Continuous-Flow-Based Microfluidic Systems for Therapeutic Monoclonal Antibody Production and Organ-on-a-Chip Drug Testing

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Continuous-flow processing in the manufacturing of modern biotherapeutics represents a great potential and could significantly improve productivity and product quality as well as reduce operating costs. Microfluidic perfusion systems are not only capable for producing therapeutic proteins but also suitable for organ-on-a-chip based drug testing and toxicology studies. Integrating modular unit operations for protein purification in the microfluidic cell culture device can lead to point-of-care therapeutic protein production. The multi-organ microfluidic platforms that integrate several organ-on-a-chip microfluidic units will help in preclinical testing of drug substances and toxicological studies by producing highly reliable preclinical pharmacokinetic data. In this perspective, the current state of the art and future trends of continuous flow systems are summarized for biopharmaceutical production and organ-on-a-chip drug testing.

Keywords: Monoclonal antibody production, cell culture, microfluidic perfusion system, organ-on-a-chip

1. Introduction

In the past decades, the sales of biotherapeutics, including monoclonal antibodies, grew rapidly and biologics became the dominant product class in the pharmaceutical market [1–3]. Based on the prognosis, the global worldwide sales of approved monoclonal antibodies, the largest product type of biologics, was estimated to reach approximately $125 billion by the year 2020 [4]. The current rapidly changing market environment and the growing competition induced by biosimilars push the biopharmaceutical companies increasingly into developing innovative, highly flexible, and cost-effective manufacturing processes [5].

Process intensification through conversion from batch-based production to continuous manufacturing has been applied effectively by the pharmaceutical industry for small molecule drugs. Continuous applications for biologics manufacturing on the other hand have their limitations due to the complexity of automation and validation challenges. For the time being, the biopharmaceutical industry predominantly employs batch processes for recombinant protein manufacturing, whereas only less than 10% of the approved biologics are produced through continuous (or perfusion) processing [6]. Recently, the field of continuous bioprocessing is gaining greater acceptance and there is a growing interest to realize the benefits of continuous biomanufacturing [7]. The paradigm shift in biologics production from batch to continuous bioprocessing technology can result in significant benefits from standardizing on common terminology, as well as from the alignment of expectations and goals. It is easier to implement process standardization in a continuous process, so all biological drugs could be manufactured in a common manner. Full process standardization can be realized throughout the different phases of manufacturing process (e.g., process development, clinical production, commercial manufacturing) with identical equipment and control systems. Processes standardization also help the regulatory approval of biologics [8]. The US Food and Drug Administration (FDA) is encouraging manufacturers to switch to these newer and more efficient continuous processes that can enable faster production and more reliable products [9].

Flow processing in monoclonal antibody production has similar yield and purity compared to batch processing but with increased productivity and much smaller manufacturing footprint [8]. Continuous manufacturing of recombinant therapeutic proteins offers several advantages over batch processing: improved and consistent product quality, reduced equipment size, streamlined processes, low process cycle times, reduced capital and operating cost, increased flexibility, high volumetric productivity, higher level of automation and consequently less human interaction, an increased use of single-use equipment, and reduced inventory and storage needs [10].

Flow process development is focused on strategies to minimize intermediate sample purification to ensure a continuous stream of material between different unit operations. This approach also requires better process knowledge, equipment, and technological advances. Successful adaption of continuous processing by the industry requires the integration of process components involved in the manufacturing [11]. Product titer and cell productivity increase, improved intensification, smaller equipment size, maximum capacity utilization, and smaller processing lots show a trend towards the prevalent application of integrated continuous processing [12].

The newest developments in tissue engineering, biomaterials, and microfabrication methods enabled the construction of biologically relevant models of human organs on a chip. The so-called continuous perfusion organ-on-a-chip systems can help to speed up drug discovery and improve disease modeling. It may also facilitate drug testing by producing more reliable preclinical pharmacokinetic and pharmacodynamic data [13].

