Lab on a Chip

RSC Publishing

FOCUS

View Article Online
View Journal | View Issue

NutriChip: nutrition analysis meets microfluidics

Cite this: *Lab Chip*, 2013, **13**, 196

Qasem Ramadan,*[a] Hamideh Jafarpoorchekab,[a] Chaobo Huang,[a] Paolo Silacci,[b] Sandro Carrara,[c] Gözen Koklü,[c] Julien Ghaye,[c] Jeremy Ramsden,[d] Christine Ruffert,[a] Guy Vergeres[b] and Martin A. M. Gijs[a]

This focus article introduces the concept of NutriChip, an integrated microfluidic platform for investigating the potential of the immuno-modulatory function of dairy food. The core component of the NutriChip is a miniaturized artificial human gastrointestinal tract (GIT), which consists of a confluent layer of epithelial cells separated from a co-culture of immune cells by a permeable membrane. This setting creates conditions mimicking the human GIT and allows studying processes that characterize the passage of nutrients though the human GIT, including the activation of immune cells in response to the transfer of nutrients across the epithelial layer. The NutriChip project started by developing a biologically active *in vitro* cellular system in a commercial Transwell co-culture system. This Transwell system serves as a reference for the micro-scale device which is being developed. The microfluidic setup of NutriChip allows monitoring of the response of immune cells to pro-inflammatory stimuli, such as lipid polysaccharide (LPS), and to the application of potentially anti-inflammatory dairy food. This differential response will be quantified by measuring the variation in expression of pro-inflammatory cytokines, including interleukin 1 (IL-1) and interleukin 6 (IL-6), secreted by the immune cells, and this is achieved by using a dedicated optical imager. A series of dairy products will be screened for their anti-inflammatory properties using the NutriChip system and, finally, the outcome of the NutriChip will be validated by a human nutrition trial. Therefore, the NutriChip platform offers a new option to evaluate the influence of food quality on health, by monitoring the expression of relevant immune cell biomarkers.

Received 25th July 2012,
Accepted 10th October 2012

DOI: 10.1039/c2lc40845g

www.rsc.org/loc

Milk and the modulation of the inflammatory response

It is now generally accepted that food is not only a source of nutrients but also a modulator of the physiological functions of the body. The response of the body depends on the type of diet and it is believed that each meal provokes a kind of inflammatory response. The cellular metabolism of nutrients, culminating in the synthesis of biochemical energy, produces side effects that include the generation of oxidative stress. These effects accumulate at the level of the organism to produce a phenomenon, called postprandial stress.[1] Under normal conditions, postprandial stress is of low magnitude and disappears within a few hours post-ingestion. An unhealthy diet may, however, increase the magnitude of postprandial stress and/or delay the person's recovery to the initial state.[2] A repetition of postprandial inflammatory stress over prolonged periods of time may significantly contribute to the development of chronic inflammation. Therefore, the inflammatory stress imposed by inadequate or inappropriate diets may contribute negatively to the maintenance of health by reducing the metabolic plasticity of the organism. A quantitative analysis of the postprandial presence and kinetics of inflammatory biomarkers provides information on the quality of the interaction between specific foods and the organism. This idea is at the conceptual basis for the biological module of the NutriChip, as explained in ref. 3.

Milk and dairy products have long been promoted as healthy nutrients, rich in proteins, vitamins and minerals and providing the necessary components in the food standard pyramid for both children and adults. Recently, milk has been promoted as not only a nutrient source but also as a health promoter. Indeed, milk is a source of immuno-modulatory effects, produced through the action of probiotic bacteria, proteins and bioactive peptides in the fermented milk products. A potentially important action is based on modulating the production of pro-inflammatory cytokines.[4] Fasting-related lower levels of pro-inflammatory cytokines have been documented in epidemiological[5] and intervention[6] studies in human adults consuming dairy products. Decreased postprandial levels of pro-inflammatory cytokines after the consumption of dairy products were also reported.[7] Taking into

[a]*Laboratory of Microsystems 2, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland. E-mail: qasem.alramadan@epfl.ch*
[b]*Agroscope Liebefeld-Posieux Research Station ALP, Berne, Switzerland*
[c]*Integrated Systems Laboratory, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
[d]*Centre for Molecular Recognition, Collegium Basilea, Basel, Switzerland*

account the vast possibilities of transformation technologies of milk, in particular fermentation by lactic acid bacteria, and the importance that gut microbiota have on human metabolism,[8] it appears that milk can be a strategic vector to deliver immuno-modulatory nutrients for improving metabolism and health.

Biological model

In the human body, food undergoes complex processes that involve adsorption, distribution, metabolism, and excretion (ADME). Nowadays, human intervention studies are clearly the gold standard in nutritional science,[8] because all factors that influence the ADME processes are present. However, it is impossible to identify the influence of individual factors, and the resources necessary to screen a large number of products for specific properties are prohibitive. Furthermore, the use of human subjects as a screening tool to test products that are not bio-active or even unsafe poses ethical problems, even if the trials involve a low grade of invasiveness by limiting analyses to blood samples. As differences between human and animal metabolism are significant, human *in vitro* cell culture models are needed for the efficient screening of bio-active food products. Use of such models would increase the predictability of human responses to nutrients and lead to higher success rates in follow-up clinical studies, significantly reduce expenses associated with the clinical studies and speed up the screening processes.

