Advancing the cell culture landscape

Chapter X - Valorization

Steven Vermeulen[1,2] and Jan de Boer[2]

[1] Laboratory for Cell Biology-Inspired Tissue Engineering, MERLN Institute, University of Maastricht, Maastricht, The Netherlands

[2] BioInterface Science Group, Department of Biomedical Engineering and Institute for Complex Molecular Systems, University of Eindhoven, Eindhoven, The Netherlands.

Introduction

In this chapter, we discuss the relevance of our research findings in a clinical/commercial context. Besides broadening our fundamental understanding of mechanobiology, an additional aim is to translate the scientific output into practice by exploring the commercialization possibilities of our research. This, we define as "**Valorization**". In **Chapter 4**, we discussed how the phenotype of tenocytes could be improved by culturing them on micro-topographies. In **Chapter 5**, we found that micro-topographies attenuate TGF-β signaling in MSCs, which can lead to improved differentiation protocols. Furthermore, we found small molecules that mimic biomechanical signaling, which could be applied in a clinical setting. We also mention that the longer retainment of multipotency in MSCs described in **Chapter 7** might be useful for harvesting secreted growth factors or creating appropriate co-culture conditions. In **Chapter 8**, we demonstrate that natural surface structures expand the design space of our regular micro-topographies while eliciting distinct cell and bacterial behavior. In this **Valorization Chapter**, we therefore explore how these natural and artificial surface topographies can be commercialized.

Social relevance of the research results and target audience

The research results described in this thesis do not document if micro-topographies can be directly applied in a clinical setting. For example, would tenocytes cultured on micro-topographies give rise to better tissue-engineering constructs for clinical applications compared to tenocytes cultured on regular polystyrene? Does a similar possibility exist for cells differentiated on micro-topographies? In addition, can coating natural and artificial topographies on medical implants inhibit bacterial infection? These questions are not answered in this thesis since clinical trials involving medical implants can take a significant amount of time [1]. Nevertheless, the results do show the potential of micro-topographies eventually being utilized in such clinical settings. Here, we would like to refer the reader to an article published in our group that demonstrated improved osteo-integration of titanium implants in rabbits when coated with micro-topographies [2]. Micro-topographies also allow guiding cell behavior, such as retaining the phenotype of cells or improving the differentiation of stem cells. This is not only supported by the research performed in this thesis, yet also by other research groups, which we discussed in **Chapter 2 and 3**. A possibility would be to harness micro-topographies to improve cell culture protocols in the culture dish for tissue-engineering purposes. For example, autologous stem cells can be harvested and cultured on micro-topographies for maintaining their phenotype for prolonged times. Work performed by Gilbert and co-workers nicely demonstrate this concept by utilizing hydrogels as a biomaterial [3]. In this work, muscle stem cells are isolated and put on an elastic hydrogel that promotes self-maintenance. This allowed growing them in sufficient cell numbers *in vitro,* after which implanting them in injured muscle improved tissue regeneration compared to cells cultured in standard conditions. If micro-topographies can play

a similar role, with direct clinical applications, then this would increase their commercial and social value. However, additional research is required through animal models and eventually in humans, to assess the clinical feasibility of the surface structures utilized in this thesis. Therefore, we will mainly focus on describing the possibility of commercializing surface structures for research purposes.

The product

A key feature in commercializing surface structures for research purposes is creating a platform that offers the most optimal structural diversity, while ensuring the creation of phenotypical diversity. As described in **Chapter 8**, natural surface structures expand the design of our micro-topographical library [4], while creating novel phenotypical diversity. Therefore, when creating a novel platform, some of these natural surfaces should be included so that the design space is optimally expanded **(Figure 1)**. However, despite the 2176 unique micro-topographies from the TopoChip and the natural surfaces explored in this thesis, such a platform would still fall short in representing true surface diversity. For example, grooves are not present in this design, which are known to induce specific and distinct phenotypes compared to our micro-topographies. For example, as shown in **Chapter 7**, micro-topographies lower histone acetylation levels, while micro-grooves are known to elevate them [5]. Furthermore, surface roughness, wells, or curvature are also not included. In addition, structures in nanodimensions [6], a combination of both [7], and disorder instead of ordered structures [8], are not included.

