

Cells in the round – scaffolds for tissue engineering

The development of three dimensional cell culture scaffolds for tissue engineering applications is a key activity for Dr. Alex Welle, who leads the biomaterials group at the Institute for Biological Interfaces at the Forschungszentrum Karlsruhe in Germany. The institute has a strong focus on organotypic in vitro cultures of cell lines as well as primary hepatocytes and embryonic stem cell lines (mouse). Welle's group is using 3d scaffolds based on polymeric materials. The goal is to develop artificial stem cell niches and other long-term cell culture systems for applications such as bio-artificial organs.

Welle's group initially used physisorbed proteins to stimulate cell adhesion. As he notes, "While this might be the method of choice for everyday's cell culture, the limitations of this approach are obvious." Today, genetics, molecular biology and nano technology raise the bar in relation to the need for precise control of the composition, the localization and the nano scaled orientation of functional groups on artificial substrates. This needs integrated processes starting from the polymerization of designed monomers, scaffold preparation, potentially surface modifications and patterning, and the attachment of ligands combined all the way with powerful analytics. As a consequence, high efforts of the interdisciplinary team are put into the chemical immobilization of peptides, synthesized technically or by genetically modified organisms.

The materials now used for the development of tissue engineered devices are inert or biodegradable polymers like the well known poly(styrene) (PS) and PMMA, as well as novel biodegradable poly(carbonates). Scaffolds are produced using a variety of technologies developed in house (micro thermoforming) or provided by collaboration partners. Examples of production technologies include thermal induced phase separation or porogen leaching to produce foams, and electro-spinning to produce non-woven nano fibres.

The group in Karlsruhe carries out photochemical modifications of polymer surfaces by deep UV irradiation and similar techniques. This tends to lead to altered surface

chemistries that are often initially too minute to have a bulk and hence a mass effect. However, QCM-D is widely used as part of the biomaterials' group's activity. Actually, the group has been using the technology since 2001 and was the first organization in Germany to own a QCM-D system. As Welle explains, "QCM-D helps us to avoid to high UV doses which usually affect polymer bulk properties in an unwanted way, e.g. if UV-irradiation times are too long surface ablation of polystyrene takes place. This is easily measured in a window chamber purged not by liquid but by oxygen or nitrogen at fixed flow. The upper window is quartz and therefore UV transparent down to 185nm."

Fig. 1

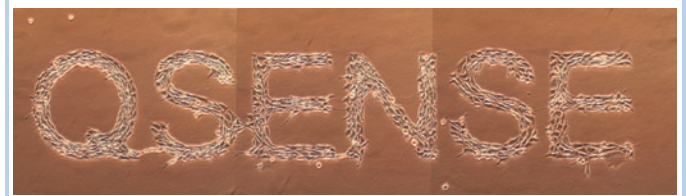


Fig1: L929 fibroblasts on patterned poly(styrene) surface. Character height: 475µm.

Fig. 2

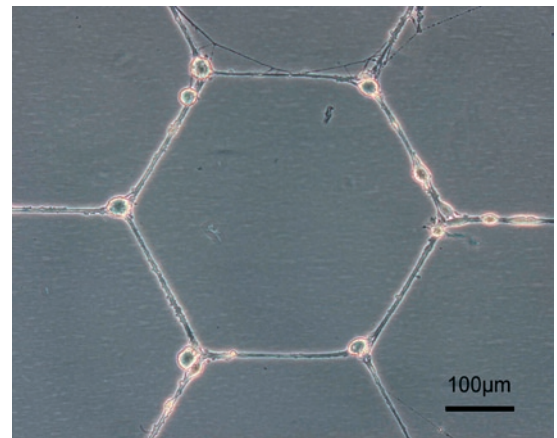


Fig2: PC12 cells (neuronal cells) on patterned poly(styrene) having a albumin/laminin pattern. After cell differentiation neurons follow distinct pathways and build a neuronal network.

The next step in the process is the adsorption of several proteins onto the modified surfaces. Welle points out, “I guess everybody in the QCM-D community is well aware of the tremendous versatility of the flow chamber system for these studies. We’ve learned from QCM-D data that, dependent on the photo modification, different protein films are formed by competitive protein adsorption which translates in case of masked irradiations into micron scaled patterns of cells (see Fig.1 and Fig.2) or gradients to control migration and orientation of cells.”