In analogy to pharmacokinetics, the absorption of nutrients through intestinal cells is a key factor for their bio-availability. The mucosal surface of the intestinal tract is covered by a thin layer of epithelial cells (ECs) underlined by a local immune system.[9] This biological construction plays a dual role: (i) it transfers nutrients into the circulation for further metabolism, while maintaining immune tolerance to these nutrients, and (ii) prevents entry of pathogenic organisms into tissues and regulates local inflammatory and immune responses to combat such intrusions. Communication between intestinal ECs and immune cells is thus essential, and takes place either *via* soluble factors or by direct cell–cell interaction at the crossroads of nutrient metabolism and immuno-modulation.[10,11] *In vitro* intestinal nutrition transport is most often measured using confluent layers of intestinal cell lines, and Caco-2 cells are the most popular cellular model in transport studies. Even though these cells are derived from a human colorectal adenocarcinoma, they express the majority of the morphological and functional characteristics of small intestinal absorptive cells in culture.[12] For example, they differentiate into polarized intestinal cells, possessing an apical brush border and tight junctions between adjacent cells. Therefore the core biological component of the NutriChip is a co-culture of a confluent layer of Caco-2 cells, which allows the application of *in vitro*-digested food on its apical side, and a basolateral culture of a monocytic cell line (in our case U937 cells) differentiated into macrophages. The two cell types are separated by a membrane that is permeable to the nutrients added on the apical side of the EC culture. U937 is a histiocytic lymphoma cell line with monoblastic characteristics and can differentiate into macrophage-like cells after treatment with phorbol esters, such as phorbol-12-myristate 13-acetate (PMA).[13] This co-culture setting mimics the *in vivo* human GIT.

Fitting the biological model into a microfluidic setup

A microfluidic cell-based system can potentially provide a better, faster and more efficient characterization of a nutrients' absorption and immuno-modulatory functions. Considerable effort has already been made to provide *in vitro* surrogates that are designed to mimic the physiological architecture and dynamics of human organs. In particular, we mention here the Ingber group at Harvard University,[14–16] who did pioneering work on ''organ-on-a-chip'' devices and the Shuler group at Cornell University who have pioneered the development of *in vitro* analogues to physiologically-based pharmacokinetic (PBPK) models.[17–21] Such micro-scale devices have the potential to accurately produce physiologically realistic parameters and can closely model the desired *in vivo* system to be tested. In a recent publication, Kim *et al.*[16] reported a ''human gut-on-a-chip'' microdevice, which was composed of a confluent layer of intestinal epithelial (Caco-2) cells cultured on a porous membrane. The gut microenvironment was mimicked by flowing fluid at a flow rate of 30 μL h^{-1}, producing low shear stress on the cells (0.02 dyne cm^2) and by exerting cyclic strain that mimicked physiological peristaltic motions. Under these conditions, well-defined epithelial monolayers were formed in three days in the microfluidic device, much faster than obtained in macroscopic conventional cell culture devices, which require 7–21 days of culture to achieve the same cell morphology. To study physiologically relevant human intestinal epithelial cell microbe interactions, the authors co-cultured a strain of *Lactobacillus rhamnosus* on the luminal surface of the cultured epithelium without compromising the epithelial cell viability, which demonstrated the possibility of mimicking the dynamic physical and functional features of the human intestine.

Fig. 1 illustrates schematically the biological and microfluidic aspects of the NutriChip platform. The human GIT is approximated by a monolayer of epithelial cells interacting with immune cells (Fig. 1a). The intestinal epithelium is considered a natural gatekeeper that controls the uptake of nutrients and potentially harmful substances by different passive and active mechanisms of transfer of these molecules from the mucosal to the serosal side. The immune cell layer underlying the epithelial barrier is highly specialized and devoted to two main tasks, namely avoiding unnecessary and potentially harmful reactivity to dietary proteins and enteric flora, while rapidly responding at the same time to episodic threats from pathogens. In a standard *in vitro* epithelial cell culture system, the transport of drugs and nutrients is