2. Batch vs. Flow-Based Production of Biotherapeutics

Therapeutic antibodies are mainly produced in mammalian host cell lines such as Chinese hamster ovary (CHO) and murine myeloma cells (NS0). The selection of expression system is based on its productivity with acceptable product quality attributes [14].

The cell culture processes for therapeutic protein production can follow three different types. The simplest mammalian cell culture process is a batch process. This is a closed system where all nutrients are added into the medium that are necessary for cell growth and production of proteins prior to addition of the cells. In the case of fed-batch process, a concentrated form of the initial culture medium is used for feeding. The feeding solution

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composition can be varied, based on the metabolic state of the cells at different culture phases. Continuous cell culturing has two options. In the case of chemostat cultures, fresh medium is continuously added to the bioreactor and the fermented medium is removed containing leftover nutrients, waste metabolites, product, and even viable cells at a constant flow rate to keep the culture volume unchanged. Perfusion culturing, on the other hand, retains the cells in the bioreactor to avoid loss of viable cells. [15]. Maximum cell densities are 1–2 × 10^6 cells/mL, 8–12 × 10^6 cells/mL, and 10–30 × 10^6 cells/mL for batch, fed-batch, and perfusion culturing, respectively [16]. The schemes of typical growth, cell viability, and mAbs production of the different processes are depicted in Figure 1.

In perfusion mode, there are different ways to keep the cells in culture, while removing the spent media. One way relies on filtration systems that keep the cells in the bioreactor by using internal filters, external loop flow-through filters, and centrifugation. Other methods for perfusion involve immobilization of the host cells on a solid support [11] such as microcarrier particles, magnetic microbeads, hollow capillary fibers, flat plates, fibrous matrices [17, 18], supermacroporous cryogel [19, 20], and membranes, which are retained or fixed in the bioreactor.

Perfusion manufacturing processes have the following advantages compared to the fed-batch processes: higher productivity, more consistent product profile, and smaller production footprint [21, 22]. Furthermore, if the product molecule is thermally instable, the product can be continuously removed during the perfusion process from the high-temperature bioreactor environments [21]. In the frame of quality by design (QbD) approach, perfusion production process can be planned and implemented efficiently and cost effectively [21, 23]. Continuous perfusion technology allows significant decrease in the residence time of the molecule in the bioreactor, which has benefits in the production of therapeutic proteins (e.g., mAbs). Modifications, such as post-secretion glycosylation changes and deamidation are minimized with continuous cultivations; therefore, it results in uncompromised product quality as well as less modified and more consistent glycoform profiles [12, 24, 25]. Yang et al. showed that continuous perfusion culture can improve the product quality by producing more active protein species (64.3%) compared to conventional fed-batch process (54.1%) [22]. Willard et al. made a comparison of the different culturing processes, namely, batch, fed-batch, and perfusion in the production of mAbs. They used two types of perfusion setups. One was equipped with an alternating tangential-flow (ATF) filtration device, which used hollow-fiber filters to retain the cells. The second one used a packed bed bioreactor, in which the cells were immobilized on a solid support. With the ATF perfusion process, they achieved noteworthy high cell density of 7.4 × 10^7 cells/mL, producing 11.4 g of antibody within 2 weeks. That represents 12-fold and 2.5-fold increases of product output compared with the batch and fed-batch processes, respectively [26]. Xu et al. compared the different production processes for mAb manufacturing. They concluded that perfusion cultures had the highest productivity (2.29 g/L/day) due to the high cell density maintained compared to the fed-batch cultures (0.39–0.49 g/L/day) [27]. An often observed disadvantage of perfusion processes is the high media cost involved, since continuous media exchange is required to maintain the desired high viable cell density. They proved that media cost for high productivity perfusion cultures could actually be lower than in the fed-batch process because of the low cell specific perfusion rates (14 ± 1 pL/cell/day) used in the continuous perfusion process [27]. These case studies also showed that perfusion production systems are getting more popular and economically feasible in biomanufacturing of mAbs.

