**Microfluidic devices towards personalized health and wellbeing**

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Abstract

To make personalised medicine a reality, there is a significant need for cost-effective methods that enable the rapid selection of optimal nutrient intake and/or disease treatment with a minimum of side effects. In this perspective, we briefly discuss the potential of merging the advances of microfluidic devices, organoid structures and advanced analytical methods to progress towards a personalised *in vitro* platform for health and wellbeing.

An *in vitro* platform that offers real-time and *in vivo* relevant analysis of cellular responses would be highly valuable for understanding the impact of small molecules on health and wellbeing (Figure 1). This includes the ability to tailor diet to personal needs or to screen drug compounds in order to aid personalized medicine to overcome metabolic imbalances. Such a platform could be based on human mini-organs, also known as organoids, which are a better mimic of the three-dimensional tissue environment than standard tissue culture modalities. Emerging analytical methods combined with a microfluidic approach would allow rapid and cost-effective understanding of individual metabolism, and rapid and tailored selection of bio-active compounds; thus they could lay the foundation for establishing a true personal diagnostic medicine system that is affordable for national health services, such as the NHS in the UK. In this perspective, we briefly discuss the benefits of such an approach and comment on recent publications that have the potential to advance in this direction.

With increasing understanding of the impact of the genetics of an individual on health, disease, and metabolism, there is a significant need for cost-effective methods that enable the rapid selection of optimal nutrient intake and/or disease treatment with a minimum of side effects. An *in vitro* platform would be ideal to achieve this task and could assist the clinicians of the future in finding optimal treatment and reducing morbidity from over-treatment. *In vitro* models can

complement or reduce ethically demanding and costly preclinical animal models, lower the amount of labor, and can be miniaturized to minimize the amount of human cells and drug compounds needed. Indeed, they can be populated with a small number of cells from an individual patient in order to enable truly personalized treatment. However, to date, *in vitro* models are unable to simulate the interactions and physiological responses of individual patient's cells to exogeneous compounds in a sufficiently realistic and translatable way as compared to *in vivo* responses. The first steps towards addressing this drawback are underway: cells can be arranged in culture devices to replicate tissue- and organ-level physiology (organ on a chip) or grown from the tissue of patients, which in culture maintain three-dimensional, physiological cell differentiation, as well as genotype and phenotype of the patient's own tissue (organoids). These organoids are therefore an ideal 'vehicle' to validate diet regimes and/or therapeutic compounds for a patient's health management, offering an exciting gateway to truly personalized medicine. However, the majority of available devices rely on end-point measurements, which limits the amount of data obtainable per culture experiment and may not accurately represent the trajectory of patient response. Furthermore, cytokines and surface markers return only the information at the exact moment when the cells are taken out of their *in vitro* context to perform the assay. Given the plasticity and flux of cells *in vivo* and in culture, single-time point measurements therefore do not accurately reflect cellular responses.

Microfluidic culture devices, with characteristic dimensions from submillimeter to submicrometers, and in which small amounts of fluids are manipulated, provide an environment conducive to cell culture growth, including 3D culture, and co-culture configurations [1,2]. These devices are designed to allow a fine spatiotemporal control over the microenvironment surrounding the cells, including the controlled delivery of nutrients and soluble biochemical factors, the implementation of signaling gradients, and control over the hydrodynamic shear stress. This level of microenvironment control is impossible to achieve in conventional tissue culture dishes [3,4]. Ultimately, the use of microfluidic devices to perform cell growth, will result in better biomimetic tissues and organ models with increased physiological relevance [2,5]. Thus, they represent a cost-effective proposition to rapidly validate the impact of nutrition and of drugs (or nutraceuticals) on a patient's diseased tissue; i.e.

to validate the efficacy of drugs, to support pharmacokinetic and pharmacodynamic modeling, to test ADMET (adsorption, distribution, metabolism, elimination and toxicity) of drugs, and even to evaluate different drug/drug combinations. Given the speed of individual developments, it is reasonable to expect that highly complex microfluidic and highly multiplexed networks will emerge soon, with which interactions of drugs and nutrition within co-cultured organoids can be probed. Therefore, as organoid and microfluidic technologies progress, our ability to mimic physiologically-relevant interactions of the human body will become very sophisticated.

