

## Horizons in Nutritional Science

### The NutriChip project – translating technology into nutritional knowledge

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#### Abstract

Advances in food transformation have dramatically increased the diversity of products on the market and, consequently, exposed consumers to a complex spectrum of bioactive nutrients whose potential risks and benefits have mostly not been confidently demonstrated. Therefore, tools are needed to efficiently screen products for selected physiological properties before they enter the market. NutriChip is an interdisciplinary modular project funded by the Swiss programme Nano-Tera, which groups scientists from several areas of research with the aim of developing analytical strategies that will enable functional screening of foods. The project focuses on postprandial inflammatory stress, which potentially contributes to the development of chronic inflammatory diseases. The first module of the NutriChip project is composed of three *in vitro* biochemical steps that mimic the digestion process, intestinal absorption, and subsequent modulation of immune cells by the bioavailable nutrients. The second module is a miniaturised form of the first module (gut-on-a-chip) that integrates a microfluidic-based cell co-culture system and super-resolution imaging technologies to provide a physiologically relevant fluid flow environment and allows sensitive real-time analysis of the products screened *in vitro*. The third module aims at validating the *in vitro* screening model by assessing the nutritional properties of selected food products in humans. Because of the immunomodulatory properties of milk as well as its amenability to technological transformation, dairy products have been selected as model foods. The NutriChip project reflects the opening of food and nutrition sciences to state-of-the-art technologies, a key step in the translation of transdisciplinary knowledge into nutritional advice.

**Key words:** Nutrition: Functional genomics: Microfluidics: Nanotechnologies

#### Current trends in human nutrition

##### Dynamic definition of health

In 1946, the WHO defined health as ‘a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity’<sup>(1)</sup>. This definition contains a static component that no longer suits current knowledge in the biomedical sciences. The conference ‘Is health a state or ability? Towards a dynamic concept of health’ has revised this definition and

proposed a more dynamic approach by defining health as ‘the ability to adapt and to self manage’<sup>(2)</sup>. In this context, the increased incidence of chronic metabolic diseases paralleling the ageing of the human organism may be due to a loss in the metabolic plasticity of the organism, i.e. its ability to respond adequately in a timely and quantitative manner to external challenges<sup>(3)</sup>. The metabolic nature of food indicates that nutrition could positively or negatively modulate metabolic plasticity and consequently the development of chronic diseases.

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### *Food is more than a confounding factor*

That nutrition is crucial to growth, development, function and the maintenance of health is obvious. In wealthy societies, the prevention and treatment of disease is most obviously associated with the widespread use of pharmaceuticals rather than dietary modification. There are many more scientific publications on pharmaceuticals than on food. Why is that so? Beyond obvious economical arguments, a comparison of the chemical and biological properties of food and drugs explains the imbalance in research activities: drugs are mostly single chemical entities possessing targeted pharmacological activity and being adsorbed in a supervised manner. Food, on the other hand, is composed of thousands of uncharacterised chemical compounds that are ingested unsupervised<sup>(4)</sup>. Thus it seems that scientists may have been so far lacking the tools to characterise the interaction of food with the human organism as efficiently as in the case of drugs.

### *Towards nutritional systems biology*

The application of functional genomics to the fields of food and nutrition sciences is about to surmount this hurdle. The molecular fate of nutrients in ingested food (composition, digestion, intestinal transport, metabolic activation, excretion) can now be monitored in humans with a precision, sensitivity and functional understanding that was previously not possible. Human tissues and fluids can be investigated for their epigenetic, genetic, transcriptomic, proteomic and metabolomic profiles and complement classical analyses to obtain holistic information on the molecular processes taking place as the response of the organism to the ingestion of food<sup>(5)</sup>. Studies that combine these analytical levels are still rare<sup>(6)</sup> but they are precursors of a nutritional systems biology, which will move nutritional science one step closer to an objective understanding of the effects of food on human physiology, in particular on the maintenance of health.

