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THE USE OF THE ARTIFICIAL DIGESTIVE TRACT MODEL IN NUTRITIONAL SCIENCES®

Wykorzystanie modelu sztucznego przewodu pokarmowego w naukach żywieniowych®

The human microbiome, especially the gut microflora, has been a research topic for many years and its influence on human health has caught a particular interest. Thus, any abnormalities are linked to the prevalence of many diseases and conditions. Due to the rapid technological development, microbiome research is often undertaken using an artificial model of the digestive tract or gastrointestinal tract, these technological developments are of significant support to the basic research performed on the microbiome. The structure of the human gut is very complicated as well as its function, thus, it is extremely difficult to build such machinery that would function correctly. This review is based on available literature about the SHIME® artificial human gut model (patented technology by the University of Ghent and the ProDigest company that act as the owner).

Thanks to the project "Food and Nutrition Center – modernization of the SGGW campus in order to create the Food and Nutrition Research and Development Center (CŻiŻ)" co-financed by the European Union from the European Regional Development Fund under the Regional Operational Program of the Mazowieckie Voivodeship for 2014-2020 [RPMA project no. 01.01.00-14-8276 / 17] The Institute of Human Nutrition Sciences has acquired the SHIME® technology artificial digestive tract.

Key words: microbiome, artificial gut, artificial gastrointestinal system, small intestine, large intestine.

Mikrobiom człowieka, w tym szczególnie mikrobiota jelitowa od wielu lat jest przedmiotem badań w aspekcie jej wpływu na zdrowie. Z kolei, zaburzenia składu mikrobioty powiązane są z ryzykiem występowania wielu chorób. Do badań nad mikrobiomem coraz częściej wykorzystuje się modele sztucznego przewodu pokarmowego, które stanowią istotne wsparcie badań podstawowych w naukach żywieniowych. Ze względu na skomplikowaną budowę i funkcjonowanie przewodu pokarmowego człowieka bardzo trudno jest skontruować taki model, który by w pełni odpowiadał wszystkim uwarunkowaniom naturalnego przewodu pokarmowego.

Niniejsze opracowanie powstało na bazie dostępnych artykułów naukowych dotyczących w szczególności działania i zastosowania sztucznego modelu przewodu pokarmowego SHIME®, (opatentowanej technologii, której twórcą i właścicielem jest konsorcjum Uniwersytetu w Gandawie i firma ProDigest).

Dzięki projektowi pt. „Centrum żywności i żywienia - modernizacja kampusu SGGW w celu stworzenia Centrum Badawczo-Rozwojowego Żywności i Żywienia (CŻiŻ)" współfinansowanego przez Unię Europejską ze środków Europejskiego Funduszu Rozwoju Regionalnego w ramach Regionalnego Programu Operacyjnego Województwa Mazowieckiego na lata 2014-2020 [Nr projektu RPMA.01.01.00-14-8276/17] Instytut Nauk o Żywieniu Człowieka wzbogacił się o sztuczny przewód pokarmowy w technologii SHIME.

Słowa kluczowe: sztuczny przewód pokarmowy, mikrobiom, jelito cienkie, jelito grube.

INTRODUCTION

The human microbiome, especially the gut microflora, has been a subject of interest to many scientists around the world for many years. Its impact on human is of high significance and the currently available scientific literature confirms that microbiome quality influences immune system, namely the formation of immunity [15, 25], it supports the digestion processes which have an impact on formation of metabolites like vitamins and hormones, [7] but also neurotransmitters like serotonin [3, 19]. Furthermore, there is a constant influx of new information and research about the abnormalities of the microbiota, usually defined as dysbiosis. Dysbiosis is a known cause of obesity, inflammation and even psychological disorders [3, 25]. Gut microbiota consists of very large numbers of microbes, approximately 0.9×10^{11} microbes/g of faeces. It is estimated that the proportion of microbes to the entire amount of cells in the human organism is 1,3:1 [14, 25].

The existing models of the artificial gastrointestinal tract, described in the scientific literature, such as Dynamic Gastric Model (DGM), Human Gastric Simulator (HGS),

TNO Gastro-Intestinal Model (TIM), DIDGI System, IViDiS [4, 10, 17, 18, 23] are considered as an important support for nutritional research. However, due to the complicated structure and function of the digestive tract, both in humans and other mammals it is nearly impossible to replace one hundred per cent of studies carried out *in vivo* models (i.e. studies carried out with the use of animal models) using these newly developed technologies. The problem that the creators of such systems had to face was often the reconciliation of the complexity of that system, reflecting the conditions that naturally prevail in the gastrointestinal tract as faithfully as possible, with its universal use for research on digestion or other physiological processes.

