Cells exist in the so-called extracellular matrix (ECM) in their native state, and numerous future applications require reliable and potent ECM-mimics. A perspective, which goes beyond ECM emulation, is the design of a host-material with features, which are not accessible in the biological portfolio. Such a feature would, for instance be, the creation of a structural or chemical gradient, and to explore how this special property influences the biological processes. First, we wanted to test if macroporous organosilica materials with appropriate surface modification can act as a host for the implementation of human cells like HeLa or LUHMES. It was possible to use a commercially available polymeric foam as a scaffold and coat it with a layer of a thiophenol-containing organosilica layer, followed by biofunctionalization with biotin using click chemistry and the subsequent coupling of streptavidin-fibronectin to it. More importantly, deformation of the scaffold allowed the generation of a permanent structural gradient. In this work, we show that the structural gradient has a tremendous influence on the capability of the described material for the accommodation of living cells. The introduction of a bi-directional gradient enabled the establishment of a cellular community comprising different cell types in spatially distinct regions of the material. An interesting perspective is to study communication between cell types or to create cellular communities, which can never exist in a natural environment.
The Influence of Structural Gradients in Large Pore Organosilica Materials on the Capabilities for Hosting Cellular Communities

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Cells exist in the so-called extracellular matrix (ECM) in their native state, and numerous future applications require reliable and potent ECM-mimics. A perspective, which goes beyond ECM emulation, is the design of a host-material with features, which are not accessible in the biological portfolio. Such a feature would, for instance be, the creation of a structural or chemical gradient, and to explore how this special property influences the biological processes. First, we wanted to test if macroporous organosilica materials with appropriate surface modification can act as a host for the implementation of human cells like HeLa or LUHMS. It was possible to use a commercially available polymeric foam as a scaffold and coat it with a layer of a thiophenol-containing organosilica layer, followed by biofunctionalization with biotin using click chemistry and the subsequent coupling of streptavidin - fibronectin to it. More importantly, deformation of the scaffold allowed the generation of a permanent structural gradient. In this work, we show that the structural gradient has a tremendous influence on the capability of the described material for the accommodation of living cells. The introduction of a bi-directional gradient enabled the establishment of a cellular community comprising different cell types in spatially distinct regions of the material. An interesting perspective is to study communication between cell types or to create cellular communities, which can never exist in a natural environment.

Introduction

The size of matter used in contemporary nanoscience can be adjusted to the range of biological entities. As nanospecies are known to harm organisms toxicologic aspects should always be considered.¹, ² Any contact with the respective nanomaterial should be avoided, in case of toxicity. However, these materials present a powerful tool regarding biological applications. As research has accomplished to manage a broad variety of chemical surface modifications and, thus, an aim of current research has become the treatment of biological systems with nanomaterials on purpose. The emerging nanomedicine field is a good example,³,⁴ where materials are used for diagnostic or therapeutic purposes. The contact between organisms and the synthetic material is still intended to be short in duration for the vast majority of cases reported in literature, like in nanomedicine. A tempting perspective is to design synthetic materials in such a way, they can be integrated with living cells in a more sustainable way, ultimately leading to persistent composites between living and synthetic matter. Such new materials may have unique properties and the synthetic constituent can be used to influence biological processes in an unprecedented way.

Cells in their natural environment are surrounded by the extracellular matrix (ECM), and huge effort was already undertaken trying to mimic it.⁵, ⁶ To support cell adhesion, proliferation, and differentiation the materials must fulfill several requirements. Matrigel®, which is a combination of extracellular matrix proteins extracted from Engelbreth-Holm-Swarm tumours in mice, is a commonly used natural substrate for mammalian cell culture.⁷ One major drawback of tissue-derived substrates is their batch-to-batch variation and the presence of undesired impurities.⁸ Furthermore, it is difficult to equip Matrigel® or other soft-matter systems used for tissue engineering with a defined structure such as open porosity. Moreover, the majority of matrices known in the literature are structurally and chemically homogeneous over macroscopic dimensions. The latter feature is clearly distinct to the situation in real biological systems since living tissue is hierarchically organized. Consequently, the generation of synthetic and hierarchically structured materials is a challenge which could open up many new possibilities beginning with the creation of emulated organs and ending with cellular communities not existing in nature.