3. Continuous-Flow Production of Monoclonal Antibodies in Microfluidics Systems

Culturing adherent mammalian cells in a microfluidic perfusion bioreactor system allows control over the microenvironment of the cells, regulating cell–cell and cell–matrix interactions, nutrient supply, removal of product, and waste product biomolecules, which all have effects on cell growth, viability, and metabolism. The highly controlled microenvironments are not available in static cultures, where concentrations of nutrients, product, and waste products are changing over time. Designing and operating a microfluidic perfusion system for cell culture are rather difficult and come with many challenges. Kim et al. summarized the guiding principles to implement a microfluidic perfusion culture system, and the essential design and operational issues are summarized in Figure 2 [28].

Several research groups studied long-term culturing of adherent cells in microfluidic bioreactors for mAbs production [29–31].

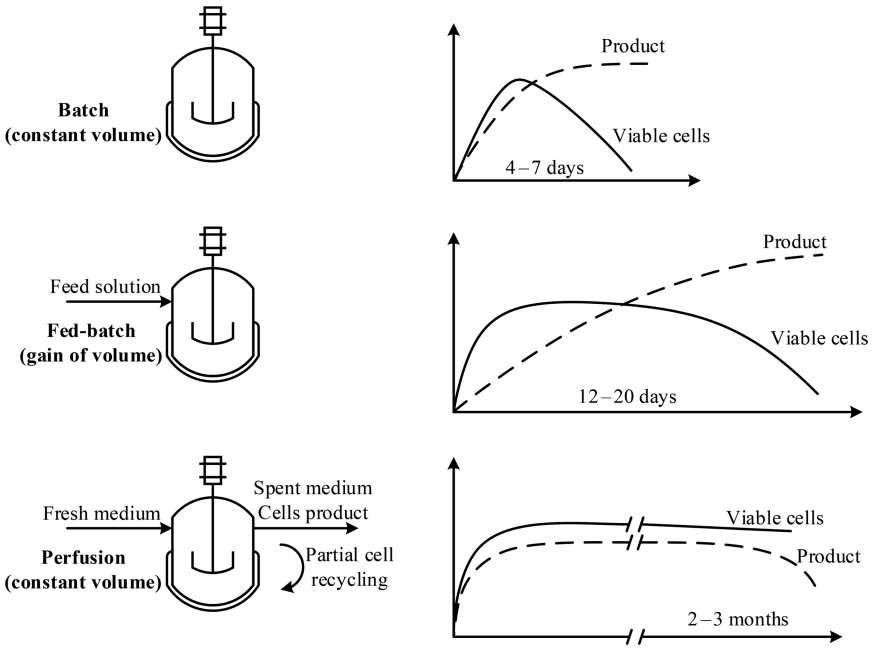

Figure 1. Overview of different operation types with their cell viability and product production over time during a typical mAb production processes