The SHIME®2 system consists of sections corresponding to the digestive system organs, i.e. stomach, small intestine and colon (ascending, transverse and descending) (Fig. 1, 2 and 3). The device is made of glass reactors placed in a double water jacket. This enables continuous and constant heating by a software-controlled water bath. Because of this configuration, it is possible to maintain a constant temperature of 37°C in the whole model. The motor functions are ensured by a number of peristaltic pumps, which are responsible for both the intestinal passage and the supply of an appropriate volume of solutions containing digestive enzymes and other necessary components. These regulate digestive functions and ensure the stability of the entire system. The system also requires feeding using the medium that ensures the optimal supply of macro-and micronutrients [5, 11]. The pumps support the process of expelling products produced in the "intestines". In order to the digestion process run smoothly at the same time maintaining the appropriate pH, the fluid in each section of the digestive tract is mixed with magnetic stirrers. This solution allows establishing a specific pH profile for each section of the gastrointestinal tract. As during normal physiological process, during digestion in SHIME® the pH in the small intestine fluctuates around the neutral pH – slightly alkaline, while in the ascending colon the pH fluctuates around 5.6 to 5.9 in the transverse part of the large intestine – in between 6.1 and 6.4, reaching a range between 6.6 and 6.9 in the descending part [8]. An important feature of the system is keeping it in an oxygen-free atmosphere. This is achieved by providing an inert gas (Nitrogen N2).

The SHIME® system has been designed to study the human gut microbiota. The quality, microbial profile and conditions of which have a fundamental effect on the human body. The research involves regulating the absorption of nutrients and other compounds formed during digestion or fermentation [25]. The system is inhabited by natural human microbiota. The *in vivo* microbiological composition of faeces differs from that of the colon. The colon has a high specificity, it acts as a bioreactor in which the transformation of the intestinal microbiota takes place. Therefore, starting an experiment using an artificial model requires time to adapt the faecal microbiome in different parts of the system. Assuming that the adult intestinal transit time is about 48 hours, the time to colonise the entire system under these conditions will oscillate in the range of 10-20 days. During this period, the inoculated faecal microbiome will adapt to the conditions in the bioreactors [13, 23]. The adaptation period is adjusted to the composition of the microbiome used, but can also be
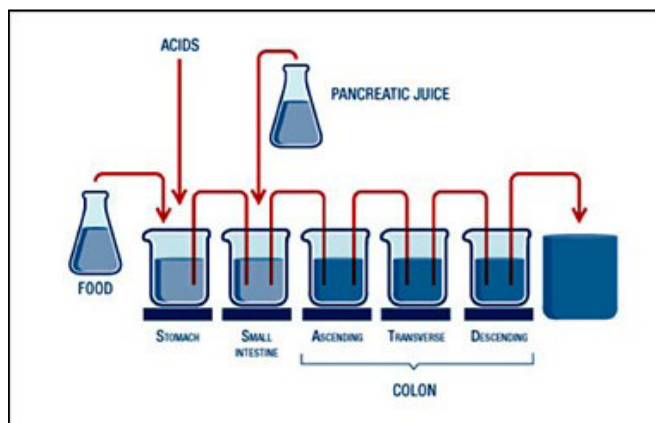

Fig. 1.   The diagram of the reactors system. Source: Pro-Digest Gastrointestinal expertise
Rys. 1.   Schemat układu reaktorów. Źródło: ProDigest Gastrointestinal Expertise

Fig. 2&3.   System of reactors. Photograph provided. Source: Own study
Rys. 2 i 3.   System reaktorów. Źródło: Opracowanie własne

controlled by the scientist depending on the research needs. As mentioned, the system is inhabited by the faecal microbiome collected from one person. This solution causes a very high convergence of natural conditions with those prevailing in the model conditions, which has been confirmed by other studies [1, 22]. The results indicate that the metabolism of microorganisms in the intestines e.g. selected polyphenolic compounds (isoxanthohumol, daidzein, catechins, etc.) is dependent on a microbial makeup of a person. Therefore, there is no pooling of samples from multiple individuals. This helps to maintain the microbiological phenotype of the person from whom the biological material was collected and allows for long-term research in the metabolism of specific polyphenols [2, 12].