A less noted element of hierarchy is the occurrence of directionality in functional gradient materials.¹⁰ A sharp boundary is replaced by a transition from one feature to the next. The gradient can either be a structural one, for instance the change of pore-size over a certain a distance or characterized by a transition of chemical/ surface properties. Thus, graded host materials offering different compartments for cells appear to be highly interesting. Before we report about our results going into this direction, information has to be given on, how to modify the surfaces of inorganic materials with groups making them biocompatible, how to structure the materials with special emphasis on gradient generation and what kind of gradient materials already exist in literature. Since, in nature there are plenty of materials that possess a functional gradient,¹⁰-¹² it is desirable to establish porous biomaterials which imitate these properties.¹³ There is only limited literature on functionally graded biomaterials. Oh et al. have been able to synthesize a polycaprolactone (PCL) scaffold with gradually increasing pore size by a centrifugation method.¹⁴ They could show that different cell and tissue types have individualized pore size ranges for their effective growth. Sobral et al. used 3D plotting to establish a fibrous scaffold made from PCL and starch.
and found out that the type of gradient has an influence on the cell seeding efficiency. Cichocki et al. have already been using the versatility of PU foams in creating various porous networks. By thermal pre-treatment and application of pressure, they were able to give the foam a new form. They succeeded in creating porous alumina with different size gradients by infiltrating an aluminium oxide precursor and calcination of the foam. Even though this approach is a simple way to create every conceivable porous structure it has not been combined with a functional surface.

The simplest method for making a surface attractive for cell adhesion is physical adsorption of appropriate proteins. Next to proteins as laminin and vitronectin, fibronectin is part of the extracellular matrix (ECM) and crucial for the attachment of mammalian cells. The amino acid sequence RGD (Arg-Gly-Asp) is the entity in the protein which is responsible for cell adhesion. However, covalent attachment of the biomolecules is preferred, because desorption and leaching is avoided. Furthermore, undesired events such as conformational changes or incorrect orientation on the material thereby presenting an inactive site to the cell can be minimized.

A valuable candidate as the synthetic constituent in the matrices is organosilica. Not only, there is a range of functional groups available for the attachment of biopolymers, it also has been proven to be non-toxic as indicated by multiple applications in a biological context. Organosilica can be structured in different ways from microporous, mesoporous to macroporous materials. The pore-size is a crucial factor regarding the possibility to host cells. So-called aerogels can be prepared by a sol-gel process, but their pore-size, though in the macroporous regime (>100 nm), is too small for cells. Others have used templates for the generation of hierarchically ordered macro-/mesoporous systems with inverse opal morphology. For instance, Zhou et al. were able to immobilize lipase which can catalyse the esterification of levulinic acid into alkyl levulinates. Nevertheless, these systems are still not able to function as a scaffold for cell growth since their pore system is limited in its size. A promising approach is to use a polymer foam as a template and infiltrate the precursor solution thereby creating a composite material of polymer and organosilica. Such materials have for instance been investigated for bone tissue engineering. Chen et al. were able to synthesize a glass which replicated the structure of the polymer foam by immersing the foam in a glass slurry and pyrolysis of the foam. By this method and the addition of a block copolymer as a co-template it is also possible to create hierarchical macroporous and mesoporous glass scaffolds. The reader interested more in bioactive glasses is referred to the review articles by Jones et al. or Polo-Corrales et al.

The methodologies for organic modification of silica materials is highly progressed and has been featured in numerous excellent review articles over the years. Because almost all of the work published has been concentrated on mesoporous materials, it seems the full potential of organosilica chemistry is not yet exploited for materials with other, and in particular larger pores like aerogels or foam-templated glasses. For instance, our group established an entire family of silsesquioxane sol-gel precursors and the corresponding materials. We were able to introduce functional groups into the organosilicate material like azides and thiols, which are capable to undergo further modifications by click-chemistry like the 1,3-dipolar Huisgen cycloaddition or the thiol-ene reaction.

The first of our tasks is to apply the organosilica methodology developed in our group to prepare materials, with pores large enough to host living cells (Scheme 1). The next step in order to generate a material with biologically relevant surface properties by exploiting click-chemistry.

![Scheme 1](image)

Scheme 1. Overview about materials prepared in this study. (a) Polymer foam structure as a template. (b) Surface modification with thiophenol organosilica materials. (c) Attachment of a biotin moiety by click-chemistry. (d) Cells hosted by the material. (e) A material possessing a single structural gradient. (f) A bidirectional gradient material leading to an adjustable structural barrier. (g) Bidirectional gradient material as hosts for spatially separated co-culture of two human cell lines.

However, we also want to go one step further, by creating a graded material and thereby creating a barrier for cell diffusion and develop a material which is a promising candidate for co-culture of cells.

**Materials and Methods**

**Materials**

The synthesis that acquired inert gas atmosphere was performed using general Schlenk techniques under argon atmosphere. The solvents were dried according to the standard literature and stored under argon. All starting materials used for the synthesis were purchased from commercial sources unless stated differently.
**Synthetic procedures**

**Scheme 2.** Synthesis steps of thiophenol organosilica to Biotin-modified and Streptavidin-Fibronectin modified surfaces (PDB ID 1MM9).