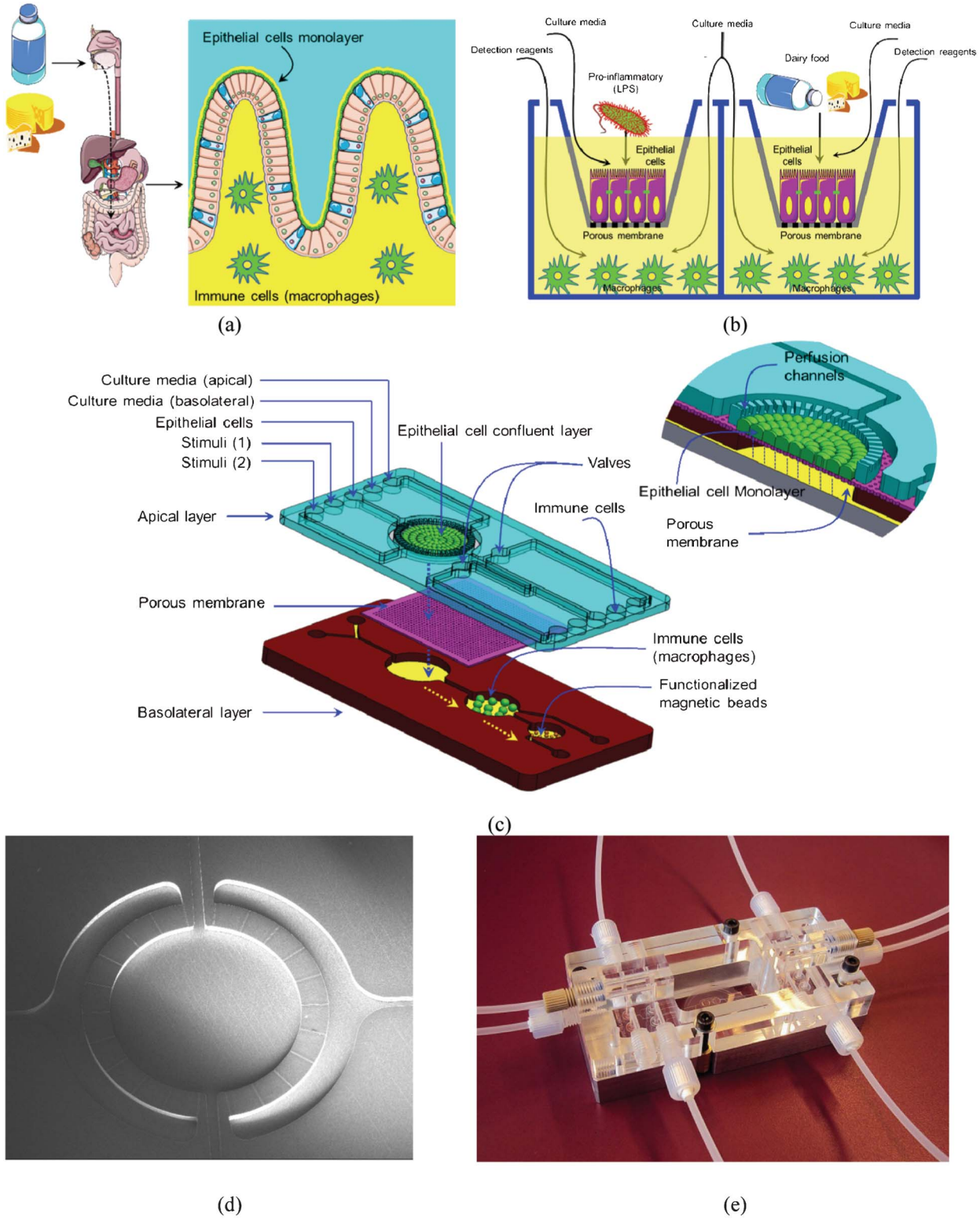

Fig. 1 The NutriChip platform concept. (a) The human GIT is approximated by a biological model consisting of a monolayer of confluent epithelial cells interacting with immune cells. (b) First co-culture is done in a macroscopic Transwell device: a layer of confluent epithelial cells is grown on a porous membrane that forms the bottom of an insert, which is placed inside a well containing the immune cells. (c) Schematic of the microfluidic chip forming the miniaturized GIT. (d) A top view of a single apical fluidic chamber with small perfusion channels. (e) The miniaturized GIT inserted in a prototype microfluidic interface unit.

measured through a confluent layer of epithelial cells.[12,22] A confluent Caco-2 cell monolayer is commonly used in studies of the mechanism of drug transport and its absorption[23] and, similarly, such a system can be used to identify metabolite transport originating from a variety of digested food types.[24] When going for a cell co-culture model, this allows additional measuring of the activation of immune cells in response to the transfer and processing of nutrients across the epithelial cell layer, and such system can therefore potentially be used to screen food for specific physiological properties of nutrients, in particular in the context of the immuno-modulatory function of the latter. In a classical Transwell (Millipore, USA) co-culture device, a layer of confluent epithelial cells is grown on a porous membrane that forms the bottom of an insert, which is placed inside a well containing the immune cells (Fig. 1b). Such a system allows access to the cells from both the apical and basolateral side. In the NutriChip project, the co-culture system has been designed to allow measurement of the differential response of immune cell stimulation through the epithelial monolayer using two parallel co-culture units. These units contain the same cell co-cultures and grow under the same conditions, however, the cells in the first unit are stimulated by a pro-inflammatory stimulus, for example lipo polysaccharide (LPS), which is an endotoxin found in the outer membrane of Gram-negative bacteria and is known as a powerful activator of immune cells,[25] while the cells in the other unit are treated with digested milk and dairy products for investigating the potential anti-inflammatory properties of the latter.