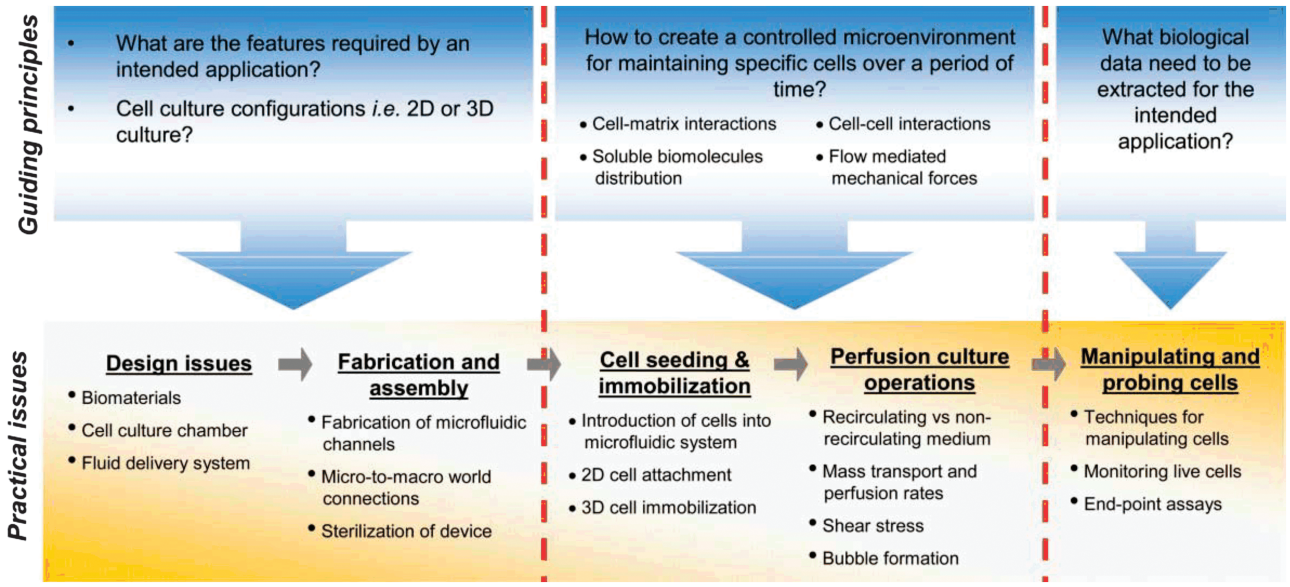

Figure 2. Guiding principles and technical issues for the robust operation of a microfluidic perfusion culture system. Reproduced with permission from Ref. [28]

They used different types of cell adhesion agents such as fibronectin [30] and poly-D-lysine [31] to improve the adhesion and viability. Green et al. tested the effect of different microchannel geometries and thicknesses, as well as the influence of flow rate [29]. Their results demonstrated that these parameters had great influence in cell adhesion and growth. Garza-Garcia et al. tested a fibronectin-coated microfluidic device with zigzag channel (length = 44 mm) for the production of Infliximab (Remicade) [32]. The single microfluidic bioreactor produced 14.4 μg of mAb in 24 h at a flow rate of 5 μL/min. The outlet concentration was 2 mg/L at residence times of 0.42 min. The productivity of the continuous flow microfluidic bioreactor was more than three orders of magnitude higher compared to fed-batch processes. Alvarez and coworkers further optimized the geometry of the microfluidic device for mAbs production [30]. The most efficient configuration was the use of a series of donut-shaped reservoirs, which yielded mAb concentrations of 7.2 mg/L at residence times lower than 1 min.

Perez-Pinera et al. developed a portable, fully integrated, and closed microbioreactor platform for programmable production of biologics shown in Figure 3 [33]. As a proof of principle, they produced interferon-α2b (IFNα2b) and recombinant human growth hormone (rhGH) using a *Pichia pastoris* strain 255B cells. The microbioreactor produced 19.73 ± 0.72 μg IFNα2b and 43.7 ± 6.3 μg rhGH in 20 h, respectively. Integrating the system in a modular microfluidics setting for protein purification can advance the treatment of human diseases in the point-of-care fashion [34].

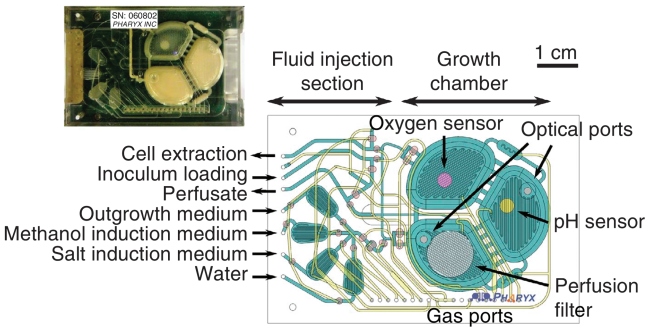

Figure 3. The principal components of a microbioreactor for programmable production of biologics. Reproduced with permission from Ref. [33]

Besides the cell culturing methods, cell-free protein synthesis (CFPS) is one of the widely used methods for the production of therapeutic proteins. CFPS method reduces the production time of proteins to a few hours compared to production systems in cells, which typically takes days and sometimes even weeks [35, 36]. CFPS uses cell extracts containing the biological machinery (DNA for encoding a specific gene and additional metabolites) necessary for in vitro protein production. Current trend applies CFPS in continuous microfluidic-based bioreactors [37]. The serpentine channel microfluidic bioreactors developed by Timm et al. shown in Figure 4 found their way in applications requiring the on-demand synthesis and subsequent purification of protein therapeutics [38]. Compared to conventional batch reactions, the average yield increased by nearly 40% with the microfluidic bioreactor. The developed microdevice represented a significant step toward rapid production of biotherapeutics, even at the point-of-care if necessary.