**Organosilica precursor.** 1,3-bis-(triethoxysilyl)-5-thiophenol (1) was prepared as reported in the literature. Further details are given in the Electronic Supplementary Information, (ESI).

**Thiophenol organosilica foams.** Prior to use, the commercially available PU foams are precleaned in boiling toluene, acetone and ethanol for 2 h to dissolve any remaining soluble components. The foams are washed extensively with ethanol and dried in the drying chamber. A typical preparation of PU-organosilica foams is as follows: A total of 0.52 g of the thiophenol-organosilane precursor (1) were dissolved in 3 mL of Ethanol and 75 µL of 1 M HCl were added under stirring. The mixture was prehydrolyzed for 3 h at 60 °C. The pre-treated PU foam was cut into suitable pieces and the prehydrolyzed solution was infiltrated into the foam. To remove any air in the pores the foam was compressed while in solution. The material was aged for 1 d.

**Biotin-Maleimide** was synthesized according to a previously reported process. Further details are given in the Electronic Supplementary Information, (ESI).

**Click-modification of the organosilica surfaces.** The PU-organosilica material was weighted before and after infiltration with the sol-gel precursor (1). The weight of the attached organosilica was calculated. 1 eq of PU-organosilica material and 1 eq of Biotin-Maleimide were dissolved in 4 mL of 0.1 M Sodiumphosphate buffer (pH = 8) and shaken for 24 h. The resulting material was washed with water for several times.

**Ellman test** was carried out according to literature.

**Fibronectin coating of the biotinylated surface.** A fibronectin streptavidin fusion protein was prepared according to an established protocol. Biotinylated thiophenol organosilica foams were incubated with 10 µM fibronectin streptavidin fusion protein in PBS for 30 min at 37 °C followed by washing once with PBS and 3 times with cell culture medium.

**Variation of the porosity.** To modify the porous structure of the polyurethane foams the washed PU foam is cut into suitable pieces and compressed between two glass slides. To generate gradients, the materials are put into a mould with an angle according to the steepness of the gradient. The compressed foams are heated to 150 °C for 5 h. The heat treatment ensures that the foams retain their form even after removal of the glass slides or mould.

**Cell culture experiments.**

**HeLa cell culture.** HeLa cells were maintained in HeLa culture medium (DMEM, high glucose, GlutaMAX™ Supplement, pyruvate supplemented with 10 % fetal calf serum and 1 % Penicillin/Streptomycin) at 37 °C and 5 % CO₂. Cells were passaged every 3-4 days. To detach cells from the plate, cells were washed once with PBS and enzymatically dissociated with trypsin. Cells were washed off with culture medium. For maintenance, cells were reseeded in lower density and defined cell numbers were used for experiments.

**LUHMES cell culture.** tRFP-overexpressing LUHMES were created by infecting the LUHMES cell line with a lentivirus overexpressing tRFP according to Schildknecht et al. Cells were grown at 37 °C and 5 % CO₂ and maintained and differentiated according to previous protocols.

**Cytotoxicity assessment** was performed using HeLa cells and standard Lactate dehydrogenase (LDH) release assay was performed. Further details are given in the Electronic Supplementary Information, (ESI).

**Assessment of biocompatibility of organosilica foams.** Onto each organosilica foam, 2x10⁶ HeLa cells were seeded in HeLa culture medium. After 17 h, viable cells were stained with Calcein-AM (1 µM) for 30 min. Imaging was performed at excitation wavelengths 350 nm and 488 nm.

**Co-Culture in bidirectional structural gradient organosilica material.**

tRFP-overexpressing LUHMES were differentiated in LUHMES differentiation medium (Advanced DMEM/F12, 1x N2 supplement, 2 mM L-glutamine, 1 mM dbcAMP (Sigma), 1 µg/ml tetracycline (Sigma) and 2 ng/ml recombinant human GDNF (R+D Systems). After 2 days, cells were enzymatically dissociated with trypsin, collected in Advanced DMEM/F12 and centrifuged at 300 xg for 5 min at RT. The cell pellet was resuspended in differentiation medium and 2x10⁶ cells were seeded onto one side of biofunctionalized bidirectional structural gradient organosilica material. 18 hours after seeding of LUHMES cells, HeLa cells that were pre-stained for 30 min with Calcein-AM (1 µM) were seeded on the other side of the organosilica material. After another 6 h, imaging was performed at excitation wavelengths 350 nm, 488 nm and 535 nm.