### *Postprandial stress: a surrogate of metabolic plasticity?*

As the analytical tools are available, the challenge is now to translate the concept of metabolic plasticity into parameters that are measurable and nutritionally relevant. The cellular metabolism of nutrients, culminating in the synthesis of the biochemical energy embodied in ATP, produces side effects that include the mitochondrial production of reactive oxygen species (oxidative stress), the synthesis of misfolded proteins (endoplasmic reticulum stress) and the activation of defence mechanisms by immune cells (inflammatory stress). These effects accumulate at the level of the organism to produce a phenomenon named postprandial stress<sup>(7)</sup>. Under normal conditions, postprandial stress is of low magnitude and transient in nature so that it disappears within a few hours post-ingestion. An unhealthy diet, permanent snacking and/or a diseased organism may, however, increase the magnitude of postprandial stress and/or delay its recovery so that fasting baseline values for markers of postprandial stress may increase over time and contribute to the development of

chronic metabolic diseases<sup>(7)</sup>. This concept has already been demonstrated for glucose in human blood as the glycaemic index, a measure of the postprandial glucose response, emerges as a potentially important determinant of the risk of cardiovascular diseases<sup>(8)</sup>. We propose that postprandial stress can be used as an indicator of metabolic plasticity and, consequently, of health.

### *Blood as a source of biomarkers*

Biomarkers in blood can provide specific information on the health status of organs, such as the heart, liver or kidney. The diagnostic strength of clinical chemistry is due to the ability of blood to serve as a sentinel of the body carrying molecular information between organs<sup>(9)</sup>. As clinical chemistry informs physicians about the ability of patients to respond to drugs, the same strategy should be used by nutritionists to monitor the quality of the interaction between consumers and food products. The assessment of metabolic and immunomodulatory biomarkers (insulin, glucose, TAG, cholesterol, hormones, cytokines, etc.) already belongs for decades to the arsenal of tools used by nutritional scientists. This arsenal is, however, in the process of being dramatically augmented with the advent of nutrigenomics<sup>(5)</sup>. Blood, as a connective fluid tissue, is very appropriate for the monitoring of biomarkers indicating postprandial stress in order to assess the impact of food on metabolic plasticity.

### **Probing the impact of nutrition on postprandial systemic inflammation in humans**

#### *High-fat meals are associated with postprandial systemic inflammation*

Inflammation is a major component of metabolic diseases. Subjects ingesting high-fat meals experience a postprandial systemic rise in pro-inflammatory cytokines, the level of these cytokines being higher in obese than in lean subjects<sup>(10)</sup>. A repetition of postprandial inflammatory stress over prolonged periods of time may significantly contribute to the development of chronic inflammation. In that context, epidemiology has established a positive correlation between a dietary regimen rich in *trans*-fatty acids and fasting markers of systemic inflammation<sup>(11)</sup>. Clear evidence for a relationship between the consumption of high-fat meals and chronic low-grade inflammation has, however, not been demonstrated, taking into account the fact that carbohydrates may also contribute to this phenomenon<sup>(12)</sup>. 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 kinetics of inflammatory biomarkers in the blood of human subjects thus provides information on the quality of the interaction between specific foods and the organism. This proposition forms the conceptual basis for the biological modules of the present NutriChip project (Fig. 1).

