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High-throughput screening of cell–biomaterial interactions

Biomaterials are increasingly employed in a broad range of medical applications, such as drug-eluting stents, cell delivery vehicles, or as *in vitro* templates for tissue regeneration. In all cases, when a foreign material is presented to the cells of the body a dynamic and reciprocal interaction occurs as the cells modify or foul the biomaterial and the biomaterial, in turn, influences cell function. Despite the prevalent use of biomaterials in the clinical setting, we still only possess a basic understanding of how cells interact and communicate with such materials. Ultimately, a more rigorous understanding of how to design biomaterials that synergize with the body during healing or drive specific biological processes during cell culture would lead to improved clinical success of biomaterials. At the core of our limited knowledge of cell–material interactions is the inability to predict how cells will respond to features, such as surface chemistry or nanotopography, of the most commonly used biomaterials. Cells can also respond to multiple cues from a biomaterial in a nonlinear fashion, as signals combine synergistically or antagonistically. Furthermore, the cell–material interaction often takes place in a complex biological milieu with chemical factors and mechanical forces, such as shear stress. High-throughput approaches are required to investigate cell–material interactions and to develop a knowledge base of how cells sense and integrate multiple, biologically relevant signals. Recently, Mei *et al.* used arrays of polymeric biomaterials to probe how surface chemistry influences embryonic stem cell fate.[1] This approach is representative of the high-throughput technologies that have enabled researchers to begin exploring cell–material interactions in a systematic manner within the vast biomaterials landscape.

Building upon this paradigm and the recent development of a high-throughput chip to study the influence of surface topography on cell function,[2] Unadkat *et al.* reported a broadly applicable microfluidic chip carrier for high-throughput cell culture of homogeneous cell populations in isolated wells of a perfusion bioreactor.[3] This bioreactor, comprised of thousands of cell-laden microwells, enables long-term cell viability and culture while ensuring that chemical communication is kept to a minimum. Further, the device is amenable to modification of the surface chemistry and topography of each well. In addition, the ability to perfuse the cells in this system allows control over the shear stress that the cells experience (Fig. 1). In this manner, this chip carrier comprises a tool to investigate the individual or combinatorial effects of biomaterial properties, such as surface chemistry, topography, chemical factors, and shear stress on cell function.

To demonstrate the ability of the perfusion chip carrier to seed homogeneous populations of cells into each microwell, or TestUnit, Unadkat and co-workers seeded the chip with three different cell suspensions (125 000; 250 000; and 750 000 cells per ml). In all cases, each TestUnit was filled with a relatively uniform number of cells ($\sim$10, $\sim$20, and $\sim$40 cells per well, respectively) and the cell number distributions followed a binomial distribution, as predicted. Cells were then cultured within the TestUnits under perfusion and cell viability remained >95% over the course of 5 days. In these

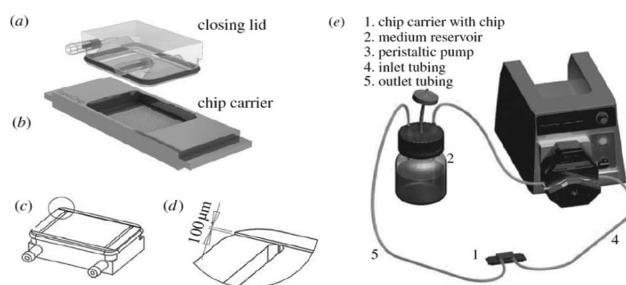

Fig. 1 Schematic illustration of the perfusion bioreactor used for high-throughput studies of cell–material interactions. Figure reprinted with permission from Unadkat *et al.*[3]

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experiments, the perfusion rate was set so that the Péclet number for small molecules in cell medium would be in excess of one over the whole device. Under these conditions, mass transfer is dominated by convection and there should be efficient exchange of nutrients and metabolites between the cells and the medium. Computational flow modeling was employed to demonstrate that while gradients of model biomolecules, such as BMP-2 or lactate, can be induced by the flow (100 μl min^{-1}, in this case) the highest concentrations observed are several orders of magnitude below physiologically relevant concentrations, indicating that despite perfusion the TestUnits remained chemically isolated. Finally, similar flow modeling was employed to demonstrate, *in silico*, control over the shear stress that the cells experience when cultured within the TestUnits (0.0001 Pa at 10 μl min^{-1} to 0.028 Pa at 100 μl min^{-1}).

