electroactive poly(vinylidene difluoride) fibers", S. Florczak et. al., 2018*