4. The Organ-on-a-chip Concept for Drug Testing

Organ-on-a-chips are continuously perfused microfluidic devices for culturing living cells to model physiological functions of tissues and organs such as skin, liver, kidney, etc. The simplest system contains only one kind of cell (e.g., kidney tubular epithelial cells) in the perfused microfluidic chamber that exhibits functions of one tissue type. In complex organ-on-a-chip microfluidic systems, porous membranes are installed between two or more microchannels, lined up on opposite sides by different cell types, to recreate interfaces between different tissues such as lung alveolar–capillary interface as described in

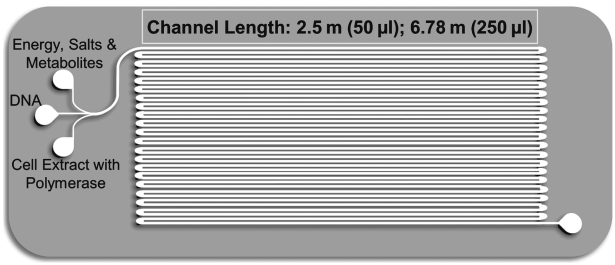

Figure 4. Serpentine channel microfluidic bioreactor design for cell-free production of biotherapeutics. Reproduced with permission from Ref. [38]

Ref. [39]. Advanced 3D tissue engineered constructs are applied more frequently instead of two dimensional (2D) cell culture to better mimic the complex three dimensional (3D) in vivo microenvironment of an organ, where the cells and the extracellular matrix (ECM) exist in a well-defined structure. 3D cell culture organ-on-a-chip systems can provide a better platform for preclinical testing of drug substances [40, 41]. These systems can be perfused to create dynamic culture environments and can be exposed to concentration gradients of drugs at the same time to perform drug metabolism and toxicology studies (Figure 5) [42, 43].

Bioprinting as an extension of 3D printing offers the ability to create a 3D biomimetic tissue by depositing cells and, in some cases, multiple cell types into a 3D construct with precise and reproducible spatial distribution. Applying bioprinting, organ-on-a-chip with 3D biomimetic tissues can be created and with continuous perfusion the tissues can be supplied with nutrients and growth factors and the metabolism products can be removed. Nowadays, bioprinters and bioprinting services are commercially available (e.g., www.biobots.io, www.organovo.com) [44]. Precise cell/ECM positioning and creation of complex biological structures are possible with the 3D printing technologies such as micro-extrusion, inkjet, and laser-assisted printing. 3D bioprinting accurately controls the spatial distribution and layer-by-layer assembly of cells, ECMs, and other biomaterials. Organ-on-a-chip with heterogeneity, proper 3D cellular arrangement, and tissue-specific functions can all be produced [45]. Wevers et al. developed an OrganoPlate® microfluidic platform for culturing three-dimensional networks of active neurons and supporting glial cells. This novel microfabricated device can help in the development and testing of new drug candidates for brain diseases, and it is a promising tool for in vitro brain modeling [46].

Microfluidic models of organs allow real-time and non-invasive monitoring of cell-based assays with tissue- and organ-level complex physiological processes that are especially useful in cancer research. Microfluidic models have several advantages over in vitro and in vivo models as listed in Figure 6, but the main advantage is the high human relevance and precision that cannot be achieved with conventional methods. [47].

Microfluidic devices provide great opportunities in cancer biology and clinical oncology research, as well as high-throughput drug screening. With their help, multiparameter studies on isolated single cancer cells that are useful for clinical and diagnostic laboratories can be performed. Furthermore, reconstruction of the cancer microenvironment with microfluidic perfusion system can support clinical diagnostic and drug testing [48].

