NUTRICHIP: AN INTEGRATED MICROFLUIDIC SYSTEM FOR *IN VITRO* INVESTIGATION OF THE IMMUNOMODULATORY FUNCTION OF DAIRY PRODUCTS

Q. Ramadan[1]*, H. Jafarpoorchekab[1], K. Bolanz[2], F. Schwander[2], C. Egger[2], R. Portmann[2], P. Silacci[2], S. Carrara[3], J. Ramsden[4], G. Vergères [2] and M. A. M. Gijs[1]

[1]*Laboratory of Microsystems, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
[2]*Agroscope Liebefeld-Posieux Research Station ALP, Berne, Switzerland*
[3]*Integrated Systems Laboratory, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
[4]*Centre for Molecular Recognition, Collegium Basilea, Basel, Switzerland*

ABSTRACT

We introduce the concept of an artificial human gastro-intestinal tract (GIT) for investigating the potential of immunmodulatory function of milk. More specifically, we are investigating the effect of milk on immune cells activation by creating conditions close to those of the human GIT, employing an *in vitro* model of GIT epithelial cells (Caco2) in co-culture with immune cells (THP1) on a Transwell. The cytokines (IL1/6) and Toll-like receptors (TLR2/4) expressions by the immune cells are monitored upon the application of milk on the epithelial cell layer. We also present initial results of a Lab-on-a-Chip-based *in vitro* GIT model (the NutriChip).

KEYWORDS: Gastro-intestinal tract (GIT), Dairy products, Cytokine, Toll-like receptors, *In vitro*, Microfluidics.

INTRODUCTION

The GIT plays a key role in the adsorption, distribution, metabolism, and excretion of nutrients, xenobiotics as well as other molecules originating from commensal and pathogenic micro-organisms. In light of the importance of gastro-intestinal immuno-modulation, cell culture *in vitro* models were developed consisting of a confluent layer of epithelial cells (EC) separated from a co-culture of immune cells by a permeable membrane to investigate the transport of molecules through the epithelial barrier and the subsequent activation of the underlying immune system [1-2]. Such *in vitro* systems can potentially be used to screen food for specific physiological properties of nutrients, in particular in the context of the immunomodulatory function of the latter. Inflammation is the major biological response by our immune system to harmful stimuli. Inflammatory response in humans is modulated by macrophages together with epithelial cells by production and release of small regulatory proteins called cytokines, such as tumor necrosis factor alpha (TNFα), interferon (IFN) and interleukins (ILs), as a reaction to invading micro-organisms or their constituents such as lipopolysaccharide (LPS) [3]. The signal transduction cascade leading to the production of inflammatory cytokines by immune cells is initiated by TLRs, such as TLR2 and TLR4, that are major receptors for molecules present on the surface of pathogens [4]. Because of their ability to activate inflammatory cells, TLR2/4 are important targets for drug development [5]. In fact, activation of TLR2/4 is beneficial in situations of acute infection, in which our organism must efficiently and quickly identify and inactivate pathogens.

The aim of the current study is to develop an *in vitro* experimental system to investigate the inflammatory response in monocytes (THP1)/macrophages upon the application of dairy food on the epithelial cell layer by monitoring a set of biomarkers, including cytokines (IL1/6) and the Toll-like receptors (TLR2/4), which are expressed by the monocytes/macrophages. The conceptual design of the Nutrichip is illustrated in Fig. 1. The system will be used to screen a series of dairy products for their anti-inflammatory properties and then will be validated by assessing the ability of a selected dairy product to modulate inflammation in a human trial. Existing analytical methods that interface with the Transwell system mostly analyze bulky volumes. In an effort towards miniaturization, we also present initial results of a Lab-on-a-Chip-based approach for the development of an efficient miniaturized *in vitro* GIT model , which in term will allow high-throughput culturing and analysis of the main two cell types present in the human GIT close to physiological conditions.

THE MICROFLUIDIC GIT CHIP

The basic unit of such microfluidic system is the cell co-culture chip, which comprises two fluidic compartments separated by a porous membrane as shown in Fig.2a. The upper compartment hosts a confluent epithelial cell layer and the lower compartment hosts the surface-immobilized immune cells. The Nutrichip hereby allows the major processes that characterize the passage of molecules, in particular nutrients, through the GIT and can respond to stimuli (e.g. bacterial) by increased production of inflammatory markers by the immune cells. Using the soft lithography technique, the upper (apical) and lower (basolateral) parts of the chip were molded in poly(dimethylsiloxane) (PDMS) employing a master SU-8 negative photoresist which was previously fabricated on a silicon wafer. The porous membrane was obtained from a Transwell insert (Millipore, USA). The three parts were then assembled by treating the three surfaces with an O_2 plasma through a multiple-step bonding process. A dedicated chip holder was machined to facilitate introducing cells and culture media into the chip (Fig. 2b&c).