Although such insert systems are easy to deal with and represent a logical option for starting our research, they require a large amount of cells, reagents and culture media, which makes this a rather expensive approach. Furthermore, the culture environment within these inserts does not closely mimic the *in vivo* microenvironment due to the absence of the fluid flow and shear stresses.[26] Also, they are less suited to providing a dynamically controlled flow of cell nutrients and stimuli and, additionally, the accumulation of waste within the insert leads to a pH drift in the static culture. Other drawbacks of the classical cell culture systems are the long growth times needed for the intestinal cells to differentiate into functional enterocytes and to reach confluence, and an external signal detection system that requires the manual manipulation of samples. It is therefore highly desirable to downscale such cell cultures and to make them more amenable to automation in order to promote efficient *in vitro* screening of the physiological properties of selected food products. Microfluidic culture systems can provide the steady-state culture conditions that mimic the *in vivo* fluid flow and shear stress in a controllable manner, thus bringing the GIT *in vitro* model closer to the physiological micro-environment. Fig. 1c illustrates the structure of a basic miniaturized GIT, which comprises two microfluidic layers made of polydimethylsiloxane (PDMS), which are termed here as the 'apical' and the 'basolateral' layers, respectively. These two layers sandwich a polyester membrane with a pore size of 0.4 μm. The apical layer contains a single chamber (Fig. 1d), which hosts the epithelial cells and is interfaced by a chamber made in the basolateral layer, which receives the chemicals and nutrients transported through the epithelial confluent cell layer grown on the membrane. It was found that the culture conditions of the epithelial and the monocytic cells are incompatible, therefore, a dedicated culture chamber was created for the monocytes next to the EC basolateral chamber. The two chambers are separated by a normally-closed valve, which is opened for the monocyte stimulation process. Downstream of the monocyte chamber, there is another chamber dedicated to performing an immunomagnetic assay, within which magnetic beads functionalized with antibodies against the targeted cytokines dynamically capture the latter. Utilizing magnetic beads allows *in situ* cytokine capturing and simultaneous washing[27] before the final fluorescent detection.

The apical and the basolateral layers were individually molded in PDMS employing a master structure of SU-8 negative photoresist, which was previously micropatterned on a silicon wafer. The porous membrane was obtained from a Transwell insert. The three parts were then assembled together by treating the three surfaces with O_2 plasma in a multiple-step bonding process. A cell culture chamber is designed to be nested inside a larger chamber with permeable side walls implemented by an array of small perfusion channels, which only allows the perfusion of the chamber with media while preventing cells to escape (Fig. 1d). Injecting the media through these small channels allows for a uniform radial fluid flow from all directions and uniform fluid transport over the cells. In this way, the perfusion chamber protects cells from elevated shear stresses and only supplies a mild shear stress which is beneficial for the natural culture conditions. The NutriChip is mounted in a custom-fabricated chip holder to facilitate cell loading as well as continuous infusion of the culture media (Fig. 1e).

First experimental results

Our first approach has been to create a biologically active *in vitro* co-culture cellular system in Transwell devices. This allowed the modeling of the GIT and it's response to pro-inflammatory stimuli, such as LPS by the activation of receptors on the surface of immune cells underlying the epithelial layer. The transport function of the epithelial layer plays a vital role in the inflammatory response of the immune cells. The epithelial cells are joined at their apical surface by tight junctions (TJs), and mass transfer from the apical side to the basolateral side of the epithelium is dependent on the integrity of the epithelial monolayer. TJ permeability is regulated by various factors, including bacteria toxins and cytokines[28] (Fig. 2a). Recent studies have shown that the intestinal functions are also affected by food factors that modulate the TJ.[28,29] The integrity of the confluent epithelial cell layer was shown by measuring the Trans Epithelial Electric Resistance (TEER) which reached a maximal level after one