Loskill et al. developed a Lego-like plug and play system referred to as μOrgano, which enabled to create integrated multi-organ microphysiological systems with the connection of a single

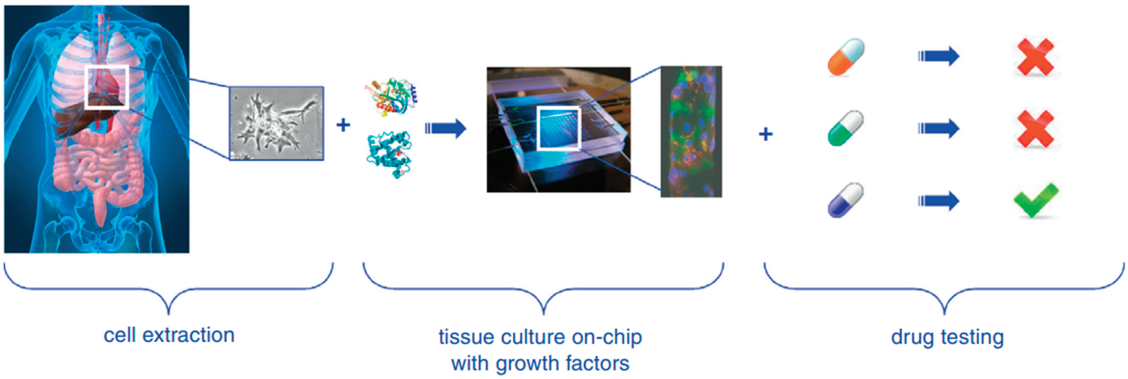

Figure 5. Concept behind the organ-on-a-chip modules for drug metabolism and toxicology studies. Reproduced with permission from Ref. [42]

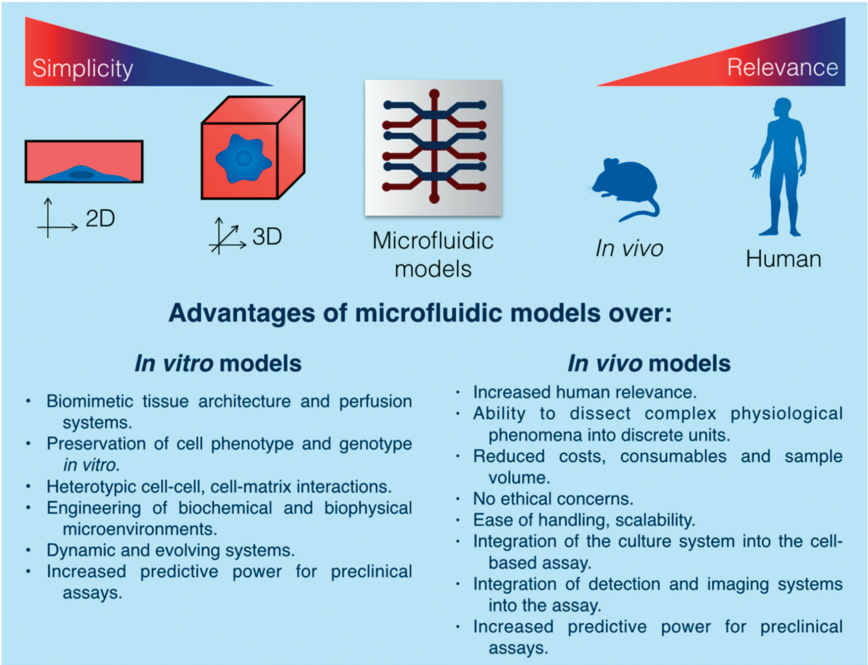

Figure 6. Advantages of microfluidic models over conventional in vitro and in vivo models. Reproduced with permission from Ref. [47]

organ on a chip module. The plug and play μOrgano system has the following features: (1) separate loading of different cell types; (2) temporal control of individual cell cultures for differentiation and tissue development; and (3) subsequent temporal control of fluidic connections of the individual tissues [49].