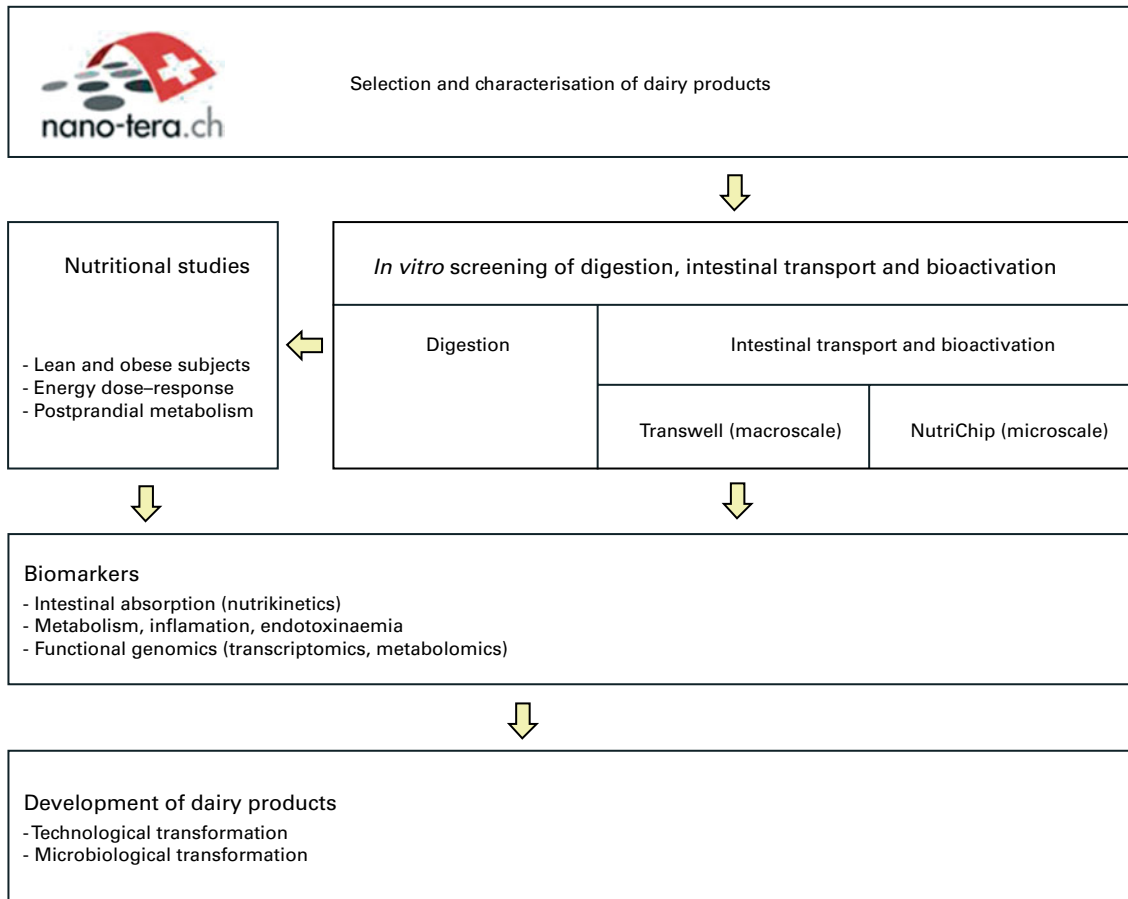


Fig. 1. Overview of the biological modules of the NutriChip project.

### *Anti-inflammatory properties for dairy products?*

Several food products, including orange juice<sup>(13)</sup> and tomato<sup>(14)</sup>, have been demonstrated to reduce the postprandial inflammatory response to high-fat meals. Dairy products may also have anti-inflammatory properties in human adults. Lower levels of fasting pro-inflammatory cytokines have indeed been documented in epidemiological<sup>(15)</sup> and intervention<sup>(16)</sup> studies in human adults consuming dairy products. Decreased postprandial levels of pro-inflammatory cytokines after the consumption of dairy products have also been reported<sup>(17)</sup>. We have investigated the postprandial kinetic response of healthy human subjects to the ingestion of dairy products by measuring the genome-wide expression of genes in the subjects' blood cells. This analysis revealed a common postprandial kinetic pattern for the expression of inflammatory genes, thus illustrating the tight link between nutrition and the immune system<sup>(18)</sup>.

Milk is a unique food; its immunomodulatory properties result from two hundred million years of evolution of the mammary gland<sup>(19)</sup>. Milk can also be transformed technologically in an almost infinite number of ways. In particular, the fermentation of milk by lactic acid bacteria clearly adds to its immunomodulatory potential<sup>(20)</sup>, even more since recent analytical breakthroughs highlight the importance of the gut microbiota on human metabolism<sup>(21)</sup>. Milk thus

appears to be a strategic vector to deliver immunomodulatory nutrients to the human organism in order to improve its metabolic health.

### *Comparative analysis of postprandial inflammatory stress in humans*

High-fat meals can be used as 'positive controls' eliciting the postprandial production of inflammatory cytokines in humans, against which other products can be compared. In order to identify suitable conditions for carrying out such comparative studies, the relationship between the energy dose of the selected high-fat meal and the postprandial production of inflammatory cytokines should be investigated. High-energy doses may induce a significant inflammatory response and consequently allow the identification of statistically significant changes even with a relatively small number of subjects. On the other hand, low-energy doses may be nutritionally more realistic and provide more flexibility in designing interventions that evaluate the ability of co-ingested food products to inhibit the postprandial inflammation induced by a high-fat meal. The present NutriChip project measures the postprandial inflammatory stress of subjects having ingested three different energy doses of a fatty meal. Pro-inflammatory cytokines, such as IL-6, are measured as markers for inflammation.

The pro-inflammatory environment is exacerbated in humans with metabolic diseases<sup>(10)</sup>. The NutriChip project thus compares the postprandial stress of lean healthy subjects to subjects with metabolic diseases. As the biological processes regulating inflammation and the metabolism of macronutrients are interconnected<sup>(22)</sup>, measuring the postprandial metabolic response (insulin, glucose, TAG, lipoproteins) in addition to the inflammatory markers should provide further insight into the impact of food on the organism.