QCM-D highlights the differences in protein adsorption on native and photomodified polymer surfaces:

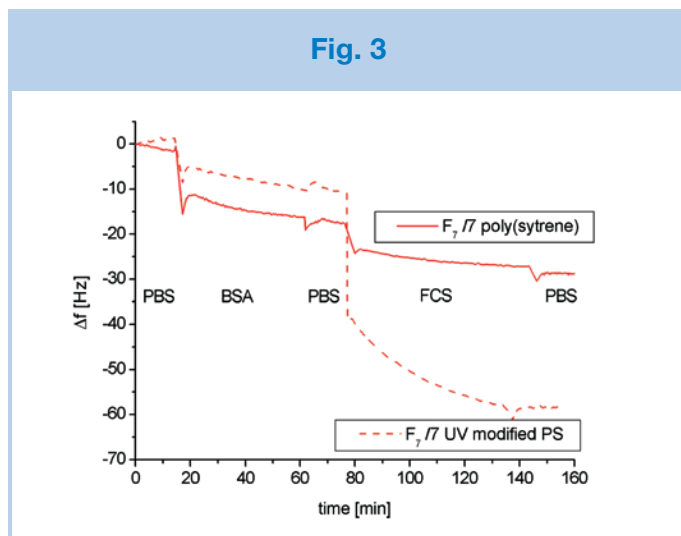


Fig3: Frequency changes during adsorption of bovine serum albumin (BSA) on native (solid line) and photomodified poly(styrene) (dashed line), followed by the exposure to fetal calf serum (FCS). Baseline recording and rinsing steps with phosphate buffered saline (PBS).

As shown in Fig3 a bovine serum albumin layer formed on native PS blocks quite effectively subsequent deposition of numerous other proteins from serum. In contrast, the albumin layer on irradiated PS is thinner and, as calculated from ΔD data, softer. This allows the deposition of proteins from serum or from some pure protein solutions like laminin. Finally, cells will be able to attach, spread and proliferate only on the irradiated areas. Also, in case of these cell/surface interactions, QCM-D helps to follow for instance the kinetics of cell adhesion. Again the window chamber is employed. In this research, increasing dissipation values reflect to adhesion of cells onto the conditioned surfaces. The sour note here is of course the lack of any systematic understanding of the highly complex interface phenomena and to combine living organisms with materials science of

viscoelasticity. Nevertheless, using QCM-D here speeds up the experiments significantly, helps save biological material and the QCM-D response can be correlated with ligand densities on the polymer surface.

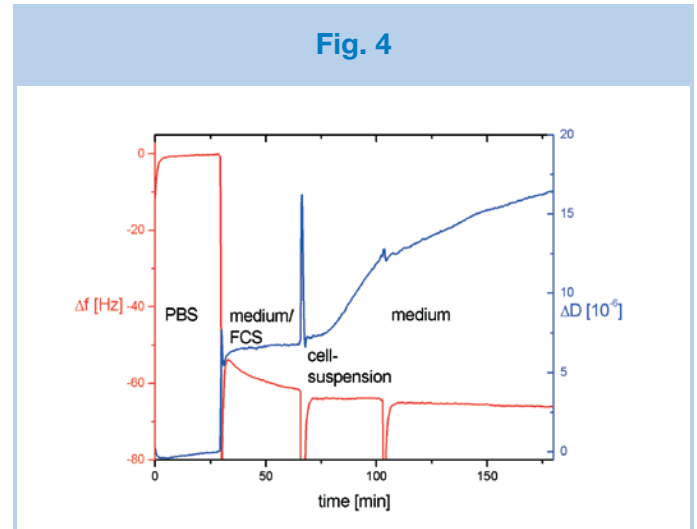


Fig4: Δf and ΔD showing protein adsorption on modified polymer followed by cell adhesion. As cells sediment down in the window chamber and attach to the formed protein layer the dissipation increases. This increase continues after flushing the chamber with cell culture media as the adherent cells continue to form firm attachment points and reorganize their cytoskeleton during cell spreading.

As an experienced user of QCM-D, the biomaterials group does some collaboration work which exploits their QCM expertise. “Our main collaborators are Rolando Barbucci at the University Siena in Italy and Ioannis Chronakis from the Swedish Institute for Fibre and Polymer Research. In both cases some QCM-D related work has been published. Apart from these two-party collaborations we are involved in the STREP “Vascuplug”, being a EU project with contributors from Germany, Greece, United Kingdom, Sweden, Hungary and Spain, aiming at vascularized large scale implantable tissue constructs”, explains Alex Welle.

References:

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2. Competitive protein adsorption on micro patterned polymeric biomaterials, and viscoelastic properties of tailor made extracellular matrices: Alexander Welle, Antonio Chiumiento, and Roland Barbucci, *Biomolecular Engineering* 24 (2007) 87-91.
3. Photo-chemically patterned polymer surfaces for controlled PC-12 adhesion and outgrowth: Alexander Welle, Siegfried Horn, Jutta Schimmelpfeng, and Dorothee Kalka, *J. Neurosci. Meth.* 142 (2005) 243-250.

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