As a product suitable for commercialization, we would like to present surfaces in a 24-well format size to enable sufficient cell numbers for multiple experimental setups such as qPCR/microarray/RNAseq, western blot/ELISA, and ICC. Researchers can perform screens

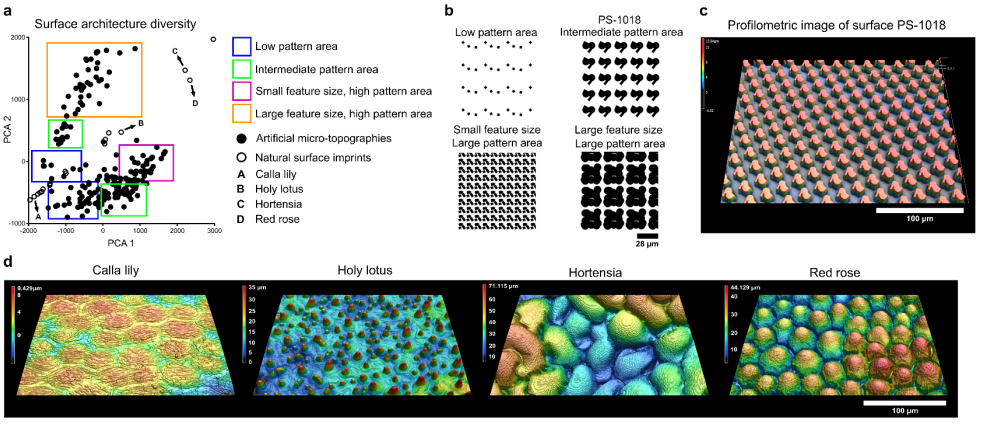

Figure 1: Figure from chapter 8 of this thesis, which illustrates how natural surface structures expand the design space compared to the micro-topographical library we commonly use.

with this setup, after which it would be possible to order a single sheet of a surface structure in a 100 mm dish format for in-depth follow-up experiments. This can be made possible by constructing a silicone mould of each surface structure. Afterwards, polystyrene sheets are fabricated according to the method described in the work of Zhao *et al.* [9]. Microwells can be constructed through thermoforming techniques [10], while for the natural surface structures a positive PDMS imprint can be generated through double PDMS casting [11] instead of using a silicon mould as a starting material. An automatic laser cutter can be applied to cut a single PS sheets in suitable 24-well format dimensions.

Next to the classical PS material, also other materials can be offered such as polyurethane (PU) to enhance the biological readouts or to test surface structures that were fabricated with materials used in the clinic.

The proposed library would contain the following 22 unique structures, which are known to induce specific cellular phenotypes **(Figure 2)**:

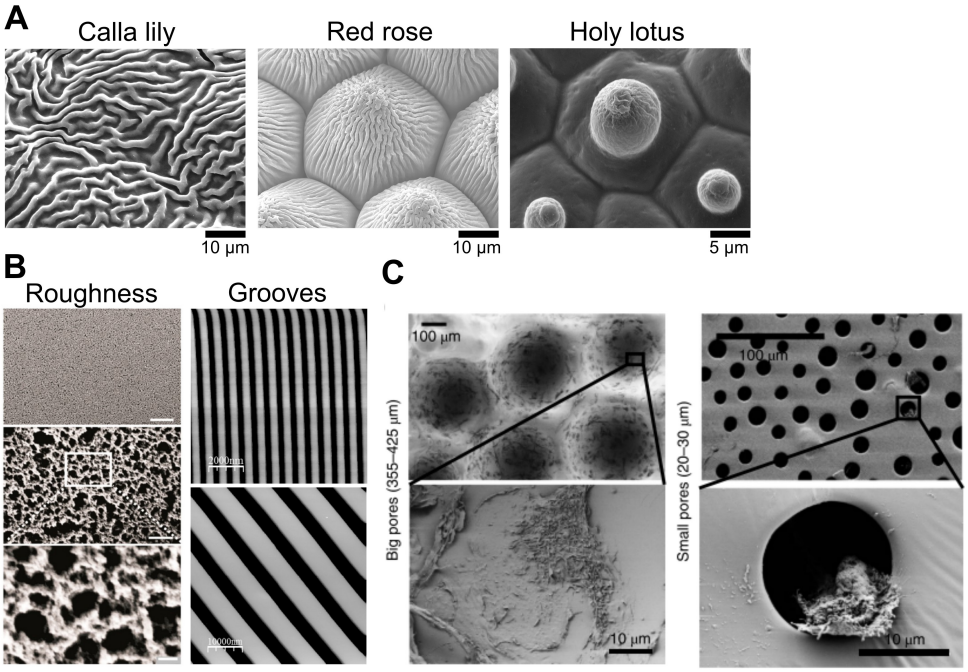

Figure 2: Sample of the distinct surfaces present on this new platform. A) SEM images of the natural surfaces. **B)** Roughness and groove patterns. **C)** Micro-wells in different dimensions.

