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Human Relevant In Vitro Tools: Microfluidics and the Enhancement of Mammalian Cell Culture

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New cell culture devices with augmented features are populating life science and pharmaceutical R&D benches at escalating rates. These efforts are driven by the need to both improve and better utilize human relevant experimental systems earlier in the drug development process.

These new systems could arguably be termed “benchtop bioreactors” and include features such as 3D culture spaces and dynamic fluidic flow capabilities. There are numerous configurations for benchtop bioreactors that commonly include a culture chamber or multiple culture chambers containing 3-dimensional scaffolds or linked to fluid pumps using tubes and valves. While these designs are elegant from an engineering perspective, many times the functionality falls short of what biologists, toxicologists, and drug development researchers really need or find valuable.

Some of the most important considerations in designing these benchtop bioreactors still need to be addressed by the end users to determine the applicability of these systems. First, an end user should ask whether drugs and toxicants are adsorbed to the various materials. If so, how will the user know and quantitate this? Other questions include: How robust is the process of getting cells into the system or to remove cells from the system for evaluation? How hard is it to achieve reproducible results? Will the user need to design,

develop, and validate completely new assays in order to get value from this system?

The purpose of this article series is to address these considerations in turn and afford new users of these advanced technologies the opportunity to see past the elegance, complexity, and sophistication of the design and really learn to address the functionality, ease-of-use, and applicability before selecting a system to meet their needs.

Part I: Getting Real with Materials

Most pharmaceutical companies have qualified and validated their cell culture systems using cell culture units manufactured out of polystyrene polymers in a wide range of configurations. Qualification of cell culture-based assays requires significant amounts of time, money, and resources to establish standard operating procedures that dictate critical ‘go and no-go’ development decisions.

Many fabricated bench-top bioreactors use a variety of other materials that are chosen based on fabrication considerations. However, when put into practice, chemical loss due to material absorption is a huge problem. For example, within a hollow-tube bioreactor advanced previously by our company founders ([Methods Mol Biol. 2013;1001:215-26](#)), the loss of the chemical 7-ethoxycoumarin due to material absorption was >90% within 30 minutes of application to the reactor (**Figure 1A**). Subsequent investigation of the four main materials used in

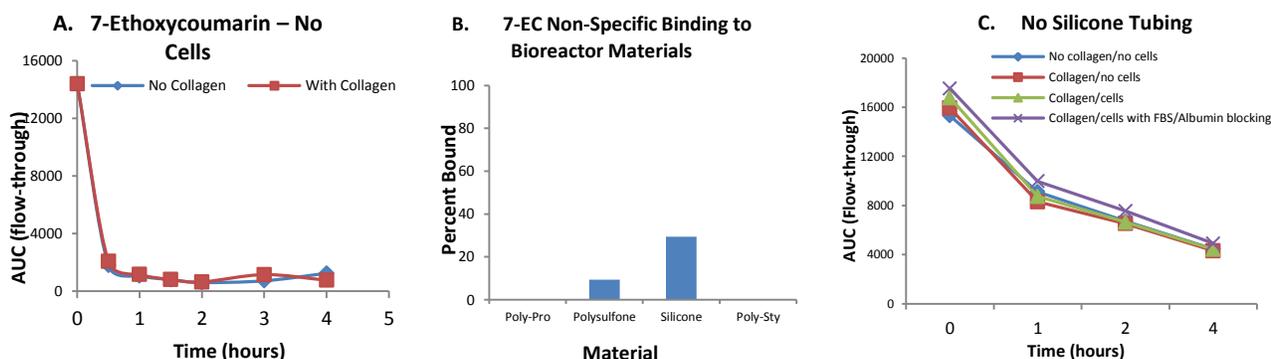


Figure 1. Non-specific binding of 7-Ethoxycoumarin (7-EC) within a hollow fiber bioreactor. A. HPLC analysis 7-EC in media passed through bioreactor over indicated times. Results show 90% loss of 7-EC in the absence of cells and independent of whether collagen matrix was present. B. Independent analysis of non-specific 7-EC binding to four of the individual materials. The 4 main materials could only account for 40% of the non-specific binding. C. When silicone tubing was removed, loss still exceeded 75% within 4 hours of application regardless of the whether reactor contained cells, collagen, or had been pre-treated with FBS/Albumin to block non-specific binding.

