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Analysis of high-throughput screening reveals the effect of surface topographies on cellular morphology

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ABSTRACT

Surface topographies of materials considerably impact cellular behavior as they have been shown to affect cell growth, provide cell guidance, and even induce cell differentiation. Consequently, for successful application in tissue engineering, the contact interface of biomaterials needs to be optimized to induce the required cell behavior. However, a rational design of biomaterial surfaces is severely hampered because knowledge is lacking on the underlying biological mechanisms. Therefore, we previously developed a high-throughput screening device (TopoChip) that measures cell responses to large libraries of parameterized topographical material surfaces. Here, we introduce a computational analysis of high-throughput materiome data to capture the relationship between the surface topographies of materials and cellular morphology. We apply robust statistical techniques to find surface topographies that best promote a certain specified cellular response. By augmenting surface screening with data-driven modeling, we determine which properties of the surface topographies influence the morphological properties of the cells. With this information, we build models that predict the cellular response to surface topographies that have not yet been measured. We analyze cellular morphology on 2176 surfaces, and find that the surface topography significantly affects various cellular properties, including the roundness and size of the nucleus, as well as the perimeter and orientation of the cells. Our learned models capture and accurately predict these relationships and reveal a spectrum of topographies that induce various levels of cellular morphologies. Taken together, this novel approach of high-throughput screening of materials and subsequent analysis opens up possibilities for a rational design of biomaterial surfaces.

1. Introduction

High-throughput screening has become a valuable tool for biologists. Examples of such tools range from drug discovery platforms [1] to microfluidic cell-based assays [2]. In situations where biological mechanisms are unknown and/or hard to simulate, such screenings can help identify promising directions for new research.

Recently, we reported on the development of such a screening platform for biomaterial surface topographies [3]. Biomaterials play an important role in tissue engineering, as their surface has been shown to affect cell behavior [4–7]. Such behavior can, for example, lead to non-optimal functioning of implants (e.g. by encapsulating an orthopedic implant with fibrous tissue). By control of cell morphology through surface-based adhesion contrasts, it has moreover been shown that surfaces can affect cell proliferation and even cell differentiation. For example, the available space for spreading [8] and the shape of this space [7,9] can affect the differentiation of respectively human and rat mesenchymal stem cells (hMSCs/rMSCs), steering them towards either osteogenesis or adipogenesis.

Cell morphologies can also be modulated through modification of the surface topography. Topographies have been shown to affect cell attachment (e.g. [10,11]), cell viability (e.g. [12]) and also cell differentiation (e.g. [13–15]). Another example of the use of surface topographies was given in Ref. [16], where microgroove surface topographies were used to align cells, allowing them to form a

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healthy collagen matrix. This is relevant to the healing of ligament and tendons, in which unaligned cells negatively affect the structure of the collagen matrix. Similarly, in Ref. [17], the focus was on replacement materials for bladder tissue. It was shown that the use of a nanostructured material enhanced cell adhesion and cell growth as well as elastin and collagen production. Surface topographies are thus important tools in the engineering of replacement tissues, such as muscles, bone and blood vesicles.

Finding surfaces that instruct cells to show a certain behavior is, however, a challenging task, as the number of ways in which materials can be modified is virtually unlimited and the biological mechanisms behind cell–material interactions are not yet fully understood. This implies that there is a need for a screening approach in which one can measure the reactions of living cells to a large set of material surfaces. The main focus to date has been on the creation of surface topography gradients (e.g. [11,13,14]). These gradients allow one to explore cellular responses across the full range of a certain fabrication parameter within a single experiment. More complex gradients can also be constructed, such as the 2-D gradient explored in Ref. [18]. However, the range of possible surface topographies is large, and many design parameters and their interactions can be considered. Such high-dimensional parameter spaces become difficult to explore using gradients. Combinatorial (high-throughput) screenings [19] have been identified as a possible solution to these problems, and have successfully been applied for polymer-based biomaterial development [20].

In this regard, we have developed a chip (called TopoChip) on which cells are grown within units (TopoUnits), each containing a different patterned surface topography. In total, the chip contains 2176 distinct surfaces, each measured by two TopoUnits. High-throughput fluorescence microscopy is used to determine cellular responses at the single-cell level.