The key for this venture and the realization of viable personalized medicine and nutrition platforms will undoubtedly rest with the development of suitable analytics. The analytical methods should allow the non-invasive and real-time monitoring of the highly dynamic cellular responses. However, implementation is challenging due to the small dimensions and closed nature of the devices. Furthermore, as *ex vivo* tissue cultures increase in complexity and functionality, it becomes increasingly difficult to probe the core of an organoid structure, and to spatially and temporally resolve the cellular responses. Therefore, to develop an *in vitro* platform capable of using organoids to select therapies for the diseased patient or advice about healthy food choices and eating patterns, it is imperative to create novel (and likely multi-modal) analytical methods that can cope with the challenge [4,6]. Additionally, the microfluidic platforms need to be cost-effective and compatible with the typical sizes and footprints of microfluidic systems. This pre-empts the use of techniques like magnetic resonance imaging (MRI), which require tissue sizes too large for microfluidic devices, or imaging modalities that rely on expensive equipment, such as multi-photon microscopes. We speculate that it is necessary to combine different analytical approaches to cost-effectively assess cell proliferation, metabolism and uptake of small molecules like nutrients and therapeutic molecules.

Image processing and automated quantitative assessment of microscopy images of cell cultures has been demonstrated for cell tracking, culture growth, and cell morphology [7] and has been applied in microfluidic devices to obtain time-course data of mouse embryonic stem cell cultures, e.g. for confluency [8]. These have however been mainly limited to 2D cultures, making their

application to organoids challenging, where at least 10 to 15 layers of cells should be monitored simultaneously. Focus stacking may enable the delineation of growth of organoids in 3D structures. The implementation of charge-coupled devices (CCD) technology on chip could in the future reduce costs. Alternatively, optofluidic imaging (i.e. the simultaneous control of fluidic conditions and optical beams e.g. to enhance live cell imaging [9]) could be substituted for conventional microscopy equipment to enhance detection capabilities [10].

Oxygen and pH monitoring are state-of-the-art for suspension culture systems [6], and have been reported for adherent cell cultures in microfluidic devices [11]. The quantification of oxygen kinetics in real-time, label-free and non-invasively allows unprecedented insight into cellular metabolism by accurately determining the oxygen uptake rate (OUR) or the specific oxygen uptake rate (sOUR). In a previously developed autoclavable and re-sealable cell culture microfluidic device [12], Super *et al.* [11] quantified cell growth from phase contrast microscopy images, and respiration using optical sensors for dissolved oxygen. Time-course data for both bulk and peri-cellular oxygen concentrations were obtained for Chinese hamster ovary (CHO) and mouse embryonic stem cell (mESC) cultures. Additionally, cellular responses to rapidly changing culture conditions were quantified by exposing the cells to mitochondrial inhibiting and uncoupling agents. For both cell lines, the non-invasively measured sOUR compared favorably with literature values where cell cultures were sampled. We expect the capability to monitor oxygen tensions, cell growth, and sOUR, of adherent cell cultures, non-invasively and in real time, will be of significant benefit for future studies in personalized health and well-being, such as respiration and anaerobic metabolism.

Fluorescent oxygen and pH sensors are commercially available and real-time monitoring has recently also been implemented with nanoparticles [6], which can be embedded in 3D matrigel cultures or injected into the lumen of the organoid [13] and combined with live-cell imaging. This will yield real-time information on the oxygen/pH distribution around and in organoid structures, which is interesting, for example to study the Warburg effect [14]. Immunofluorescent staining can give information regarding sensing and nutrient transport [15]. Nonetheless, there is a strong need to

develop further optical probes which would detect online specific target molecules, such as proteins and biomarkers.

