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An end-user perspective on Organ-on-a-Chip: Assays and usability aspects

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The field of Organ-on-a-Chip is rapidly shifting from academic proof-of-concept studies to real-world solutions. The challenge is now to enhance end-user adoption by improving user friendliness, compatibility, assay ability and product readiness of these solutions. This review evaluates Organ-on-a-Chip efforts published over the last two years in light of such end-user adoption aspects. Elegant platforms have been reported including a microtiter plate-based 3D cell culture platform and a platform of cantilevers with integrated gauge sensors for contractility measurement. Also functional assays for angiogenesis, calcium imaging of neurons and neuro-muscular contractility were reported. Compatibility with standard analysis techniques such as sequencing, fluorescent activated cell sorting and mass spectrometry were reported only in rare cases. It is concluded that the elements that enable the leap towards end-user adoption are in place, but only few systems have managed to incorporate all aspects, and are able to answer biological questions.

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Introduction

Organ-on-a-Chip has recently emerged as a new paradigm in enhanced cell culture [1]. The field builds on almost 25 years of developments in microfluidic and associated microfabrication techniques on the one hand and an urge towards ever more physiologically relevant cell culture on the other hand [2,3]. Application of microengineering techniques in cell culture enables the use of flow and associated sheer stress, mechanical strain and allows integration of sensors and systems such as, sample preparation aspects, automated dosing and dilution series preparation. It also facilitates co-culture, 3D culture and application of controlled gradients.

Earliest work in microfluidic cell culture appeared around the turn of the century and includes perfused Transwell systems, multi-organ systems and 3D liver tissue [4–7]. Although many applications have been developed over the last 15 years, it was not until the paradigm shifting Lung-on-a-Chip publication of the Ingber group in 2010 that one could identify Organs-on-Chips as a field in its own right [8]. Since then, the field has expanded tremendously, both in terms of academic publications as well as commercial offerings.

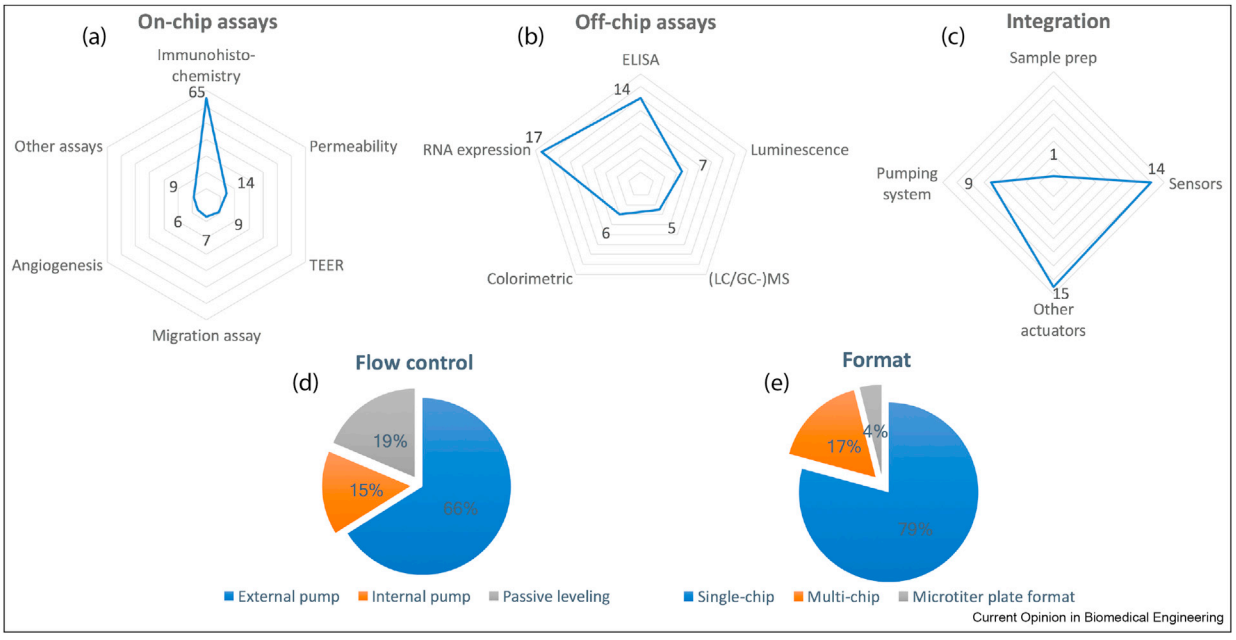
In our 2015 review article, we concluded that the field is currently shifting from a technology focus, aiming to develop prototypes and concepts, towards a biology focus, whereby validation of culture systems and integration of state-of-the-art stem cell and cell culture techniques are key [9]. With this transition towards an application focus, the question poses itself: what efforts are ongoing to promote end-user adoption?