1) Micro-topography - Small feature size, small pattern area (TopoChip library) [4]
2) Micro-topography - Large feature size, large pattern area (TopoChip library) [4]
3) Micro-topography - Large feature size, small pattern area (TopoChip library) [4]
4) Nanotopographies - Large pattern area (NanoTopoChip library) [6]
5) Nanotopographies - Small pattern area (NanoTopoChip library) [6]
6) Calla lily - Complex structures with low elevation (Natural surface library)

7) Red rose - Hierarchical micropillars with nanofoulds (Natural surface library)
8) Holy lotus - Hierarchical micropillars with surface roughness (Natural surface library)
9) Microgrooves - 1 μm depth, 40 μm wide [5]
10) Microgrooves - 1 μm depth, 20 μm wide [5]
11) Microgrooves - 1 μm depth, 10 μm wide [5]
12) Nanogrooves - 200 nm wide and 100 nm deep nanochannels [12]
13) Hierarchical micro-nanogrooves - 10 μm wide and 1 μm deep channels in combination with 200 nm wide and 100 nm deep nanochannels [12]
14) Microwells/pits - 30 μm diameter [13]
15) Microwells/pits - 400 μm diameter [13]
16) Nanopits - 120-nm-diameter, 100-nm-deep nanopits (ordered) [8]
17) Nanopits - 120-nm-diameter, 100-nm-deep nanopits (disordered) [8]
18) Convex curvature - $\varkappa = 1/175\ \mu m^{-1}$ [14]
19) Microroughness - Sa = 2 μm
20) Nanoroughness - Sa = 900 nm
21) Nanoroughness - Sa = 70 nm [7]
22) Hierarchical micro-nano roughness - Sa = 900 nm + 70 nm [7]
23) Flat 2x

Innovation

Offering scientist platforms to study mechanobiology or perform screens is not novel, yet demonstrates that also our platform has commercialization potential. An example of such a successful commercialization concept is the biotech company CYTOO. This company offers high-throughput screens including those in an adhesive island format. Our design would fill a surface structure niche in the market. Here, we emphasize that even though our design space is very broad, it still does not encompass potential designs that can have distinct phenotypical readouts. For example, podocytes have a unique morphology, and biomaterials that mimic this cellular shape *in vitro* induce beneficial phenotypical effects in this cell type [15]. Furthermore, not all micro- or nanogroove dimensions are covered in our setup, as demonstrated by the polyimide platform [16]. Therefore, it is essential that efforts are continued to improve the design space and the platform. In the future, if many novel and functional design spaces are discovered, a 96-well format can be offered in plates. We previously demonstrated this for the TopoWellPlate [17], yet this platform only contained micro-topographies. Options for further improving the design space would be to apply basic functional assays such as cell morphology, focal adhesion staining, and differentiation experiments on novel natural surfaces. Furthermore, applying artificial intelligence, as recently demonstrated by our group, can predict novel surface designs with interesting functionality [18].

Additional research will allow novel innovations such as the possibility to implement these structures for clinical applications such as for medical implants. Also in the culture dish, investigating how these structures can promote cell differentiation or phenotypical maintenance

protocols will further add to its commercialization potential. If a particular topography indeed gives rise to an intriguing phenotypical readout, then a collaboration could be considered with other companies to create commercial kits. These kits can then contain an appropriate cell culture media supplemented with growth factors and a surface substrate of interest to obtain a cell phenotype. Such companies could be Stem Cell Technologies, Lonza, or Thermo Fisher.

Schedule & Implementation

Since this platform is strongly associated with the possibility of performing fundamental research, a plausible format for this product would be to develop a spin-off company around the concept. This can be achieved through university funding and local research groups. Furthermore, equipment, technical, and biological expertise are available there enabling a quick realization of the product. This also ensures a limited risk in commercializing this product since the research value can also be translated into research publications. As such, additional funding should be able to be acquired through research grants.

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