To compare the behavior of cells in 2D and 3D culture in different modalities and to better understand cellular behavior within the organoid, genetic biosensors of metabolism, cell signaling, and cell stress responses can be used, some of which have already been developed. Metabolite biosensors based on Förster Resonance Energy Transfer (FRET), where the signal is calculated from two fluorophores, thereby allowing robust quantification of metabolite concentrations in single cells, in real-time without destruction of samples have been developed for a number of metabolites including glucose and amino acids [16,17]. Cell signaling and cell stress biosensors based on the production of a fluorophore in response to changes in gene expression, either to adjust to environmental conditions or as part of cellular differentiation pathways, have been developed. These are similarly non-destructive and the fluorophore can be destabilized to prevent its accumulation in cells to allow for more sensitive detection of changes [18]. Although use of such sensors requires the genetic modification of cells, they can be used to gain information on changes in metabolism and signaling within patient samples by first transducing the cells with the gene circuits similar to procedures for engineering of CAR-T cells as therapies.

Raman spectroscopy is compatible with microfluidic devices but has to date not been used to analyze organoids. The penetration depth of a simple Raman system with a low-cost laser is a few hundred microns, sufficient for initial testing of the concept. Furthermore, drug compounds or cell culture nutrients will have a different Raman footprint than cells. Given their difference in metabolism, it is also likely that healthy and cancerous cells can be distinguished from each other.

Currently, the quantification of protein production and secreted biomarkers is performed with enzyme-linked immunosorbent assays (ELISA) both at-line and offline. While the throughput of such an approach is relatively high, the analysis is still limited to the culture media. This approach does not enable the real-time monitoring of the highly dynamic cellular responses. There have been considerable efforts to miniaturize ELISAs and integrate the assays on chip [19] and to develop

electrochemical immunobiosensors to quantify soluble protein biomarkers, e.g. albumin [20]. Multi-parameter monitoring systems have been developed to monitor oxygen, pH, temperature and biomarkers (albumin, GST-α and CK-MB) simultaneously in cultures of human iPSC cardiomyocytes and hepatocellular carcinoma cells [20]. This system was used to study the effect of chemotherapeutic drugs on the liver cancer organoid. The proposed integrated microfluidic device, however, lacked online and real-time monitoring capabilities which could increase the complexity of device fabrication and daily operations dramatically.

Gastrointestinal (GI) organoids can serve to evaluate the impact of nutrition on human health. While these systems on their own cannot model the entire complexity of the digestive tract, they can offer complementary high quality data [21]. This will enable metabolic studies, i.e. analysis of the impact of common dietary nutrients [22], probiotics, prebiotics and synbiotics, and ultimately human health. In the future, gut bacteria must be co-cultured with GI cells [23,24] to effectively study the gut microbiome in order to enhance the treatment of or to prevent diseases [25].

Advances in analytical technology such as those described above have the potential to enable an unprecedented understanding of the metabolism of individual human cells both in the presence of different nutrient additives and with respect to different drug molecules. Combined with microfluidic technology this will enable truly personalized understanding of the health and wellbeing of individuals. We believe that standardization of device components will reduce costs and facilitate the implementation of automation. This would remove operator-induced variability and increase throughput while increasing data quality. However, the integration of analytical technology will undoubtedly also increase the system complexity (Figure 2). The envisioned platform will allow physicians in the future to develop tailored dietary advice, therapeutic regimes, and general health maintenance for each patient based on a small number of cells such as that obtained from a biopsy tissue, fine-needle aspirations, or even resection specimens [26–28]. This can truly create a revolution that maximizes the total health of the population.