The perfusion chip carrier presented in this work comprises an important advance in the development of high-throughput technologies to decipher how cells interact with and respond to biomaterial surfaces. This device particularly enables experiments that depend on homogeneous cell populations, chemically isolated wells, or defined shear stresses. Further, the device has been tuned to independently vary these parameters and interface with a library of surface chemistries and topographies. In brief, this perfusion bioreactor should enable improved screening of combinatorial effects of extracellular signals on cell function during cell–biomaterial interactions.

Heterogeneous catalysis-on-chip

The use of catalytic reactions are central to numerous industrial applications as diverse as petroleum refining[4] and manufacture of commodities such as methanol and ammonia, in large part due to the potential to replace hazardous chemical reactions with more environmentally friendly ones and to provide more energy efficient manufacturing processes.[5] Here, particularly heterogeneous catalytic reactions–notable for the difference in phase of the catalyst and the reactants–are of interest, as industrial catalysts tend to be solid and the reactants tend to be gaseous or liquid materials. The development of low-energy and non-hazardous chemical reactions based on such heterogeneous catalysts then necessitates a detailed investigation of the reaction dynamics and kinetics as well as the reactants and the catalytic materials. A time- and cost-efficient way to explore these factors involves miniaturizing the experimental platform, in particular using microfluidic approaches.

The team led by Klavs Jensen has recently utilized their microfluidics expertise to develop such a platform by miniaturizing the traditional packed-bed approach, in which liquid or gaseous reactants are introduced into a tube filled with solid catalyst particles. Reduced to a lab-on-a-chip scale, these tubes were transformed into long, straight microchannels on a silicon–Pyrex device, with a microfabricated physical filter at one channel end to keep the catalyst particles confined inside the structure (Fig. 2). Liu, Ünal and Jensen[6] introduced glass beads into the channels to serve as an additional barrier, followed by a thick suspension of catalyst in water. The dense packing of the catalyst particles (on mg scale) served to increase the surface contact area between the reactants and the catalyst. The gaseous and liquid reactants were pumped into the channels through separate gas and liquid inlet ports and the device was placed on a temperature stage to generate two well-defined regions at low (reactant input/output area) and high (catalytic reaction area) temperatures. The entire structure was coupled with an external pressure and temperature control system.

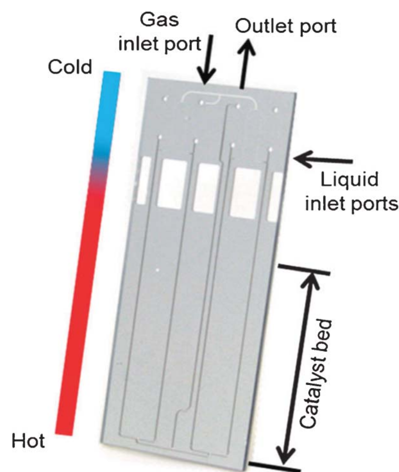

Fig. 2 Schematic detailing the structure of the packed-bed microreactor for heterogeneous catalysis. Figure reprinted with permission from the Royal Society of Chemistry from Liu *et al.*[6]

The functionality of this microscale catalyst bed platform was tested on two reactions. In the first, an aerobic oxidation of 4-isopropylbenzaldehyde (IBA), various catalysts based on Pt and Pd were tested for efficiency; factors that were used to optimize the catalyst included a gas chromatograph analysis of the reaction products (at only 100 μl) and the time needed for the reaction to reach a steady state. This reaction was chosen as an example of generating cumic acid, a compound often used in pharmaceutics as an intermediary. In the second type of reaction, hydrogenation (also a key reaction in the pharmaceutical and petroleum industries), temperatures of up to 350 °C and pressures near 100 atm could be established. In addition to the multiphase packed bed microreactor (Fig. 2), the authors developed a separate silicon–Pyrex microfluidic device with an attenuated total reflectance (ATR) section (not shown). In this device, infra-red windows were microfabricated into the silicon layer to enable probing of reactions in the silicon microfluidic channel by Fourier transform infrared spectroscopy. Using this unit, they monitored reactor effluents in-line, downstream of the multiphase reactor. This new low volume (2 μL) microfluidic ATR flow cell offered wider operating range than any other similar devices reported previously.

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Compared to bench-top scale optimization experiments, the catalytic reactions conducted using the microfluidic device required orders of magnitude smaller reagent volumes, which made the disposal of hazardous chemicals easier and less costly and helped contain any air-sensitive reagents in a sealed environment. In addition, the small channel dimensions effectively offered isothermal operations and eliminated any undesirable mass transfer effects in the catalytic reactions. In addition to the precise control over pressure and temperature inside the chip, the microfluidic approach with the ATR unit also enabled continuous monitoring of the reaction products and, as a result, steady readjustment of the control parameters. Thus, unlike the traditional macro-scale platforms, this device allows for a feedback loop that can be used to optimize experimental conditions.