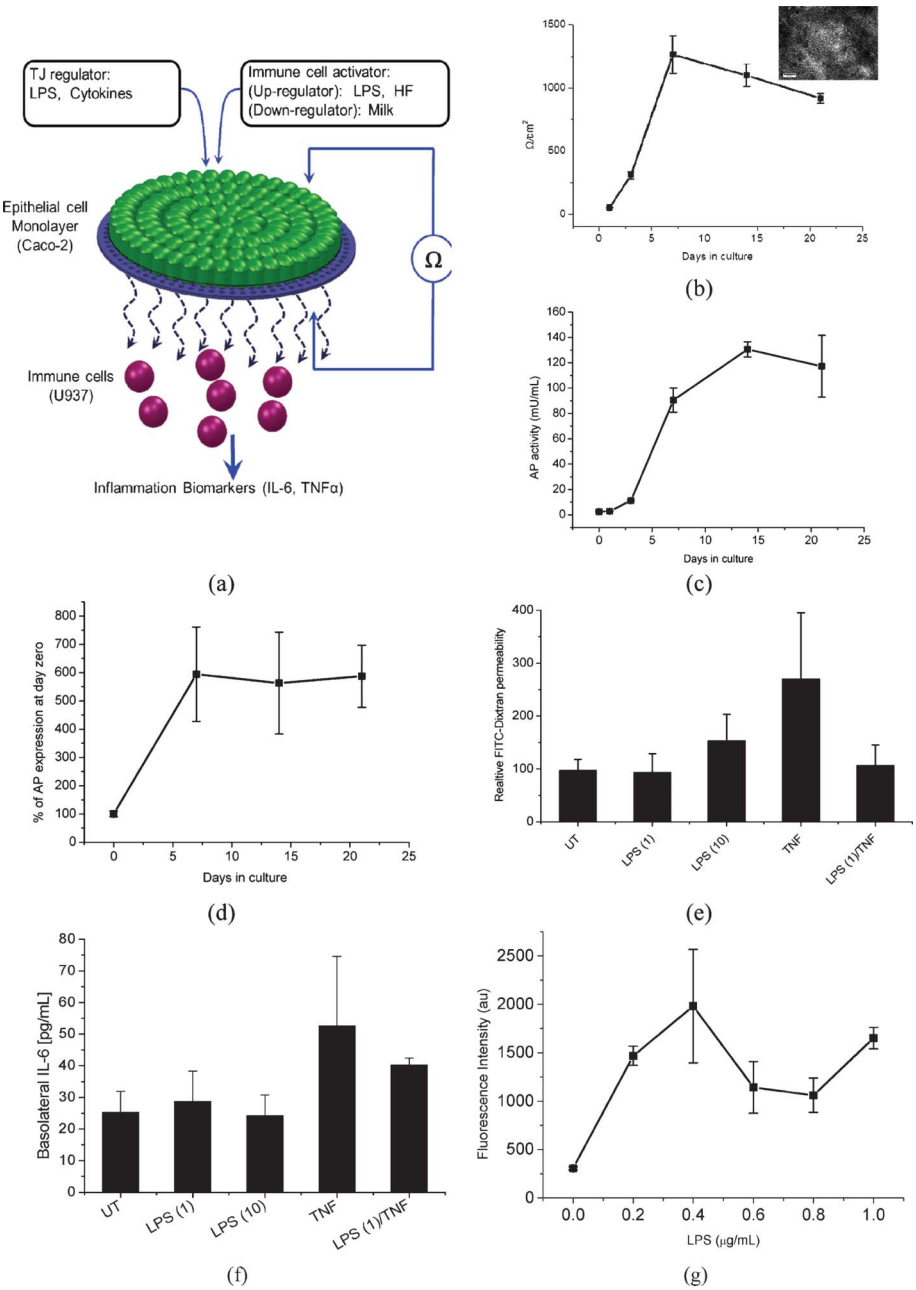

Fig. 2 (a) Schematic of the experimental design of the GIT model, showing the cells' co-culture and the setup for Trans Epithelial Electric Resistance (TEER) measurements, the stimuli applied to the epithelial cell layer, and the detection biomarkers. (b) TEER measurements in a Transwell device indicate that the Caco-2 cells form a confluent monolayer (inset) after one week of culture. (c) Alkaline phosphatase (AP) activity, *i.e.*, the conversion of the substrate *p*-nitrophenylphosphate into phosphate and *p*-nitrophenol, as detected by measuring the *p*-nitrophenol concentration, indicates correct Caco-2 cell differentiation. (d) The percentage of AP expression with reference to day zero of the differentiation process, as measured using western blotting. (e) Caco-2 monolayer permeability to FITC-dextran, modulated by applying LPS (1 μg mL^{-1} [LPS(1)] and 10 μg mL^{-1} [LPS(10)]) and TNFα, or a combination of these stimuli; UT: untreated. TNFα appears to be the strongest permeability modulator. (f) IL-6 expression measured at the basolateral side as a response to apical stimulation with LPS and TNFα, or a combination of both stimuli. (g) Increase of IL-6 secretion after treating differentiated monocytes (U937) with LPS, as measured using an immunomagnetic assay. Error bars indicate standard deviations from the average of at least three measurements.

week (Fig. 2b). Caco-2 cell differentiation was confirmed by measuring the alkaline phosphatase (AP) activity, which is a digestive brush-border enzyme up-regulated during small intestinal epithelial cell differentiation; detection was done using the AP determination kit from Roche (Roche Applied Science, Switzerland). The assay is based on the conversion of the substrate *p*-nitrophenylphosphate into phosphate and *p*-nitrophenol by alkaline phosphatase. The amount of *p*-nitrophenol is assessed by measuring the absorbance using a spectrophotometer at 405 nm at the end of the reaction. In

parallel, AP expression was followed by western blotting using a specific antibody (Sigma, Switzerland). AP activity reached maximal levels after two weeks of differentiation (Fig. 2c), whereas the expression was already maximal after one week (Fig. 2d). The apical-to-basolateral transport flux (correlated to the paracellular transport route) has been quantified by measuring the permeability of the epithelial monolayer to fluorescein isothiocyanate (FITC)-4 kDa dextran (Fig. 2e). IL-6 was selected as the primary measure in our experiments. Nevertheless, the final selection of the inflammation marker to be monitored will depend on the results of the running human trial. Other biomarker candidates are tumor necrosis alpha (TNFα) and interleukin-1β (IL-1β). A significant increase of IL-6 secretion was observed in the basolateral media after treating the apical side of Caco-2 cells with LPS and TNFα for 24 h (Fig. 2f). Since the Caco-2 intestinal barrier protects macrophages from LPS very well, more than a thousand-fold increased concentration of LPS had to be added on the apical side to reach a level of pro-inflammatory cytokine production that is similar to the response induced when LPS is added basolaterally. No cell de-sensitization was observed after LPS and TNFα treatment.

