

P25 – Elastic Fully Three-Dimensional Microstructure Scaffolds for Cell Force Measurements


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The rhythmical contraction of the heart that drives the cardiac cycle involves the coordinated and synchronized action of a large number of cardiomyocytes. Determining the force contribution of an individual cardiomyocyte to overall heart contraction requires sensitive cell force measurement devices. To measure the contractile force of a single cell, we have produced elastic 3D cell culture scaffolds by means of direct laser writing (DLW) into a biocompatible photoresist (Ormocomp). These 3D scaffolds contain flexible beam elements of submicron thickness which can be rhythmically deformed by single beating cardiomyocytes. To obtain a quantitative measure of the involved cellular contraction forces, the cell culture substrates were calibrated using the cantilever of an atomic force microscope as a micro-indenter. Matching cell-induced beam deflections required applying external forces of about 50 nN, indicating that cellular contraction forces are of similar magnitude. Furthermore, by adjusting the DLW write parameters, and thus the beam diameter (0.66 to 1.33 μm), the beam stiffness could be fine-tuned over a range of almost one order of magnitude (0.05 N/m – 0.4 N/m). In conclusion, we have demonstrated that DLW can be used to fabricate 3D cell culture substrates with tailored stiffness to measure a wide range of cellular contraction forces. In future, this method could be expanded to systematically investigate the influence of three-dimensionality and elasticity on other cell functions, such as the differentiation of individual cells and on tissue formation.



Tailored three-dimensional microstructure scaffolds for cell culture



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Introduction

Three-Dimensionality

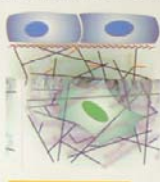
Cells grown on 2D tissue culture substrates often differ considerably in morphology, cell-cell and cell-matrix interactions, and differentiation from those growing in more physiological 3D environments (K.M. Yamada and E. Cukierman 2007). We have successfully produced **arbitrary flexible 3D structures** to study cell growth and function.

Topography

In vivo, cells will encounter many topographical features ranging from protein binding to collagen bundling. Human MSC differentiation, for example, can be controlled using nanoscale symmetry and disorder (M. J. Dalby 2007).

ECM Ligands

The biochemical composition of the cellular environment and the geometric distribution of adhesive cues has a fundamental impact on cellular function.

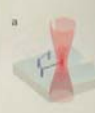
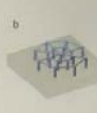




Mechanical Properties

The stiffness of the environment has emerged as an regulator of cell behavior and fate. It has been shown that cell organization and differentiation changes dramatically as cells are plated on increasingly softer 2D substrates (A. J. Engler 2006). Flexible 3D structures were made possible by the use of Ormocer® as a photoresist.




Method

Direct laser writing (DWL)

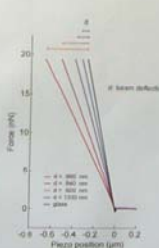
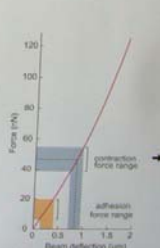





DWL by multiphoton polymerization is an established technique to produce arbitrary 3D structures of adjustable flexibility.

Calibration by Atomic Force Microscopy (AFM)


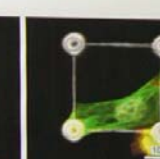
The mechanical properties of the flexible Ormocer® scaffolds are calibrated using AFM indentation measurements. In these measurements the beam deflection is determined in response to a given indentation force.






Cellular contraction forces ~40 to 80 nN



Flexible 3D Ormocer® Substrates

Beam deflection by single beating cardiac myocytes


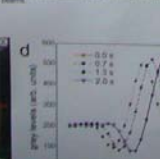



3D-microstructures of cardiac fiber spanning microtissue stacks depicting cardiac myocytes suspended in different Ormocer® cell culture scaffolds.

Phase contrast and image (left) and line-scan image (right) showing the myotonic contraction of the cell culture scaffold. Red arrows indicate deflected beams.

Interferometrically high-resolution scanning confocal microscopy demonstrates that Ormocer® scaffolds have elastic properties within the force range produced by single cells.

Modelling beam bending lines

Theoretical beam bending lines at an area load of 20 nN




$E = 800 \text{ MPa}$

Summary

- Bio-compatible scaffolds can be fabricated by direct laser writing (DWL) and subsequent surface functionalization
- In-vitro production of flexible 3D cellular environments is the prerequisite to nanoscale length
- Ormocer® scaffolds can be mechanically deformed by single beating cardiomyocytes
- Quantitative AFM evaluation demonstrates that contraction forces are in the range of 40 nN
- Cellular contractile forces down to ~10 nN can be measured

The method introduced will be the platform for future studies systematically investigating the influence of these microstructure and elasticity on the differentiation of individual cells and on tissue formation.