In addition to the discrete set of metabolic and immunomodulatory parameters, the blood cell transcriptome and the serum metabolome of the lean and obese subjects are measured. A comparative analysis of the fasting and postprandial samples then allows the identification of biomarkers modulated by nutrition that may have an impact on the development of metabolic diseases.

### Screening food products in an *in vitro* model of digestion

#### *In vitro* screening strategy

Despite their complexity, human intervention studies are clearly the 'gold standard' in nutritional science<sup>(23)</sup>. Their limitations are logistical in nature, as 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 bioactive or even unsafe is unethical even if the trials involve a low grade of invasiveness by limiting analyses to blood samples. *In vitro* models are thus needed for the efficient screening of bioactive food products. In the NutriChip project, a panel of dairy products is digested *in vitro*, the digested nutrients are transported through an intestinal cell monolayer, and the ability of the transported nutrients to modulate the inflammatory activity of immune cells is finally measured. The immunomodulatory properties of these products are compared to those of the high-fat meal and the most active dairy products are tested in human intervention trials.

#### Characterising the molecular composition of the test products

If one wants to follow the fate of the nutrients present in food during digestion, intestinal transport and metabolic activation, the composition of the undigested food should first be characterised at the molecular level. Recent developments in the fields of proteomics/peptidomics<sup>(24)</sup> and metabolomics/lipidomics<sup>(25)</sup> now allow food scientists to go significantly beyond the macronutrient composition of food, thus opening the path to the identification of specific bioactive molecules in food products. As the delivery of high-density data increases, sharing this information by creating interactive data banks that can be used by the scientific community has the potential to boost the efficacy of food and nutrition research. In the context of the NutriChip project, several hundred milk and bacterial proteins have been separated by two-dimensional PAGE and identified by liquid chromatography coupled to MS (LC-MS) in a variety of dairy products. The reference

maps will be made freely available in the form of an interactive online platform, the Dairy Protein Atlas (R. Portmann, private communication).

#### *In vitro* digestion

*In vitro* models that mimic human digestion are the subject of intense research<sup>(26)</sup>. The European COST Action FA1005 (Improving health properties of food by sharing our knowledge on the digestive process) was initiated in 2011 and aims to harmonise research by defining a reference model for *in vitro* digestion. Most models are composed of three steps that represent digestion along the gastrointestinal tract. Appropriate buffer composition, identity and concentration of digestive enzymes, pH, and digestion times are selected accordingly. The first step mimics digestion by saliva in the mouth. The second step mimics gastric digestion in the presence of pancreatic juice. The third step models intestinal digestion in the presence of bile salts. The most advanced models introduce dynamic features such as mastication, peristaltic movements or the controlled secretion of digestive juices. Whereas dynamic models are closer to human physiology, the less elaborate static models are still widely used in most research laboratories<sup>(26)</sup>.

The NutriChip project has validated a static three-step model for the specific digestion of dairy products using pasteurised whole milk as a standard<sup>(27)</sup>. Interestingly, a comparison of the amino acids released from milk proteins during the course of the *in vitro* digestion revealed that the fraction of essential amino acids released by digestion was significantly higher than the total fraction of these amino acids present in the entire pool of milk proteins. This observation suggests that the digestive system has evolved in a way that allows an efficient extraction of the essential amino acids present in food, in particular in milk.

#### Transport through confluent intestinal cells

In analogy to pharmacokinetics, the concept of nutrikinetics becomes more and more important in nutrition science. The absorption of nutrients through intestinal cells significantly contributes to their bioavailability. *In vitro*, the intestinal transport of nutrients is most often measured with the confluent layers of intestinal cell lines<sup>(28)</sup>, although intestinal tissues isolated from animals are also used<sup>(29)</sup>. Proteomics and metabolomics will dramatically increase our knowledge of the intestinal transport of nutrients. For example, while the transport of amino acids, dipeptides and tripeptides is well characterised, the transport of larger peptides, even of intact proteins, remains poorly characterised. In particular, the population and fraction of large peptides being transported through the gut, the mechanisms mediating this transport and even its biological significance are current research challenges<sup>(30)</sup>. High-density ('omics') data gained from *in vitro* experiments will allow the identification of bioavailable nutrients, which will then be specifically traced in humans. Such data will contribute to the identification of functional food components as well as to a better understanding of the mechanisms mediating

oral tolerance<sup>(31)</sup>. A high-density analytical strategy (metabonomics, peptidomics) can thus be applied to identify the metabolites transported through a confluent layer of Caco-2 cells. The identified dairy nutrients can then be investigated for their presence in the postprandial sera of human subjects participating in intervention studies.