Differentiated monocytes (macrophages) were also treated on-chip with different concentrations of LPS and the secreted IL-6 was quantified using an immunomagnetic assay. To this purpose, magnetic beads of 2.8 μm in diameter (Invitrogen, Switzerland) were conjugated with human IL-6-specific antibody (Invitrogen, Switzerland) and mixed with the supernatant of the stimulated cells to capture the released IL-6. Phycoerythrin-conjugated detection antibody against IL-6 (BioSciences, Switzerland) was then introduced to complete the sandwich immunoassay under magnetic trapping of the beads. After washing, the fluorescence intensity due to the phycoerythrin-conjugated detection antibody was measured using a fluorescent microscope and analyzed using ImageJ software (see Fig. 2g). Our preliminary results show a significant increase of the IL-6 concentration after treating the macrophages with LPS, which demonstrates the possibility of quantifying the induced cytokines using an on-chip immunomagnetic assay. Also, a human intervention study is being conducted to validate the *in vitro* co-culture model system for measuring the pro-inflammatory activity of a high-fat meal, followed by comparison with the effect of the application of anti-inflammatory dairy products.

Fluorescence detection and imaging system

Microfluidics and optical detection have been shown to be matched due to the non-invasive nature of optical measurement techniques and the transparency of the microfluidic fabrication materials (*e.g.* glass and PDMS). The Nutrichip platform will also encompass a dedicated fluorescence camera and image processing system. The fluorescence intensity correlated to the captured cytokines on the surface of magnetic beads will be acquired by a dedicated complementary metal oxide semiconductor (CMOS) imager. The CMOS imaging sensor is made using a conventional CMOS semiconductor manufacturing process. Several transistors next to each photosensor convert the light energy to a voltage. Additional circuitry on the chip may be included to convert the voltage to digital data. These imaging sensors have gained popularity recently as powerful and low-cost devices for cell imaging applications. A special CMOS design will reduce the imager noise while decreasing the pixel area of the sensors. This approach enables an image quality comparable with state-of-the-art charge coupled device (CCD) cameras.[30] The ''smart'' aspect of the NutriChip imager lies on the interpretation of the collective information gathered by the output of the imaging system. This task will be carried out by a dedicated image-processing unit, which will enhance the quality and the detection sensitivity thanks to the target sub-resolution and generation of synthetic images of fluorescently stained cells.[31] The related algorithms will be implemented in a Digital Signal Processor or Field Programmable Gate Array Integrated Circuit in order to have on-line image processing and data analysis. Therefore, the final optical detection system will combine a CMOS imager and a CMOS processing unit for real-time quantitative analysis.

As we have seen before, an increased concentration of LPS induces pro-inflammatory cytokine. Bogunovic *et al.*[32] showed that the Toll-like Receptor 2 (TLR2) is found by *in vitro* immunofluorescence staining of intestinal cells. Moreover, Chen *et al.*[33] demonstrated that LPS, in addition to its classical receptor TLR4, induces the expression of TLR2 in intestinal cells and that TLRs appears to be important for regulating the intestinal epithelial barrier integrity by regulating the expression of proteins involved in TJs. In our preliminary studies, we confirmed similar findings by inducing TLR2 expression on Caco-2 cells treated with LPS. Therefore, the ultimate goal of our fluorescence detection and imaging system is to provide imaging and quantitative analysis of both cytokine and expressed TLRs, including TLR2. In both cases, fluorescent staining using specific antibodies provides imaging of the biomarkers. For example, Fig. 3a shows TLR2 induced by LPS on Caco-2 cells. These images are acquired in the NutriChip system in order to get an on-line quantitative estimation of the TLR2 receptors. In analogy, images like the one shown in Fig. 3b are acquired using fluorescent microbeads in order to obtain an on-line quantitative estimation on pro-inflammatory cytokines.

Future: nutrikinetic studies

Intestinal calcium absorption is an important process that is involved in the maintenance of calcium hemostasis.[34] Calcium is absorbed in mammals along two routes: using a transcellular mechanism, predominantly taking place in the duodenum and a passive paracellular mechanism, where the concentration is dependent on the diffusional processes. The importance of pharmacokinetics has long been recognized in medicine and pharmacology and ADME studies are essential components for the development of efficient and safe drugs.