**Analytical methods**

NMR-spectra were acquired on a Bruker Avance III 400 spectrometer using CDCl₃ or DMSO-d₆ as a solvent. The ESI-MS data were recorded using a Bruker microOTOF II spectrometer. UV/VIS spectroscopic analysis was performed using an Agilent Cary 60 spectrometer. SEM measurements were performed using a Zeiss FESEM Auriga 40™ Crossbeam and a Hitachi TM3000 Tabletop SEM with a Bruker Quantax EDX Detector for the EDX measurements. EDX line scans were performed at a Zeiss Gemini 500 equipped with an Oxford EDX Ultim Max 100 detector. FT-IR spectra were recorded by using a Perkin Elmer Spectrum 100 spectrometer using ATR unit. TGA measurements were measured on a Netzsch STA449 F3 Jupiter. All measurements were performed under oxygen atmosphere with 80 mL/min flowrate and a heating rate of 10 K/min. Fluorescence microscopy was performed at labelled excitation wavelengths using a Zeiss Axio Observer Z1 microscope.

**Results and Discussion**
Thiol-functionalized organosilica and aerogels as impractical host materials.

Due to the high versatility of the thiol-ene click-chemistry, we focus on the organosilica system containing thiophenol as an organic constituent (see Scheme 1). Since aerogels possess a macroporous structure and high porosity, we hoped the pore-size can be made large enough to host cells. The preparation of the aerogels using 1,3-bis-(triethoxysilyl)-5-thiophenol as a sol-gel precursor was successful, so was the modification of the surfaces with biotin groups followed by streptavidin-fibronectin attachment (data are summarized in Electronic Supporting Information Fig. S1; see also Scheme 2). Comparison of the sizes of the pores ($D_p \approx 100-200\,\text{nm}$) according to scanning electron microscopy (SEM) micrographs with the size of HeLa cells (10-50 $\mu\text{m}$) indicates the size of the voids is by a factor of 100 too small for hosting the cell. As a consequence, cells can only sit on top of the material, but they cannot enter at all. As we saw no possibility to increase the pore-size of the aerogels that much and still have a mechanically stable material, the aerogel-approach was dropped immediately.

Preparation of Thiol Functionalized Organosilica Coating of Polymer Foams and Structural Gradients

Given the arguments described in the previous paragraph about aerogel materials, our next approach was to use a material as a scaffold, whose surfaces can be coated with thiophenol-based organosilica. Polymer foams appeared as ideal candidates due to the following arguments. A large variety is commercially available with different chemical composition, different pore-sizes and three-dimensionally connected pore-systems. Many are toxicologically safe and, if intended, the polymer can be removed easily from the hybrid material. Last but not least, unlike inorganic solids as silica materials, polymer-foams can be deformed without taking structural damage. However, it cannot be taken for granted that coating of a polymer foam with an organosilica layer works, as the surface of the foam is hydrophobic and hydrolyzed sol-gel precursors are hydrophilic due to their silanol groups (see Scheme 2). It was expected that the partial hydrophobic character of the thiophenol precursor is an advantage. The result of our experiments on commercially available poly-urethane (PU) foams are shown in Fig. 1.

In scanning electron microscopy, there is no structural differences between the materials before and after the sol-gel process visible (Scheme 2). Element-specific maps recorded by energy-dispersive X-ray spectroscopy, however, prove Si and S originating from the organosilica matrix are present. The organosilica has formed a homogeneous and dense film on the polymer (Fig. 1d), which is clearly confirmed by EDX line-scan measurements. The latter conclusion is confirmed by infrared (IR) spectra, which contains the band at 1052 cm$^{-1}$ characteristic for the Si-O-Si vibration and thermogravimetric (TGA) data shown in Electronic Supporting Information Fig. S2. The method described in the previous paragraph and in the experimental section can be applied to other polymers foams too, which allows to prepare the corresponding organosilica materials with different texture and pore-size. Two representative examples are given in Electronic Supporting Information Fig. S3,4 and were analyzed by an analogous set of methods.

As mentioned before, one advantage of using polymer foams as scaffolds is the possibility for deformation by mechanical force (see Fig. 2). Following deformation, the organosilica coating process can be performed. As a result, one obtains a material characterized by a persistent structural gradient with large pores on one side and almost closed pores at the other end.
that no harmful substances were washed out of the material (Fig. S6). As cells attached poorly to plain thiophenol organosilica foams (Fig. 4c), we aimed at the ECM mimicry of this three-dimensional scaffold to enhance cell attachment. To achieve this, it is necessary to coat the surface with protein epitopes known to promote cell adhesion. Consequently, the next step involved the modification of the thiol-groups by click-chemistry (Scheme 2). The pairing between biotin and streptavidin is a well-established tool in biological chemistry for the attachment of proteins. Therefore, we aimed to modify the organosilica surface using biotin-maleimide first, followed by the attachment of a fibronectin streptavidin fusion protein\(^\text{15}\) of which the fibronectin moiety confers binding to integrins like αvβ3 of HeLa cells\(^\text{65}\). The procedure can be applied to all organosilica materials but is discussed here for the non-graded material shown in Fig. 1 as a proof-of-concept.