### Inflammatory action of nutrients on immune cells

Once transported and metabolised by the intestinal cellular barrier, the primary fate of nutrients is to interact with receptors on their target organs and cells. Immune cells are obvious cellular targets to demonstrate the pro- or anti-inflammatory potential of the translocated molecules. The design of the immunomodulatory *in vitro* system depends on the mechanism inducing the postprandial activation of inflammatory cytokines in response to a high-fat meal. Several mechanisms can be put forward to account for this activation effect<sup>(7,12,13,32–34)</sup>: (i) long-chain fatty acids activate the Toll-like receptor pathway of immune cells; (ii) fat promotes the translocation of bacterial endotoxin from the gut into the circulation by increasing paracellular intestinal permeability; (iii) the fatty acid particles undergoing intestinal transcytosis are intracellular cargos for bacterial endotoxin; (iv) the metabolism of the energy-dense fat macronutrients induces the production of a non-specific cellular stress response. The NutriChip project uses a co-culture Transwell system composed 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 differentiated to macrophages, which will react to the presence of pro-inflammatory molecules in the basolateral media by producing inflammatory cytokines, such as IL-6. The Transwell system allows a wide range of

variations in the experimental set-up that will enable the different inflammatory models proposed here to be tested.

### The NutriChip

The Transwell co-culture system suffers from several drawbacks, in particular its inability to provide a dynamically controlled flow of cell nutrients and stimuli to the *in vitro* intestinal model, the long growth times needed for the intestinal cells to differentiate into functional enterocytes and to reach confluence, the high costs of the system (since it demands relatively large volumes of media to grow the cells), and an external signal detection system that requires the manual withdrawing and manipulation of samples. The NutriChip project takes a micro-engineering strategy to alleviate these hurdles. The NutriChip is a miniaturised replica of the Transwell system integrated into a micro-system (Fig. 2).

### The gut-on-a-chip

Working with a microfluidic chip allows significant reduction of the liquid volumes of cell culture chambers (down to the 10 µl range), when compared to a classical co-culture system. Therefore, the use of microfluidic devices will lead to reduced reagent consumption, and obviously reagent-associated costs can be diminished accordingly. Moreover, miniaturisation procedures allow the fabrication of many identical analytical devices on a given microfluidic chip surface, paving the way for parallel assays and high-throughput analyses. Microfluidic chips can also be equipped with certain functionality, at the cost of some technological effort and complexity; for example, miniaturised valves and pumps can be configured on the chip, which can be easily operated using

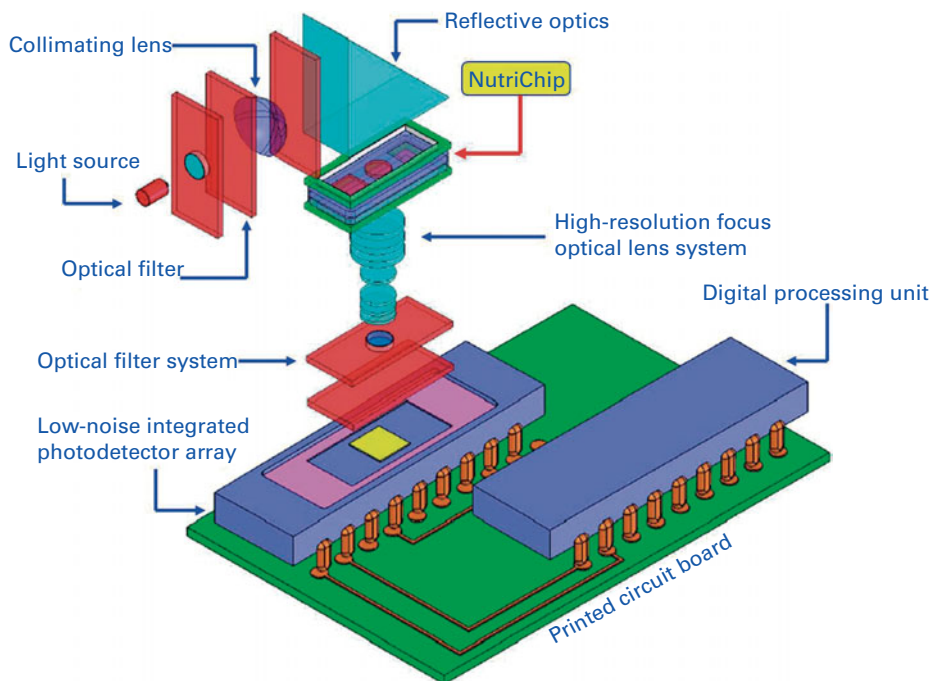


Fig. 2. A schematic representation of the engineering modules of the NutriChip project (adapted from <http://www.nano.tera.ch/projects/403.php>).