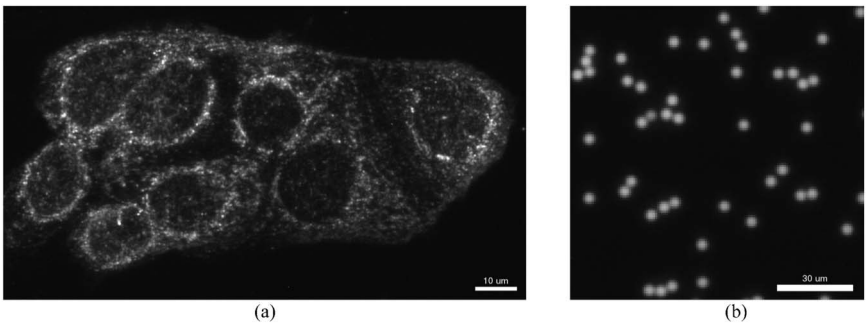

Fig. 3 (a) Fluorescent image of TLR2, as induced by application of LPS, acquired on Caco-2 cells. (b) Fluorescent image of a test sample of fluorescent micro-beads of 6 μm diameter using excitation/emission wavelengths of 505/515 nm.

In nutrition research, the concept of nutrikinetics is still in its infancy, but will undoubtedly become more important in the future. Monitoring the bio-availability of nutrients in digested food is an extremely important task in nutrition research, which aims at quantifying the contribution of a certain type of food as a source of a specific nutrient (*e.g.*, calcium). The NutriChip platform will also be used for investigating the bio-availability of calcium by quantitatively monitoring the adsorption and transport of calcium through the epithelial cell layer, as well as calcium uptake by target cells. The Caco-2 cell layer again serves here as a model for the intestinal barrier through which calcium transport will take place.[35] The NutriChip platform has the potential to significantly improve nutrikinetic measurements and alleviate some of the restrictions of classical *in vitro* systems for monitoring the bio-availability of calcium, by a combination of both microfluidic-based cell culture and manipulation with time-resolved fluorescent imaging. The setup for detecting the bio-availability of calcium includes a dedicated imaging setup, which relies on the ratio imaging technique and quantification of the fluorescent emission intensities of specific dyes, which change their absorbance spectra upon binding to Ca^{2+}. Several fluorescent probes show a spectral response upon binding Ca^{2+}, which enabled researchers to investigate changes in transport and absorption of Ca^{2+} using fluorescent microscopy, flow cytometery and fluorescent spectroscopy. Fura-2 is the most popular dye for ratio-imaging microscopy; it is an UV-excited Ca^{2+} indicator that undergoes a shift in absorption upon binding to Ca^{2+}.[36] Our system will be used for detecting both free transported Ca^{2+} through the epithelial monolayer as well as the intracellular Ca^{2+} absorbed by the target cells. The system will be interfaced by a fluidic injection unit for continuous flow media exchange and will contain a magnetic washing, purification and separation module.

An integrated platform as a paradigm for nutrient analysis

The NutriChip platform will be a first-generation integrated system centered on the miniaturized GIT. After establishing the biologically immune competent GIT model, the miniaturized GIT, and the imaging unit, we will turn our attention to integrate these components in a modular system, which allows the application od continuous liquid flows that mimic the *in vivo* environment of the human GIT. The size of the miniaturized GIT device would allow for the integration of an array of identical units, which can be addressed either with the same or different epithelium regulators or immune cell stimuli for high-throughput screening and analysis of several nutrients for faster and efficient prediction of the influence of food ingestion. In the future, the system can be further expanded to include an on-chip digestion functionality. Despite the intrinsic potential of microfluidic devices, accommodating new analysis and protocols into a micro-scale environment will require the tackling and solving of practical challenges, particularly those associated with the analysis of complex samples, such as food.[37] Additionally, integrating sample treatments, such as food digestion, co-culture of different types of cells and/or bacteria, immunoassays, *etc*, would involve designing a microfluidic network capable of manipulating the involved fluidic samples where and when desired. We believe that the prototypical NutriChip platform will be able to clearly demonstrate a new technology paradigm, and is generic enough to open other applications in the food technology area, or for the analysis of other types of food.

References

1 A. N. Margioris, *Curr. Opin. Clin. Nutr. Metab. Care*, 2006, **12**, 129–137.
2 F. Nappo, K. Esposito, M. Cioffi, G. Giugliano, A. M. Molinari, G. Paolisso, R. Marfella and D. Giugliano, *J. Am. Coll. Cardiol.*, 2009, **39**, 1145–1150.
3 G. Vergères, B. Bogicevic, C. Buri, S. Carrara, M. Chollet, L. Corbino-Giunta, L. Egger, D. Gille, K. Kopf-Bolanz, K. Laederach, R. Portmann, Q. Ramadan, J. Ramsden, F. Schwander, P. Silacci, B. Walther and M. Gijs, *Br. J. Nutr.*, 2012, **142**, 245–250.
4 Z. F. Bhat and H. Bhat, *Int. J. Dairy Sci.*, 2011, **6**, 1–12.
5 D. B. Panagiotakos, C. H. Pitsavos, A. D. Zampelas, C. A. Chrysohoou and C. I. Stefanadis, *J. Am. Coll. Nutr.*, 2010, **29**, 357–364.