The post-functionalization reaction of the organosilica surface with biotin decreases the chemically accessible thiol groups. The number of remaining free thiols can be probed by the so-called Ellman test. The test is based on the reaction of 5,5′-dithiobis(2-nitro benzoic acid) (DTNB, absorption at 325 nm) with a free thiol in the material and thus cleaving its disulfide bond to give 2-nitro-5-thiobenzoate (TNB), which has an absorption at 412 nm. Results from the Ellman test showed that the substantial number of reactive thiol groups prior to the post-functionalization reaction was reduced to nearly undetectable levels (Fig. 4a). In addition, one can use streptavidin conjugated to the fluorescent dye Cy5 as an indicator for biotinylation. The Cy5-fluorescence gives information on both the extent and distribution of biotinylation of the organosilica foam. As shown in Fig. 4b, an even distribution of biotin over the whole organosilica surface was confirmed. In a next step, the biotinylated organosilica material was incubated with a fibronectin streptavidin fusion protein. To prove that biofunctionalization of the surface was successful, HeLa cells were seeded onto the material. Attachment of the cells to the organosilica foam was evaluated by calcein-AM staining which visualized the cells and indicated their viability.

Closer inspection of the SEM images (Fig. 2c) and comparison to the homogeneous material (Fig. 1b) also demonstrates, the shape of the pores has become anisotropic. Obviously, the structural gradient can be varied easily by adjusting the angle between the two plates (Fig. 2a). It was also possible to prepare materials with a bidirectional gradient. The foam is confined and deformed by a stamp, which defines the gradient angle (see Fig. 3a and Fig. S5). Coating with thiophenol organosilica is performed as described before. The lower the angle of the kink in the stamp, the steeper is the gradient in the final material (Fig. 3b,c). By further modification of the stamp, it is also possible to change the extension of the zone characterized by small pores (Fig. 3c).

Click-Modification and Biofunctionalization

To utilize the material as a three-dimensional scaffold for cell growth its biocompatibility has to be confirmed. Therefore, we checked whether any soluble and potentially cytotoxic substances remained in the organosilica foam. For this, cell culture medium was used to produce eluates from the material and we found that such media allowed similar growth and viability of HeLa cells as control medium. This finding confirms

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**Figure 2:** (a) Deformation of the polymer foam by mechanical force. Pore-sizes determined at three different positions from SEM micrographs (b; scalebar = 500 µm).

**Figure 3:** (a) Schematic deformation procedure leading to a bidirectional structural gradient organosilica material. SEM micrographs (scalebars = 500 µm) of a bidirectional 150° gradient (b) and variation of the extension of the pore-small region of a 120° bidirectional gradient (c).
The live stain revealed complete covering of the biofunctionalized organosilica scaffold with HeLa cells (Fig. 4d), which is significantly different from cell growth observed on non-biofunctionalized organosilica materials (Fig. 4c). The drastically enhanced coverage of the material with live cells confirms successful biofunctionalization of the organosilica foam and its suitability for biological applications.

**Gradient materials as hosts for human cells**

After having successfully proven the significantly higher settlement of HeLa cells on fibronectin-functionalized organosilica materials, the graded materials were tested next. First, the materials were post-functionalized in the same way as the non-graded materials. The successful reaction was visualized again with the Cy5 assay (Fig. 5). The decreasing pore size and increasing foam density is shown by the autofluorescence of the material itself (Fig. 5, red pseudo-coloured). Seeding of HeLa cells depicted a considerable contrast in the different compartments of the material. The cells were analyzed 24 h after seeding onto the material. Staining with calcein-AM (green) confirmed the viability of the cells. A maximum of cells was found in the parts with normal sized pores (Fig. 5-1 – Fig. 5-3). The smaller the pores get, the fewer cells were found to attach (Fig. 5-4) until there were no more cells present in the material (Fig. 5-5). This effect can be explained by an increase of foam density, decrease of pore-size, and eventually a changed hydrophobic-hydrophilic balance of the material.