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the device showed that about 40% of the absorption was attributed to the use of silicone and polysulfone materials (**Figure 1B**). When silicone was removed from the device, loss was reduced, but still exceeded 75% after 4 hours (**Figure 1C**).

For many microfluidic devices currently being developed, the most common manufacturing material is PDMS (polydimethylsiloxane), a derivative of silicone, which is easy to fabricate in complex shapes and patterns and supports real-time imaging due to optical clarity. In a rigorous study of the absorption and leaching properties of PDMS, Reagher and colleagues showed the partition of hydrophobic molecules between media and PDMS was 9:1 after 24 hours ([Lab Chip](#), 2009, 9, 2132-9). Additionally, several groups have shown oligomers of PDMS can leach back into the fluidic system and be found integrated in cell membranes resulting in changes in cell signaling ([Lab Chip](#), 2006, 6, 1484-1486).

Another common problem with PDMS is that cells that normally adhere to polystyrene are highly variable with respect to adherence properties to PDMS. For each new cell type, a variety of biological or chemical coatings or other manipulations of the PDMS surface are required to simply replicate growth properties of cells on polystyrene ([Lab Chip](#), 2007, 7, 987-994).

One of the most overlooked aspects of PDMS devices is that fabrication is not amenable to mass manufacturing. As a result, most devices are expected to be re-used leaving washing and sterilizing efforts up to the end-user. These manipulations including bleach, alcohols, and/or autoclaving have been shown to change the physical properties of the polymer with

detriment to reproducibility ([Current Opinion in Biotechnology](#) 2014, 25:95–102).

SciKon’s founders have been developing bioreactors since 1997. To circumvent these problems encountered in many existing microfluidic devices, SciKon Innovation is developing the SciFlow™ line of modular fluidics devices made entirely of **polystyrene**, the most commonly used polymer in cell culture today.

Our first product, the SciFlow-1000 plate is an all-in-one device configured similar to the 96-well microtiter format. The device incorporates capillary fluid channels to establish 8 unique fluid rows with each row having 10 connected cell culture chambers (**Figure 2**). Because the SciFlow-1000 device is made entirely of polystyrene, researchers can directly adapt existing cell lines and assays and make comparisons to their previous cell culture experiments. Furthermore, the design and materials allow mass manufacturing to produce tens of thousands of plates with each device pre-treated, pre-packaged, and pre-sterilized for one-time-use. This feature will allow companies to more rapidly and reproducibly incorporate fluidics into existing assay pipelines, saving significant cost and human resources.

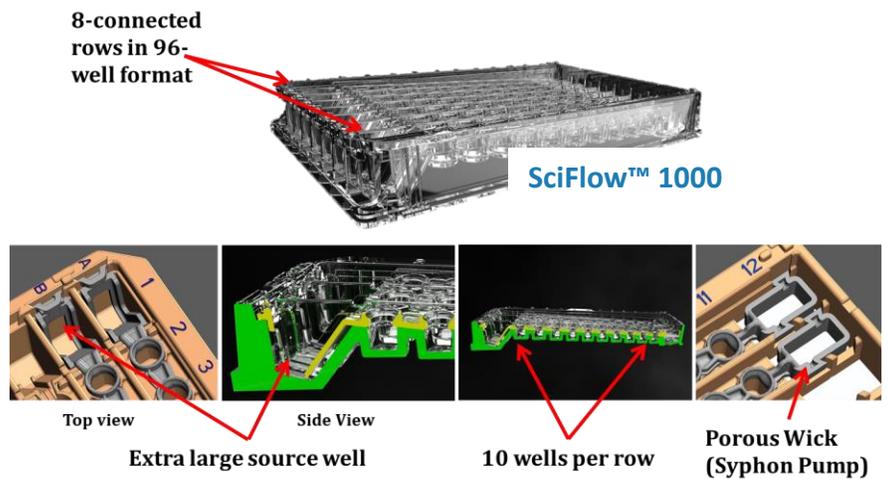


Figure 2. Features and Benefits of the SciFlow™1000 Capillary Fluidics Cell Culture Plate.

SciFlow™ Features	SciFlow™ Benefits
Each unit has 8 replicates of 10 wells connected together with capillary channel	Perform more life-like drug exposures in a user-configurable system that mimic complete target organ environments
No accessory pumps or tubes required	Maintains a small footprint with virtually no set up cost to user
Manufactured using chemically inert materials	Readily adaptable to existing safety and toxicity assays
Standard microtiter dimensions	Compatible with most existing laboratory instrumentation