Maschmeyer et al. developed a four-organ microfluidic platform for absorption, distribution, metabolism, and excretion (ADME) profiling and toxicity testing of drug candidates as shown in Figure 7 [50]. The four-organ-chip with the combination of a human skin, intestine, liver, and kidney model enabled the study of absorption and distribution of the drug molecules between the model organs and the first path metabolism in the liver tissue, secondary metabolism, and, finally, excretion through the kidney model. The developed microfluidic platform can help to obtain more reliable pharmacokinetic and pharmacodynamic data, such as effective concentration as well as intensity of therapeutic and adverse effects.

The micro cell culture analog (μCCA) device is a multi-organ-chip microfluidic system with the challenging goal to create a model for the entire human body. First of all, for wider acceptance of this technology, the drug test results obtained with μCCAs with animal cell lines should be compared and validated with the animal studies. Human μCCAs (operated with human cell lines) data should also be compared considering clinical efficacy and toxicity data [51].

5. Conclusions and Future Prospective

Utilization of continuous processing in the manufacturing of modern biotherapeutics could significantly improve productivity and throughput, and result in improved and consistent product quality at a reduced capital and operating cost. Productivity could be further increased with the application of microfluidic perfusion bioreactors. Cell viability and growth and, thus, the production of biologics can still be improved with optimization of channel geometry, thickness, and flow rate. Recently introduced bioprinting opens up new horizons to create more advanced 3D culture microfluidic devices for biologics production. Integrating modular unit operations for protein purification in the microfluidic cell culture device can lead to point-of-care therapeutic protein production. Cell-free protein synthesis method drastically reduces protein production times, i.e., to a few hours compared to cell culturing system, where it takes typically several days. Microfluidic perfusion devices in a form of organ-on-a-chips can culture living cells to model physiological functions of tissues and organs such as skin, liver, or kidney. These microfluidic devices with different organ functionalities can be integrated into one microfluidic system to model the relevant parts of the human body. However, several issues should be addressed in the future for an organ-on-a-chip microfluidic system. One is that the organ-on-a-chip must authenticly replicate the in vivo cellular behavior [51]; thus, several process parameters (e.g., culturing media, temperature, flow velocity, channel geometry) should be optimized. Another one is the integration of several organ-on-a-chip devices into one combined microfluidic system to produce a bubble free integrated platform. This latter is still challenging, and each integration method (e.g., microfluidic chamber connection with tubing, all chamber integration into one single chip) should be checked individually. Finally, using such an integrated platform, universal culture media are necessary for the different cell types [52]. These multi-organ microfluidic platforms will help in preclinical testing of drug substances and toxicological studies by producing more reliable preclinical pharmacokinetic and pharmacodynamic data.

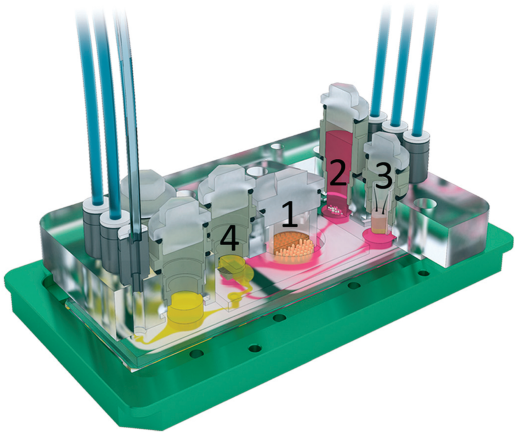

Figure 7. Four-organ-chip microfluidic platform. Numbers represent the four tissue culture compartments for intestine (1), liver (2), skin (3), and kidney (4) tissue. Reproduced with permission from Ref. [50]

Abbreviations

CHO	Chinese hamster ovary
NS0	murine myeloma cells
ATF	alternating tangential-flow
CFPS	cell-free protein synthesis
ECM	extracellular matrix
μCCA	micro cell culture analog

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