Studies optimising the operation of the SHIME® system [20] have found that the selection of a specific inoculum enables the proper colonisation of the large intestine mucosa. The mucosa microbiome is that part of the gut microbial ecosystem that is able to colonise the mucus covering the intestinal epithelium [8]. Due to the proximity of host epithelial cells, it is believed that the mucosal microbiome has a greater intrinsic power to modulate intestinal health as well as human health overall [21]. Additionally, it is known that the mucosal microbiome differs significantly from the intestinal microbiome in composition. Interestingly, the presence of important mucosal colonizers, such as *Faecalibacterium prausnitzii*, seems to be negatively correlated with the occurrence [24] and postoperative recurrence of Lesnikowski-Crohn's disease in the ileum [16]. Given the difficult access to the mucosal environment, the development of gut simulators that closely mimic mucosal microbial colonisation is considered a strong asset in gaining a better understanding of host-microbial interactions. An artificial model of the gastrointestinal tract such as SHIME® is a highly standardised system. The scientist uses software to control many parameters of the processes that are running in this system, this creates the possibility of obtaining highly reproducible results [5, 23]. It is a significant and important element of experiments that allows comparison between different products such as new probiotics, prebiotics, new plant extracts or potential drug ingredients [8].

OPERATION

According to the assumptions of ProDigest, a typical SHIME® simulator experiment involves four stages:

- stabilization period (2 weeks) – allowing the inoculated faecal microorganisms to adapt to the environmental conditions in the relevant regions of the colon;

- basic period (2 weeks) – during which the parameters in the reactors are equalised, this enables the basic parameters (output - control) to be measured in the experiment

- the period of the experiment (2–4 weeks) – the effect of the substances used on the community of microorganisms in the gastrointestinal and gas-intestinal tract is examined;

- a wash period (2 weeks) – to determine how long the changes caused by the test substance can be measured in the absence of the substance itself.

The scheme of action adopted in this way makes it possible to study the activity and stability of probiotics and prebiotics during gastrointestinal transport, bacterial conversion of bioactive food ingredients (e.g. phytoestrogens), metabolism of pharmaceutical ingredients, the effectiveness of delivery systems targeted to the colon, and the conversion and biological (in) activation of food and/or ingested environmental pollutants [23].

Due to the modularity of the SHIME® system, short-term experiments are also possible. One-week long experiments can be performed, especially in the context of monitoring the initial stages of microbial colonisation on the mucosal surface.

USES

The experiment carried out by [20] confirmed the possibility of colonisation within 5 days of the intestinal mucosa, excluding the luminal microbiome. The modular configuration of the system also allows for placebo-controlled studies, e.g. when comparing different prebiotics and probiotics [6] or when the microbiome phenotype producing a bioactive metabolite is compared with a non-producing phenotype [12]. Furthermore, it is possible to study the interindividual variability in the microbiome profile by dividing the system into several parallel sections of the colon, each of which is inoculated with faecal microbiota from different donors.

The application of the SHIME® artificial gastrointestinal model is an *in vitro* model that integrates the entire gastrointestinal transit into a single system makes it possible to study the digestibility of prebiotic substrates and their subsequent fermentation in the colon. Additionally, measuring the survival of pathogens or probiotics in the upper gastrointestinal tract before they reach the colon environment is possible. Thanks to the modularity of the system, to conduct both short-term experiments appropriately, e.g. the colonisation capacity of microorganisms, and long-term experiments gives the possibility of responding to the adaptation of the microbial ecosystem to the changing environmental conditions specified by the system operator. Another interesting application, which is a strong advantage of the SHIME® system, is the possibility of extending it to M-SHIME – enabling the assessment of mucosal microbiota [20, 23].

By using the SHIME® model, the production of SCFA or ammonia can be preliminarily assessed on the basis of the amount of NaOH or HCl, which are supplemented by pH regulators to maintain appropriate pH values in the corresponding vessels of the colon. This system also allows the microbiome composition to be assessed in the relevant vessels in the colon. For further determinations, DGGE (Denaturing Gradient Gel Electrophoresis) is usually used, which can be supplemented by quantitative analysis by q-PCR or high-throughput analysis at the phylogenetic level with next-generation sequencing [9].

SUMMARY

The artificial digestive tract SHIME® technology (by ProDigest) will allow the Institute of Human Nutrition Sciences to expand its research activities with the new scientific directions concerning microbiota. It can also contribute to the development and creation of new products or the improvement of existing formulations and the protection of the gastrointestinal tract, which play a vital role in the human body and impacts human health greatly.

PODSUMOWANIE

Podsumowując, sztuczny przewód pokarmowy w technologii SHIME® (firmy ProDigest) pozwoli Instytutowi Nauk o Żywieniu Człowieka rozszerzyć swoją działalność badawczą o nowe kierunki badań dotyczące, przede wszystkim mikrobioty. Może również przyczynić się do opracowywania nowych produktów lub ulepszania istniejących receptur oraz ochrony przewodu pokarmowego – tak ważnej części ciała odpowiedzialnej za zdrowie i prawidłowe funkcjonowanie organizmu ludzkiego.

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