6 D. C. Hunter, R. Brown, T. Green, C. Thomson, M. Skeaff, S. Williams, J. M. Todd, C. E. Lister, T. McGhie, J. Zhang, H. Martin, P. Rippon, R. Stanley and M. A. Skinner, *Int. J. Food Sci. Nutr.*, 2012, **63**, 90–102.
7 P. J. Nestel, S. Pally, G. L. MacIntosh, M. A. Greeve, S. Middleton, J. Jowett and P. J. Meikle, *Eur. J. Clin. Nutr.*, 2012, **66**, 25–31.
8 M. M. Most, A. G. Ershow and B. A. Clevidence, *J. Am. Diet. Assoc.*, 2003, **103**, 729–735.
9 J. B. Furness, W. A. A. Kunze and N. Clerc, *Am. J. Physiol. Gastrointest. Liver. Physiol.*, 1999, **277**, 922–928.
10 S. Yeruva, G. Ramadori and D. Raddatz, *Int. J. Colorectal Dis.*, 2008, **23**, 305–317.
11 G. Bisping, N. Lugering, S. Lutke-Brintrup, H. G. Pauels, G. Schurmann, W. Domschke and T. Kucharzik, *Clin. Exp. Immunol.*, 2001, **123**, 15–22.
12 E. L. Ferrec, C. Chesne, P. Artusson, D. Brayden, G. Fabre, P. Gires, F. Guillou, M. Rousset, W. Rubas and M. L. Scarino, *ATLA*, 2001, **29**, 649–668.
13 G. Rovera, T. A. O'Brian and L. Diamond, *Science*, 1979, **204**, 868–870.
14 D. Huh, G. A. Hamilton and D. E. Ingber, *Trends Cell Biol.*, 2011, **21**, 745–754.
15 D. Huh, B. D. Matthews, A. Mammoto, M. Montoya-Zavala, H. Y. Hsin and D. E. Ingber, *Science*, 2010, **328**, 1662–1668.
16 H. J. Kim, D. Huh, G. Hamilton and D. E. Ingber, *Lab Chip*, 2012, **12**, 2165–2174.
17 G. J. Mahler, M. B. Esch, R. P. Glahn and M. L. Shuler, *Biotechnol. Bioeng.*, 2009, **104**, 193–205.
18 J. H. Sung and M. L. Shuler, *Lab Chip*, 2009, **9**, 1385–1394.
19 G. J. Mahlera, M. L. Shulerb and R. P. Glahnc, *J. Nutr. Biochem.*, 2009, **20**, 494–502.
20 J. H. Sung, C. Kama and M. L. Shuler, *Lab Chip*, 2010, **10**, 446–455.
21 J. H. Sung, J. Y. Dan Luo, M. L. Shuler and J. C. March, *Lab Chip*, 2011, **11**, 389–392.
22 A. S. Sandberg, *Int. J. Vitam. Nutr. Res.*, 2010, **80**, 307–313.
23 P. Shah, V. Jogani, T. Bagchi and A. Misra, *Biotechnol. Prog.*, 2006, **22**, 186–198.
24 J. R Kanwar and R. K Kanwar, *BMC Immunol.*, 2009, **10**, 7.
25 C. R. H. Raetz, *J. Bacteriol.*, 1993, **175**, 5754–5753.
26 M. B. Esch, T. L. King and M. L. Shuler, *Annu. Rev. Biomed. Eng.*, 2011, **13**, 55–72.
27 Q. Ramadan and M. A. M. Gijs, *Analyst*, 2011, **136**, 1157–1166.
28 M. Shimizu, *Biosci., Biotechnol., Biochem.*, 2010, **74**, 232–241.
29 J. R. Turner, *Adv. Drug Delivery Rev.*, 2000, **41**, 265–281.
30 G. Köklü, J. Ghaye, R. Beuchat, G. De Micheli, Y. Leblebici and S. Carrara, *2012 IEEE International Symposium on Circuits and Systems (ISCAS)*, 2012, DOI: 10.1109/ISCAS.2012.6271688.
31 J. Ghaye, G. De Micheli and S. Carrara, *BioNanoScience*, 2012, **2**, 94–103.
32 M. Bogunovic, S. H. Davé, J. S. Tilstra, D. T. W. Chang, N. Harpaz, H. Xiong, L. F. Mayer and S. E. Plevy, *Am. J. Physiol.: Gastrointest. Liver Physiol.*, 2007, **292**, G1770–G1783.
33 J. Chen, J. N. Rao, T. Zou, L. Liu, B. S. Marasa, L. Xiao, X. Zeng, D. J. Turner and J. Y. Wang, *Am. J. Physiol.: Gastrointest. Liver Physiol.*, 2007, **293**, G568–76.
34 A. V. Perez, G. Picotto, A. R. Carpentieri, M. A. Rivoira, M. E. Lopez and N. T. De Talamoni, *Digestion*, 2008, **77**, 22–34.
35 S. Cosentino, C. Gravaghi, E. Donetti, B. M. Donida, G. Lombardi, M. Bedoni, A. Fiorilli, G. Tettamanti and A. Ferraretto, *J. Nutr. Biochem.*, 2010, **21**, 247–254.
36 G. Grynkiewicz, G. M. Poenie and R. Y. Tsien, *Biol. Chem.*, 1985, **260**, 3440–3450.
37 M. Asensio-Ramos, J. Hernandez-Borges, A. Rocco and S. Fanali, *J. Sep. Sci.*, 2009, **32**, 3764–3800.