**Bidirectional gradient materials as hosts for spatially separated co-culture of two human cell lines**

The design of materials mimicking the human body more closely is one major challenge in the design of multifunctional materials. The challenging aspect here is the culture of two or more cell types spatially separated from each other, however, keeping them indirectly connected via a shared culture medium. The shared culture medium allows the exchange of signaling molecules or metabolites between the different cell types. Such a system could be used as a model for single organs that are connected via body fluids. To tackle this challenge, we made use of the findings about cell behavior in graded materials. In the previous section we demonstrated that it is possible to build a material with a cell-free part. In a final experiment, this result was used to culture two human cell types spatially separated in one material. As a model we used HeLa and LUHMES cells. By designing a material with a bidirectional gradient (Fig. 6c), the small sized pores in the middle of the material built a barrier for diffusion due to their hydrophobic character. This barrier enabled the cultivation of HeLa cells exclusively on one side of the material (Fig. 6c-1, 6c-2) and LUHMES cells spatially separated on the other side (Fig. 6c-4, 6c-5). The barrier, depicted by the grey lines, remained free of cells (Fig. 6c-2-4).

**Conclusions**

In the present work, we report the first synthesis of giant porous organosilica materials as a 3D scaffold for cell culture.
We used commercially available PU foams as a structural template. Infiltration of the PU foam with a thiol containing benzene-bridged alkoxy silane precursor followed by sol-gel process allowed the coating of a PU foam with a functional layer organosilica layer. Since living tissue is hierarchically organized, one more challenging goal was to develop a material which displays a structural gradient. By inserting the PU foams into an appropriate mould, it was possible to create a material with any conceivable gradient. Therefore, it was possible to significantly decrease the pore diameter and create anisotropic pores. In order to make the surface biocompatible, thiol-ene chemistry was applied and a biotin-containing linker molecule was quantitatively attached to the surface. By exploiting the strong binding between biotin and streptavidin, the material was covered with a fibronectin-streptavidin fusion protein. Fibronectin is part of the ECM and responsible for cell adhesion. Comparisons of pure thiol-organosilica with fibronectin-functionalized surfaces confirmed the biocompatibility of the advanced material. One demanding task in the development of biomaterials is the urge to produce materials which represent more human in-vivo like models. In doing so, one aspect is the co-cultivation of more than one cell type. By generating a pore size gradient, it was possible to establish a cell-free domain in the area of the drastically reduced pore size. In a final experiment we were able to establish a bidirectional gradient material with small pores in the middle. The barrier did not allow any cell diffusion into the other compartment and made it possible to co-culture two human cell types with a spatial separation in one material. Having different cell types cultured in one three-dimensional material makes it possible to investigate communication of cells that are not in direct contact with each other but are still capable of exchanging information via signalling molecules. This feature is accompanied by the technical advantage of easy handling as only one material has to be handled by the operator. Furthermore, large cell numbers can be cultured in small volumes on these three-dimensional porous materials, thereby also modelling hypoxia conditions of human tissue better.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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The Influence of Structural Gradients in Large Pore Organosilica Materials on the Capabilities for Hosting Cellular Communities

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**ELECTRONIC SUPPLEMENTARY INFORMATION - PART B**

**Experimental details**

**Chemical experimental details**

1,5-Bistri(isopropoxysilyl)-benzene-3-thiol (1) was synthesized according to a previously reported process. To a solution of 6 g of 1,3-bistri(isopropoxy)silyl-5-bromobenzene (10.6 mmol) in 400 mL of dry Et₂O was added tBuLi (11.5 mL, 1.9 M, 21.2 mmol) dropwise at -78 °C. The mixture was stirred for 30 min. Then 355 mg (10.6 mmol) of S₈ was added and stirred for another 30 min at –78 °C. Afterwards the colorless solution was warmed to room temperature and stirred for 1.5 h. Then the reaction was hydrolyzed with 120 mL of dry isopropanol. After removal of the solvent a yellow oil can be obtained. For further purification column chromatography was applied (silica gel 60, CH₂Cl₂).

Finally, 5.38 g (10.37 mmol; >95 %) of a colorless oil was obtained. ¹H-NMR (400 MHz, CDCl₃): δ (ppm) 1.20 (d, 36 H), 3.42 (s, 1H), 4.24 (sept, 6H), 7.62 (s, 2 H), 7.76 (s, 1H).

²⁹Si-NMR (400 MHz, CDCl₃): δ (ppm) -62.71.

ESI-MS (m/z): calcd for C₂₄H₄₆O₆Si₂ 541.2453 [M+Na⁺] found: 541.2456.