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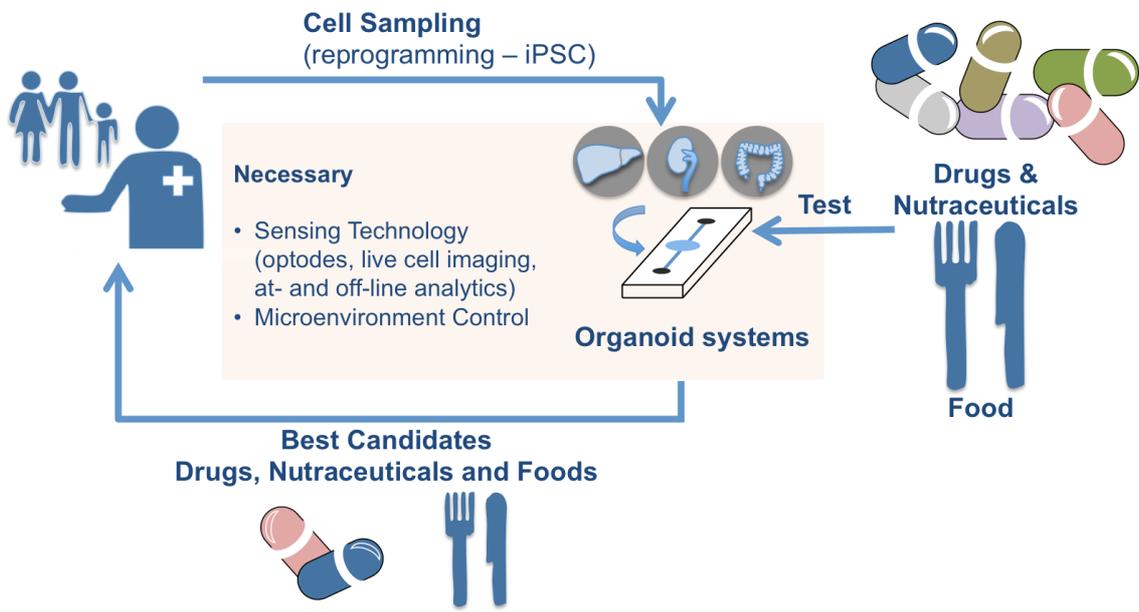
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**Figure Captions**

Figure 1 – Personalized health and wellbeing platform. Patient cells collected through biopsy or resection specimens are, for example, reprogrammed into pluripotent stem cells and derived into different organoids. These are then cultured in an integrated microfluidic device with high spatiotemporal control over the cellular microenvironment. Physicians will have at their disposal a platform to tailor dietary advice, therapeutic regimes, and general health maintenance for each patient.

Figure 2 – Criteria for a microfluidic personalised health and wellbeing platform, weighted against each other. Efficient operation of the microfluidic system and high quality data requires implementation of soft sensors, live cell imaging and complementary analytics (e.g. enzyme-linked immunosorbent assays, metabolite biosensors and microarrays) which ideally should be on-line measurements. Spatiotemporal control over the microenviroment is essential to control the delivery of nutrients and biochemical factors, to generate signaling gradients, and mimic the physiological hydrodynamic shear stress. The standardization of device components will facilitate automation with the use of robotic platforms reducing device cost and overall complexity, though are not preponderant for the effective implementation with health services.

**Figure 1**

| | | High sensitivity optodes | Live cell imaging | Compl. Analytics | Microen. control | Automation | Simplicity design | Standard. | Low cost | Weighting (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Sensing technology | High sensitivity optodes | | % | ✔ | ✗ | ✔ | ✔ | ✔ | ✔ | 69 |
| | Live cell imaging | | | ✔ | ✗ | ✔ | ✔ | ✔ | ✔ | 69 |
| Complementary analytics | | | | | ✗ | ✔ | ✔ | ✔ | ✔ | 50 |
| Microenvironment control | | | | | | ✔ | ✔ | ✔ | ✔ | 88 |
| Automation | | | | | | | ✔ | ✔ | ✗ | 25 |
| Simplicity of design | | | | | | | | ✔ | ✗ | 13 |
| Standardisation | | | | | | | | | ✗ | 0 |
| Low cost | | | | | | | | | | 38 |

**Figure 2**