In this critical review, we attempt to take an end-user perspective on Organ-on-a-Chip developments and make an inventory of instrument compatibility, ease of handling, and adoption readiness aspects. In addition, we consider the type of assays that are typically carried out in, or on samples from, these systems, providing insight in the spectrum of techniques that can be deployed for assessing biological properties and responses, and to answer biological, clinical or pharmacological questions.

Overview

In this review, we catalogued 77 research articles containing the keywords (Organ-on-a-chip) OR (“Organ on a chip”) OR (“microfluidic” AND “cell culture”), which appeared since 2014 on PubMed. Papers that were not found with the search string, but were known to the authors as highly relevant were added to the database. The articles were categorized according to on-chip and off-chip assays, integration aspects, flow control and format in Figure 1 and Supplementary Info.

Figure 1



Overview of assays and usability aspects of Organs-on-Chips since 2014. (a–c) Relative scores for the frequency of assays and integrations in Organs-on-Chips. On-chip assays: Immunohistochemistry scored the highest followed by permeability. Off-chip assays: RNA expression had the highest score, followed by ELISA. Integration: Other actuators and sensors scored the highest. (d) The distribution of different mechanisms of flow control in Organ-on-a-Chip. More than half of the developed microfluidic models had external pumps. (e) The distribution of different formats: The majority of Organ-on-a-Chip models is comprised of single chip concepts.

Although articles referenced in this paper describe many aspects of Organ-on-a-Chip systems, we have chosen to focus solely on usability and compatibility aspects of the solutions proposed. Physiological relevance of the various systems has been extensively reviewed elsewhere [1,9–11].

Figure 1a and b show spider graphs of assays performed in Organs-on-Chips, categorized into on-chip and off-chip assays. On-chip assays include immunohistochemistry, permeability, trans epithelial electric resistance (TEER), migration assays, angiogenesis and other assays (*e.g.* calcium imaging, colorimetric and luminescence). Off-chip assays consist of enzyme-linked immunosorbent assays (ELISA), luminescence, liquid/gas chromatography–mass spectrometry ((LC/GC-) MS), RNA expression and colorimetric assays. Immunohistochemical staining is the dominant on-chip analysis technique. Almost all publications used immunohistochemical staining to characterize the physiology of their tissue or organ models. We assume that also phase-contrast microscopy is generically used for on-chip assessment of cell morphology and confluence during culturing, however we omitted this from our analysis as it is usually not used as an endpoint or quantified analyses.

RNA expression analysis and ELISA are often used for assessing cellular responses to flow, co-culture or drug compounds. Although very well possible to perform such techniques on chips, in our analysis we find PCRs and ELISA to be exclusively performed off-chip. Although being a highly generic analysis technique, (LC/GC-) MS is used as a readout for Organs-on-Chips only by few [12–16].

Off-chip assays have the benefit that they are readily available and standardized. However, a disadvantage arises in conjunction with microfluidic chips. Cell culture volumes are typically quite small and dead-volumes in comparison are large. This renders the signal-to-noise ratio low in comparison to classical cell culture techniques. This problem is largely solved by performing assays on the chip. It is for this reason that immunohistochemical staining and other optical readouts are highly popular. Not only is their implementation relatively straightforward, the microfluidic environment also assures excellent imaging quality. Other on-chip assays are reported less often, as they have the disadvantage that they need to be tailored to the microfluidic environment. This puts higher constraints on the engineering skills of the research team, potentially distracting from biological developments.

Microengineering techniques offer ample opportunities to integrate actuators, sensors and complex fluid handling modules on the same chip (see Figure 1c). In recent Organ-on-a-Chip publications, this is predominantly done for sensors and actuators. The trend to