N-(tert-Butyloxycarbonyl)-ethyl-1,2-diamine (2) was synthesized according to a previously reported process. 27 mL (400 mmol) of 1,2-diaminoethane are dissolved in 400 mL of chloroform at 0 °C. 8.7 g (40 mmol) of Boc-anhydride are dissolved in 300 mL of chloroform and added dropwise over a period of 5 h. The reaction is stirred overnight and heated up to room temperature. The solvent is concentrated, and the residue dissolved in 3 N Na₂CO₃ (300 mL) solution. The mixture is extracted with chloroform (3 x 200 mL), the organic phase dried over MgSO₄ and evaporated to produce a colorless oil. ¹H-NMR (400 MHz, CDCl₃): δ (ppm) 1.43 (s, 9 H), 2.78 (t, 2 H), 3.14 (q, 2H), 4.92 (s, 1H).
1-(N-tert-Butoxycarbonyl)-2-aminoethyl maleimide (3) was synthesized according to a previously reported process. 5.1 g (31.9 mmol) of N-(tert-Butoxycarbonyl)-ethyl-1,2-diamine (2) and 6.6 mL (47.8 mmol) of NEt₃ are dissolved in Et₂O (60 mL) at 0 °C. 3.1 g (31.9 mmol) of maleic anhydride in 60 mL of Et₂O are added dropwise to the solution and stirred for 4 h during which the reaction is allowed to reach room temperature. The solvent is evaporated, and the residue is dissolved in acetone (150 mL). 8.8 mL (63.7 mmol) of NEt₃ are added and the reaction is heated to reflux. 4.5 mL (47.8 mmol) of acetic anhydride are added and the reaction is heated to reflux for 20 h. The solvent is removed, the crude product purified via silica column chromatography (EtOAc/ pentane 1:1) and resulted in a white solid. ¹H-NMR (400 MHz, CDCl₃): δ (ppm) 1.40 (s, 9 H), 3.32 (q, 2 H), 3.66 (q, 2 H), 4.71 (s, 1 H), 6.71 (s, 2 H).

N-(2-Aminoethyl)maleimide (4) was synthesized according to a previously reported process. 4.6 g (19.2 mmol) of 1-(N-tert-Butoxycarbonyl)-2-aminoethyl maleimide (3) are dissolved in 35 mL of CH₂Cl₂ at 0 °C. 27 mL of TFA is added to the solution and stirred for 1 h during which the solution is allowed to reach room temperature. The mixture is concentrated, washed with cold Et₂O and provided a white solid. ¹H-NMR (400 MHz, DMSO-d₆): δ (ppm) 2.99 (t, 2H), 3.66 (t, 2H), 7.06 (s, 2H), 7.93 (s, 3H).

Biotin-NHS-ester (5) was synthesized according to a previously reported process. 2.0 g (8.19 mmol) of biotin are suspended in dry DMF under inert gas atmosphere. 1.13 g (9.82 mmol) of N-hydroxysuccinimide and 1.88 g (9.82 mmol) of EDC hydrochloride are added and the reaction is stirred for 2 h at room temperature. The solvent is evaporated and the remaining solid washed with a mixture of water/ ethyl acetate and ethanol (95/1/4). ¹H-NMR (400 MHz, DMSO-d₆): δ (ppm) 1.36-1.71 (m, 6H), 2.59 (m, 1H), 2.68 (t, 2H), 2.82 (s, 2H), 2.86 (m, 1H), 3.12 (m, 1H), 4.16 (m, 1H), 4.31 (m, 1H), 6.36 (s, 1H), 6.42 (s, 1H).

Ellman test was carried out according to literature. 3 mL of a 20 µM solution of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) in 0.1 M Sodiumphosphate buffer (pH = 8) were added to a UKON-2j foam. After an incubation time of 2 h the supernatant was analysed by UV/Vis measurement.

**Biological experimental details**
**Cytotoxicity assessment.** HeLa cells were seeded in 96 well plates at a density of 60,000 cells/cm². After 24 h, medium was exchanged, and cells were cultured in HeLa culture medium that was used to wash the organosilica foams before. After 48 h in wash medium, cytotoxicity was assessed by lactate dehydrogenase (LDH) release assay.

**Lactate dehydrogenase (LDH) release assay.** LDH activity was measured separately in the supernatant and cell lysate. Supernatant was collected and cells were lysed using 0.5 % Triton X-100 in PBS for 1 h. The percentage of released LDH was calculated as 100xLDH_{supernatant}/LDH_{supernatant+lysate}. For the enzymatic assay, 10 µl of sample was added to 100 µl of reaction buffer containing NADH (100 µM) and sodium pyruvate (600 µM) in sodium phosphate buffer (pH 7.4, adjusted by 40.24 mM K₂HPO₄ and 9.7 mM KH₃PO₄ buffer). Absorption was measured at 340 nm at 37 °C in intervals of 1 min over a period of 20 min. Enzyme activity was calculated from the slope.
Figure S1. Thiophenol-based aerogel materials.

(a) Synthesis route: Aerogel.

In a typical synthesis 0.22 mmol of UKON-2j are dissolved in 2 mL of Ethanol. 50 µL of a 1 M HCl solution are added under vigorous stirring. After hydrolyzing the solution for 1 h, 50 µL of concentrated Ammonia was added and the solution was filled into a syringe and gelled overnight.

