TopoChip Screening of Designed Surfaces to Instruct Cell Fate

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Statement of Purpose:

Small changes in surface topology on which cells grow can have a dramatic impact on cellular parameters such as proliferation, orientation, morphology, gene expression and differentiation[1]. Identifying the appropriate surface topography to induce desired cell behavior has widespread implications for the development of the biomaterials field, as well as in cell therapy. We have developed a platform for high-throughput screening of material surface topographies, consisting of an *in silico* library of over 150 million unique multiparametric features. From this library, nearly 2200 topographical designs were randomly selected and arrayed in duplicate as 290×290 μm^2 'TopoUnits' upon a 2×2 cm^2 'TopoChip'[2] of any desired material.

High-throughput screening using the TopoChip platform facilitates rapid identification of specific topographically-defined surfaces that elicit the desired biological response in the cell type of interest on the selected biomaterial.

Our work shows that topographies indeed have striking effects on cell attachment, morphology and orientation, and therewith cell behavior. Using standardized well-plate sized surfaces featuring the found 'hit topographies' allows us to further validate these hit topographies by conventional analysis panels and investigate the underlying mechanisms that cause these cellular responses.

Methods:

One way to fabricate the TopoChip is by hot embossing of a polymer using a (silicon) mold holding the inverse pattern. Silicon master molds are fabricated by conventional clean room methodology, including photolithography, from which temporary molds of more flexible material can be fabricated when required for TopoChip fabrication process (depending on the selected TopoChip material). In addition, a secondary material can be deposited by e.g. (spin)coating to enable screening cells growing on e.g. metal or ceramics surfaces. Cultivation of the cell type of interest on the TopoChip is performed in a custom-designed cell seeding and culture device for the intended period of time. A specific bio-assay is developed to stain the cells for desired biological response analysed in the particular screen. TopoChips are imaged via automated fluorescent microscopy (Hamamatsu Nanozoomer 2.0RS) and image analysis is performed using a custom-designed analysis pipeline partially making use of CellProfiler[3]. In addition, we have produced well-plate sized samples each featuring a single hit topography identified in TopoChip screens in order to perform further validation and *in vitro* secondary screening experiments.

Results:

Quantitative image analysis shows that different topographically-defined TopoChip surfaces can have significant impact on cell morphology, such as cell shape and orientation (Figure 1), thereby instructing cell fate.

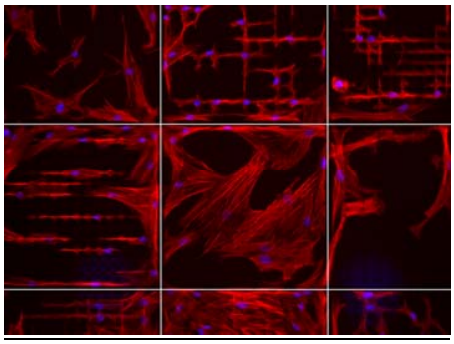

Figure 1. Morphology of human mesenchymal stromal (hMSC) cells on different surface topographies. Cells were fixed after 5 days of culture in medium without added cytokines and stained for their nuclei (DAPI, blue) and the F-actin cytoskeleton (Phalloidin, red).

As an example, TopoChip screening of topographies inducing differentiation of human bone marrow-derived MSCs towards the osteogenic lineage revealed a number of hit-topographies that enhance the induction of a well-known osteogenic screening-marker equally or even stronger compared to known stimulating compounds added to culture medium. Interestingly, the non-patterned control surface ranked as one of the lowest affecting surfaces, i.e. nearly any TopoChip topography increased expression of the osteogenic screening marker to some extent. However, hit-topographies affected marker expression significantly stronger than the bulk of topographies which demonstrates the importance of high-throughput screening of topographies to identify the exact topography design that optimally elicits desired cellular behavior.

Conclusions:

Within the TopoChip approach we combine the power of high-throughput screening with mathematical design of micro-range topographies imprinted to create a highly-versatile, automated high-throughput platform. The TopoChip is a revolutionary and powerful tool to identify those topographies that significantly improve desired biological performance, thereby demonstrating the importance of appropriately designed surfaces for biomaterials and cell therapy.

References:

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