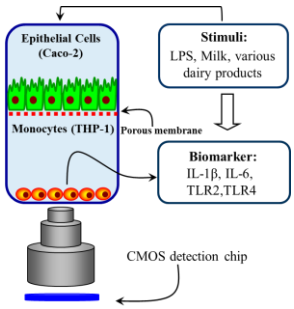
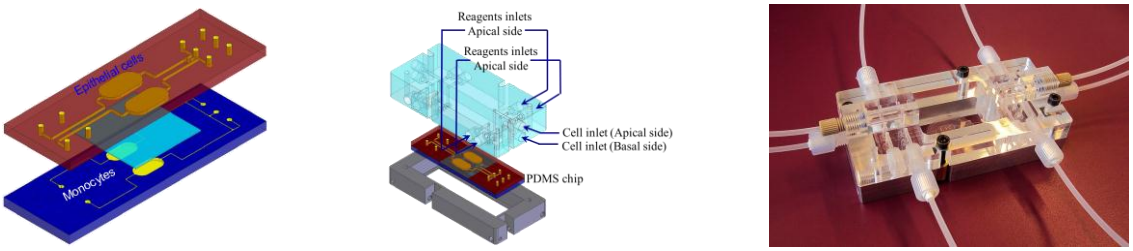


Figure 1: Schematic representation of the Nutrichip concept, in which various stimuli are applied to the Caco2 epithelial cell layer and the inflammatory biomarker response is measured in the medium of the THP1 monocytes using fluorescent detection via a dedicated CMOS chip.



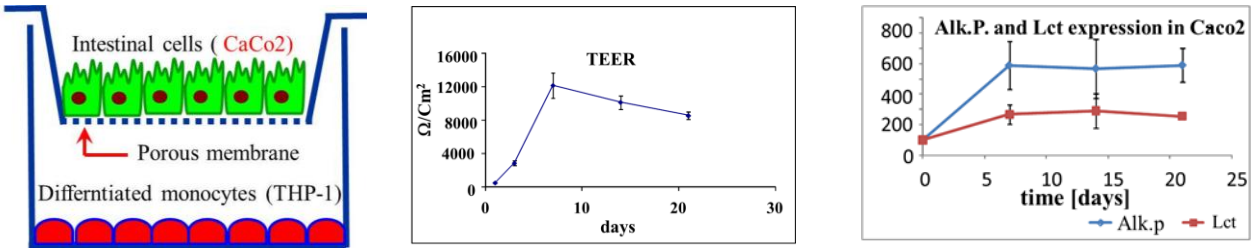
(a) (b) (c)

Figure 2: (a) Schematic representation of the basic NutriChip, which comprises two cell-culture compartments sandwiching a porous membrane. (b) The Nutrichip inserted in its fluidic interface unit. (c) The final fabricated device.

THE TRANSWELL ARTIFICIAL GIT MODEL

So far, a biologically active *in vitro* cellular system modeling the gastrointestinal tract has been developed in a Transwell co-culture system and is composed of an epithelial cell (Caco2) layer and an immune cell (THP1) layer, which are co-cultured and separated by a porous membrane, as shown in Fig.3a. The system is being used to investigate the response of immune cells to pro-inflammatory stimuli, such as LPS, by the activation of receptors on their surface. This setting mimics the major processes that characterize the passage of molecules, in particular nutrients through the human GIT, namely gastro-intestinal digestion, transport trough the epithelial cell layer, and activation of the underlying immune system.

The integrity of the confluent epithelial cell layer was demonstrated by measuring the trans-epithelial electrical resistance (TEER) as shown in Fig.3b. The fitness of the THP1 cell line was demonstrated by its ability to differentiate into macrophages. Fig.3c shows the alkaline phosphatase activity and lactase expression in Caco2 cells as indictors of correct cell differentiation. It was observed that THP1 macrophages produce significant amounts of mRNA coding for TLR2/4 in response to LPS (Fig.4a&b). We could also demonstrate a significant induction of the cytokine IL1β in the media in response to the activation of THP1 macrophages by LPS (Fig. 4c).

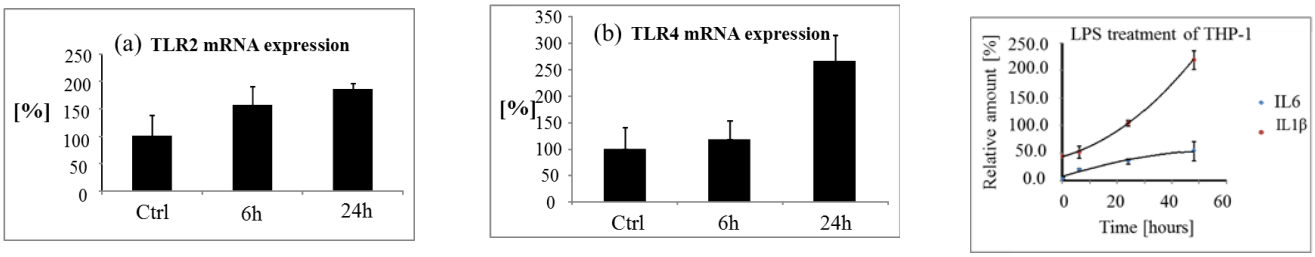


(a) (b) (c)

Figure 3: (a) Typical design of an in vitro GIT. (b) Trans-epithelial electrical resistance of a Caco2 cell layer cultured on a nanoporous membrane, reaching a maximum level after 1 week of culture. (c)Alkaline phosphatase activity and lactase expression in Caco2 cells as indicators of correct cell differentiation.