(b) Synthesis route: Biotin modification.

In a typical synthesis 0.022 mmol of UKON-2j aerogel and 0.022 mmol MalBiotin are dissolved in 5 mL of DMF. 0.21 mmol of NEt₃ are added and the material is shaken for 2 d. The postfunctionalized material is washed with DMF and Ethanol.
(c) SEM micrograph of the aerogel.

Black = UKON-2j aerogel; grey = UKON-2j aerogel post-functionalized with MalBiotin

Calculations yield in a post-functionalization degree of 83 %.
(e) FTIR-analysis.

Black = UKON-2j aerogel post-functionalized with MalBiotin, dark grey = UKON-2j aerogel; light grey = molecular MalBiotin

The IR spectra of the UKON-2j aerogel post-functionalized with MalBiotin shows several significant vibration bands (black spectrum). The NH-vibration at $\nu = 3275$ cm$^{-1}$ and the aliphatic CH-vibrations at $\nu = 3120 - 2820$ cm$^{-1}$ and the CO-vibration at $\nu = 1693$ cm$^{-1}$. These three vibrations in the composite material result from the clicked MalBiotin (compared to the identical signals in the light grey spectrum of pure MalBiotin). The Si-O-vibration at $\nu = 1020$ cm$^{-1}$ results from the UKON-2j silica vibration (compared to the identical signals in the dark grey spectrum of pure UKON-2j aerogels).

(f) Physisorption

Black = Physisorption measurement of UKON-2j aerogels; grey = Physisorption measurement of UKON-2j aerogels post-functionalized with MalBiotin

Due to the post-functionalization there is a reduction of the BET surface of 768.81 m$^2$/g (for UKON-2j aerogels) to 287.30 m$^2$/g (for UKON-2j aerogels post-functionalized with Biotin-Maleimid). There is mainly a reduction of the surface of the micropores.
(g) Application as a host material for living cells.

Cell growth of viable HeLa cells stained with calcein-AM (green) on biocompatible organosilica aerogel materials (surfaces modified by thiol-Biotin-Strep-Fib). Scalebar = 100µm.
Figure S2. Thiophenol-based organosilica PU foam composite.

(a) FTIR spectroscopy

Black = Thiophenol-silica PU composite; grey = pure PU foam as a reference.

The strong Si-O stretching vibration is visible at 1052 cm\(^{-1}\) which can be clearly assigned to the organosilica part of the hybrid material. The signals for the aliphatic C-H vibrations at 2986 – 2847 cm\(^{-1}\) and the C=O vibration at 1717 cm\(^{-1}\) which result from the PU foam are still visible in the spectrum of the hybrid foam.
(b) TGA.

Black = Thiophenol-silica PU composite; grey = pure PU foam as a reference.

3 % of the total masse remains for the pure PU foam, the composite material has a remaining masse of 33 %. Under the assumption that the UKON-2j decomposes completely to SiO₂, a mass ratio of PU foam to UKON-2j of approximately 1:1 can be calculated.
Figure S3. Thiophenol-based organosilica PU2 foam composite.

(a) SEM micrographs

Scale bar corresponds to 500 µm.

(b) EDX analysis.
Scale bar corresponds to 200 µm.

Green: carbon, Yellow: oxygen, Red: silicon, Blue: sulfur
Figure S4. Thiophenol-based organosilica Basotect foam composite. (a) SEM micrographs
(b) EDX analysis

Energy / [keV]

Turquoise = carbon; green = nitrogen; yellow = oxygen; red = silicon; blue = sulfur
**Figure S5**: Angle-dependent variation of pore size gradient.

Stamp with an angle of 90°. SEM picture of resulting PU-UKON-2j material. Scale bar 500 µm.

The angle of the kink in the stamp defines the steepness of the gradient in the final material.
Figure S6: Assessment of biocompatibility of organosilica foams before and after biotinylation. HeLa cells cultured in eluates of biotinylated (green) and unbiotinylated (blue) organosilica foams showed cell growth and viability comparable to control cells grown in pure culture medium. Cell viability was assessed by lactate dehydrogenase release assay. SD: standard deviation.
**Figure S7**: Cy5 staining of gradient PU2-UKON-2j materials that have been post-functionalized with biotin.

The pictures show Cy5-fluorescence (magenta) and autofluorescence (blue) of thiophenol organosilica foam incubated with Cy5-tagged streptavidin after biofunctionalization of the surface. Pore size decreases from left to right. Scale bar 100 µm.

The biotinylation of the surface was successful shown by Cy5 fluorescence all over the organosilica foam.