external pressure control. Such valves also permit to separate on-chip the two cell types of the co-culture system, which allows both types of cells to grow independently under optimum media conditions and permits them to correctly differentiate. If it is observed that both cell types have reached the target structure (e.g. formation of a confluent layer for the epithelial cells and differentiation of the seeded monocytic cells to macrophages), the two cell reservoirs can be put in fluidic communication by simply opening an on-chip valve and the chip can then be exploited for food testing. The microfluidic chips will be configured with a suitable microfluidic architecture, i.e. appropriate microchannels for exposing the cell solutions to culture media, washing buffer, fluorescent detection labels, functionalised magnetic beads, etc., which will allow controlled and quantitative testing of the food quality parameters<sup>(35)</sup>.

### Going 'nano' and 'tera' with the NutriChip

Subjects ingesting high-fat meals have increased postprandial levels of insulin, glucose, TAG, cholesterol and pro-inflammatory cytokines released in the blood serum as well as activation of Toll-like receptors in their immune cells. The artificial gut-on-a-chip described previously will allow the quantitative acquisition of terabytes of information related to the nanoscale metabolic and immunomodulatory biomarkers. Although electrochemical detection of insulin<sup>(36)</sup>, glucose<sup>(37)</sup> and cholesterol<sup>(38)</sup> has been already demonstrated *in vitro*, the first prototype of the NutriChip implements the direct fluorescent detection of Toll-like receptors on Caco-2 cells as well as of IL-6 in the media. Microfluidic chips are ideally suited to optical detection methods due to the non-invasive nature of the optical measurement techniques and the transparency of the microfluidic fabrication materials (e.g. glass and polydimethylsiloxane). The fluorescence intensity of light will be acquired with a dedicated complementary metal oxide semiconductor imager and fluorescent dyes as labels for the cells' receptors, while functionalised (anti-IL-6) magnetic micro-beads will be used for the capturing of the IL molecules and their quantification with an *in situ* immunomagnetic assay. Special complementary metal oxide semiconductor design will reduce the imager noise while decreasing the pixel area of sensors. This approach enables an image quality comparable with that obtained by using charge coupled device cameras<sup>(39)</sup>. Special image processing and analysis will increase the quality of the quantification of metabolites, thanks to more precise target sub-resolution and generation of synthetic images of fluorescent stained cells<sup>(40)</sup>. Therefore, the final optical detection system will combine a complementary metal oxide semiconductor imager and an integrated electronic microchip for the online quantitative analysis. The combination of the dynamic microfluidic-based cell co-culture, with prolonged time perfusion-based exchange of media and reagents, and the imaging system will offer near-real-time live-cell imaging for continuous tracking of the variation of immune cell response to perfused stimuli in a time-lapsed manner.

### Limitations and applications of the NutriChip

Keeping in mind the versatility of the immune system, the limitations of the co-culture system as a model to mimic human digestion, intestinal transport of nutrients and immune activation by the absorbed nutrients are obvious. Care should thus be taken not to uncritically extrapolate the *in vitro* data to the *in vivo* situation. A comparative *in vitro* screening of the inflammatory properties of different food products should, however, reveal quantitative differences that are likely to be retrieved *in vivo* in the postprandial inflammatory response of the subjects having ingested these products. Thus, the dairy products selected *in vitro* for a low inflammatory impact, compared to the high-fat meal, will have to demonstrate the same relative activity in the human trial presented here.

The potential of the NutriChip extends beyond the identification of food products with a low impact on inflammatory stress. We are currently adapting the NutriChip to evaluate the effect of the food matrix on the intestinal transport of Ca (Ca-NutriChip). A recent report investigating the intestinal adhesion and metabolic activity of a probiotic bacterium suggests that guts-on-a-chip have the potential of better mimicking the *in vivo* situation than their macroscopic co-culture counterparts<sup>(41)</sup>. Technologies are also available to extend the functionality of the NutriChip upwards to include the three *in vitro* digestion steps outlined earlier. Finally, in addition to food science and nutrition, the NutriChip should find applications in all fields of human biology in which gastrointestinal phenomena are important, in particular in medicine, pharmacology and toxicology.

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