Also, the decomposition of macro-nutrients like proteins, fat and carbohydrates of milk were investigated throughout the whole digestion process, i.e. by treating the milk with solutions mimicking the saliva, gastric juice, pancreatic juice and bile. As such, a physiologically-relevant *in vitro* model system for digested dairy products, including milk, cream, cheese and yogurt was developed, mimicking the major sites of human digestion towards establishment of a standard operating procedure to

prepare *in vitro* digested dairy food to be applied to the NutriChip. Fig. 5 shows the characterization using High Performance Liquid Chromatography (HPLC) of released free amino acids during digestion of milk with artificial pancreatic juice and bile.



(a) (b) (c)

Figure 4: TLR2 (a) and TLR4 (b) mRNA expression after treatment of THP1 cells with LPS. TLR2 expression up-regulation is more rapid than TLR4. Both TLR2 and TLR4 inductions are relatively strong.(c) IL1β and IL6 concentration in the medium after stimulation with LPS of THP1.The IL6 level remains relatively low, while the IL1β level is strongly increased.

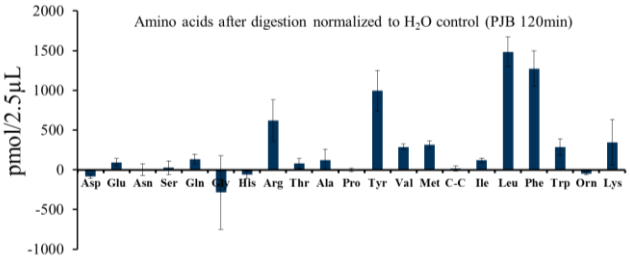


Figure 5: Characterization of the free amino acids that are released during digestion of milk with artificial pancreatic juice and bile using HPLC

CONCLUSION AND OUTLOOK

An immuno-competent artificial GIT that responds to LPS stimuli by increased production of inflammatory markers by the immune cells has been established in a Transwell co-culture system and a miniaturized microfluidic chip-based aGIT has been developed in parallel. In near future, a series of dairy products will be screened *in vitro* for their anti-inflammatory properties and the miniaturized NutriChip will be used to assess the ability of these products to modulate inflammation in human intervention trials and finally to identify dairy products with significant increased ability to inhibit the inflammatory response. The Nutrichip platform thereby has the potential to evaluate *in vitro* the influence of various food qualities by monitoring the expression of the relevant immune cell biomarkers.

ACKNOWLEDGEMENTS

This project is Financed by the Swiss National Science Foundation (SNSF) through the Nano-Tera.ch program.

REFERENCES

[1] J. R. Kanwar and R.K. Kannwar,"Gut health immunomodulatory and anti-inflammatory functions of gut enzyme digested high protein micro-nutrient dietary supplement-Enprocal", *BMC Immunol*., vol. 10, pp. 1-19, 2009.

[2] E. Le Ferrec, C. Chesne, P. Artusson, D. Brayden, G. Fabre, P. Gires, F. Guillou, M. Rousset, W. Rubas and M. L. Scarino," In vitro models of the intestinal barrier", *Altern Lab Anim*. vol 29, pp. 649-668, 2001.

[3] S. Schmitz, M. W. Pfaffl, H. H. Meyer, and R. M. Bruckmaier, "Short-term changes of mRNA expression of various inflammatory factors and milk proteins in mammary tissue during LPS-induced mastitis". *Domest. Anim. Endocrinol*. Vol. 26, pp. 111–126, 2004.

[4] V. D. de Mello, M. Kolehmainen, L. Pulkkinen, U. Schwab, U. Mager, D. E. Laaksonen, L. Niskanen, H. Gylling, M. Atalay, R. Rauramaa, M. Uusitupa, "Downregulation of genes involved in NFκB activation in peripheral blood mononuclear cells after weight loss is associated with the improvement of insulin sensitivity in individuals with the metabolic syndrome: the GENOBIN study", *Diabetologia*, vol. 51, pp. 2060-2067, 2008.

[5] C. G. Leon, R. Tory, J. Jia, O. Sivak, and K. M. Wasan, "Discovery and Development of Toll-Like Receptor 4 (TLR4) Antagonists: A New Paradigm for Treating Sepsis and Other Diseases", *Pharm Res*. vol. 25, pp. 1751-1761, 2008.

CONTACT

* Qasem Ramadan, tel: +41 21 693 6759; qasem.alramadan@epfl.ch