With the miniaturization (realized through the use of a chip-based platform), combined with an algorithmic design of surface topographies, the effects of numerous surface design parameters can be explored within a single experiment. Through the standardization of chip formats, the results of different experiments can be compared.

Such a new high-throughput platform also reveals opportunities for new computational analysis methods. The large amount of information produced enables a transition whereby surface screening is augmented by surface modeling. That is, we can begin to link the biological performance of a surface to its actual topographic design. This systematic approach to material research, in which one combines screening with modeling, has been termed "materiomics" [21]. This gives insights that open the road to a rational approach to surface discovery, which is useful, given that the potential materiome is of infinite size.

In this work, we propose a materiomics-based computational analysis for the TopoChip. To illustrate our analysis methods, we explore the cell morphological responses of hMSCs (which are known to be mechanosensitive) to different surface designs. We learn models which relate such surface designs to the measured cell morphology. In this way, we gain insight into the important surface design properties. This finally allows us to predict cellular responses to surfaces in silico.

We focus specifically on finding "hit" surfaces, i.e. those surfaces that best promote a certain specified cell behavior, as this is one of the primary applications of biomaterial testing. This is a challenging problem in a high-throughput setting, not only due to the number of material surfaces tested, but also due to the miniaturization of the surface area required for designing an efficient high-throughput platform. This means that the number of cells tested per surface type is reduced, making results more susceptible to (natural) variation. Nevertheless, across the whole chip, a large amount of data is gathered for a large number of cells. We propose a hit ranking method that makes use of this data by taking into account surface similarities and cell behavior similarities. This allows us to share information between surfaces, thereby significantly improving hit surface ranking performance and reducing the number of replicate measurements required.

We make use of machine-learning techniques to learn models that link cell responses to surface descriptions. We show how the use of robust statistics enables us to obtain high-quality hit rankings and create predictive models that are consistent across experiments. Over the years, various studies within the (molecular) biomaterials field have investigated the use of machine-learning algorithms for various applications [22], ranging from protein adsorption performance predictions [23] to drug-release kinetics predictions [24]. Their correctness and predictive quality generally depend on the number of measurements that are used to learn the model. With the TopoChip, we now have, for the first time, access to measurements across thousands of surfaces, enabling these methods to learn unprecedentedly detailed models of the cell–surface relationship.

To show how the surfaces affect the morphologies of hMSC cells, we perform in this study an eight-chip experiment, constituting ~35,000 surfaces, on which half a million hMSCs are grown. By measuring the morphology of each of these cells, we show that the surfaces consistently induce a whole range of different morphologies. We generalize across all measurements to determine how the surfaces influence these morphological responses. Based on this, we generate spectra of surfaces that induce the whole range of possible morphological property values, and show how models allow us to predict these morphological property values for new surfaces in silico.

2. Material and methods

2.1. General approach

2.1.1. TopoChip

The TopoChip, which has dimensions of 2 × 2 cm, measures the effect of 2176 different topographical surfaces on cell behavior

Table 1
Surface properties.

Surface property	Description
FeatSize	The size of the bounding square for the primitives (10, 20 or 28 μm)
NumCirc	The number of circles used
NumTri	The number of triangles used
NumLine	The number of lines used
CircDiam	Circle diameter
TriSize	Length of the shortest side of a triangle
LineLen	Line length
RotSD	The standard deviation (in degrees), is used to determine the rotation of the primitives when they are placed in the feature
Rot	The standard deviation for rotation of primitives scaled with number of line and triangle primitives (since circle primitives are unaffected by rotation)
WN0.1–WN4	The fraction of energy in the signal in sinusoids with wavenumber 0.1–4
CircArea	The area of circle primitives
TriArea	The area of triangle primitives
LineArea	The area of line primitives
DC	The number of circle primitives scaled by feature area
DT	The number of triangle primitives scaled by feature area
DL	The number of line primitives scaled by feature area
CA	The total area of circle primitives scaled by feature area
TA	The total area of triangle primitives scaled by feature area
LA	The total area of line primitives scaled by feature area
CCD	Number of color changes of the feature over the diagonal

Feature area refers to the feature size, which is a bounding square of 10, 20 or 28 μm (see FeatSize). Each feature contains primitives (circles, triangles and lines (rectangles)). Features are repeated to cover the surface of a TopoUnit.