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Non-invasive biopsy tools

Miniaturization approaches and the development of microelectromechanical devices have revolutionized not only fundamental research and applications such as electronics, but also the field of biomedical research and medicine itself. A prime example is the emergence of minimally invasive surgical procedures, which are replacing invasive procedures in a range of treatments, such as endoscopy, microsurgery or subdermal implantation. The advantage of these procedures lies in the minimal scarring and faster recovery of the patient, but the use of tethered instruments limits their application to easily accessible parts of the body.[7] Untethered tools such as pill-sized endoscopy cameras[8] or nanoprobes,[9] however, can be employed to access most areas in the body, while being delivered to the site of interest non-invasively. One challenge facing the wide-spread application of these physically unconstrained devices is keeping them small (on the nano- or micro-scale) while lending them motility and multiple functions. This challenge is particularly important to address in the development of untethered biopsy devices, which are used to extract cell and tissue samples from a patient for diagnostic purposes.

David Gracias and co-workers have recently tackled this problem and developed temperature-sensitive forceps termed μ-grippers, capable of grasping and extracting cells from a tissue sample in a static liquid environment.[10] Gultepe *et al.*[11] have then extended the capabilities of these μ-grippers to retrieve cells under dynamic, *in vivo* conditions: and in a hard to reach location in the body, namely the bile duct.

Consisting of a series of Cr–Au, ferromagnetic Ni, and thermo-sensitive polymer layers, the star-shaped μ-grippers were fabricated using conventional multilayer lithography. The choice of polymer was dictated by several factors: biocompatibility for the duration of the procedure; photo-responsiveness to allow for photo-patterning; chemical and physical stability in various tissues; and softening at the physiological temperature of 37 °C. Namely, softening of this polymer at body temperature is intended to release the stress in the pre-stressed Cr–Au layer and cause the device to close within 10 min. Conversely, at low temperatures the polymer is supposed to stiffen and keep the device arms flat and open (Fig. 3). All four requirements were met in a mixture of photoresists. Although devices as small as 300 μm were fabricated, the μ-grippers used *in vivo* were ~1 mm in size, large enough to be imaged using an endoscopic camera, yet sufficiently small to be extracted from the tissue using a magnetized catheter.

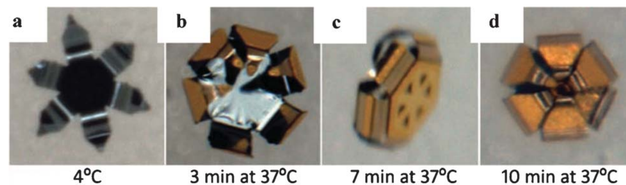

Fig. 3 Thermal actuation of the μ-gripper. While in a closed state at body temperature, the device can be opened *ex vivo*, after tissue extraction, by cooling down to 4 °C. Figure adapted and reprinted with permission from Gultepe *et al.*[11]

In the *in vivo* experiment, over 1500 μ-grippers were deployed in the bile duct of a live pig. Insertion and removal after tissue collection were conducted using endoscopic catheters, the latter of which utilized a magnet at the catheter end to attract the ferromagnetic Ni in the device structure. The deployment and collection process was imaged with an endoscopic camera and the devices could be tracked at the collection point using an X-ray based imaging method. After retrieval, the excised tissue samples were removed from the μ-grippers and stained to verify their quality for cytological analysis.

Challenges to the proper function of the microdevices involved uncontrolled muscular motion of the animal as well as the shear environment during insertion and inside the tissue, but the imaging results showed that the μ-grippers functioned as intended. Besides being able to transfer the *in vitro* μ-gripper technology to conduct biopsies in living animals, the authors have succeeded in developing a medical tool that is up to two orders of magnitude smaller than conventional biopsy needles. This guarantees a reduction in the production and handling cost of the microdevices, while the lack of incision sites and thus a reduced probability of complications due to the biopsy procedure are likely to decrease the medical treatment expenses. Although the use of the μ-grippers currently requires insertion of a catheter into the body and is therefore not entirely non-invasive, we can envision a delivery method that combines oral ingestion of the microdevices with external magnetic guides to lead them to the desired biopsy location.

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