Developing an In-Vitro Dynamic Model of the Stomach and Small Intestine for Milk Products with Rheological Monitoring

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ABSTRACT

The aim of this study is to give an overview of main factors relevant for the elaboration of a dynamic *in-vitro* model of a human stomach and small intestine. A special focus is given in the article to the flow and deformation of matter occurring in the gastrointestinal tract, as well as the biomechanics which is relevant for the design of a new *in-vitro* model. This article presents a new *in-vitro* model as a process diagram.

INTRODUCTION

The first step for the food in the digestive system is the oral cavity. The mixture then passes through the esophagus to the stomach. The stomach serves the function of mechanical processing (churning) mixing with secreted hydrochloric acid and hydrolytic enzymes, pH change and pre-digestion of the food. The small intestine serves the following purposes:

• bulk transit of digesta from the stomach to the large intestine;

• digestive reactions such as hydrolysis of proteins, lipids and starch by the pancreatic proteolytic enzymes, lipase and α-amylase, respectively.

• convection and diffusion of the nutrients, enzymes and biosurfactants.

• absorption of water across the wall of the small intestine. Water moves into or out of the intestine until the osmotic pressure of the intestinal contents equals that of the plasma.[1]

The large intestine's (colon) function is: to absorb remaining water from the by-then indigestible food matter, the microbial fermentation of undigested food, the storage of the unusable food matter (wastes) and then elimination the wastes from the body.[2]

Fig. 1 shows a diagram representing the organs to be simulated in the *in-vitro* system with the stomach and the duodenum as the main compartments.

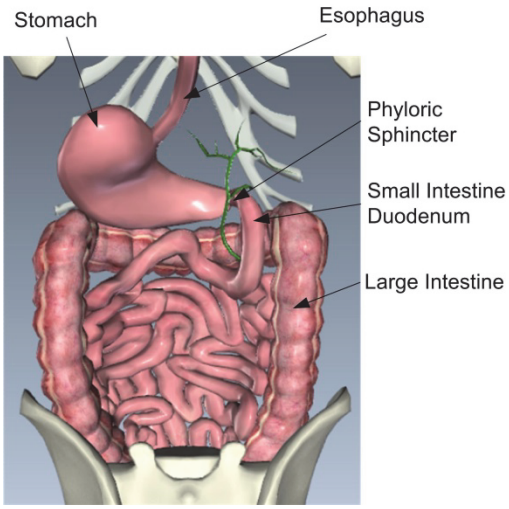

Figure 1. Drawing of a human digestive system - abdominal region, posterior view. Drawing made in BioDigitalHuman 2014.

The attempts creating *in-vitro* models can be divided into two broad categories: 1). *static models*, where the products of digestion do not have a forced dynamic motion and do not mimic physical processes such as shear, mixing and so on, and 2). *dynamic models* which include physical and mechanical processes and temporal changes in luminal conditions to mimic *in-vivo* conditions[3]. The latter are particularly useful for the change in digesta over time (for example, particle size and viscosity) and take into account some temporal effects not considered otherwise (mixing, diffusion, formation of colloidal phases).[3]

The main difference between a static and a dynamic digestion model from a chemical point of view, is whether there is removal of digestion products or not. In dynamic models there will be a (continuous) removal of digestion products. Whereas, in a closed, static system, the relationship between the different components will be determined when chemical equilibrium is reached.

There is a continuous transport (active and passive) of the degradation products during the digestion of food in the intestine. These hydrolysed products are absorbed and collected in the circulatory system of blood vessels and lymphe which drain into ductus thoracicus before it enters the blood circulatory system.

The compounds are in particular amino acids and small peptides from proteins, fatty acids and monoglycerides from the hydrolysis of lipids, mainly triglycerides and some phospholipids. Complex carbohydrates are hydrolyzed to simple monosaccharides; they are readily absorbed.

Lipoproteins are mandatory for the transport of lipids in the aqueous phase.

From the liver and pancreas, hydrolytical enzymes and emulgating substances (bile salts) are secreted through papilla Vateri. In addition, a great number of enzymes, hormones and other signaling substances are secreted from the intestinal cell wall.

Understanding the flows of fluids, mixing and diffusion of nutrients through structured foods in the small intestine is required to achieve good *in-vitro-in-vivo* correlations.[2]

Since physiological differences exist among people according to age, sex, metabolism, alimentary habits, etc., the design of an *in-vitro* model should consider operating the system at different shear stresses, shear rates and Reynolds numbers.

This work focuses on gathering literature information needed to develop a new dynamic *in-vitro* system. This work will also present a tentative *in-vitro* model at process diagram level of the stomach and small intestine (duodenal region). Once the process diagram is established, a new phase of design and prototyping will begin following this article.

FUNCTIONS OF THE STOMACH

The stomach is located in the upper part of the abdomen immediately below the diaphragm. The shape can be greatly modified during digestion, such that no form can be described as typical. [4] The functions of the stomach are:

- Storage and pre-digestion of the food for processing within the stomach itself or in the duodenum and lower parts of the gastrointestinal (GI) tract. As food enters, the stomach expands. This is facilitated by relaxation of its proximal part and is referred to as gastric accommodation.
- Mixing of food with gastric secretions containing water, hydrochloric acid, mucus and enzymes (pepsin and gastric lipase). The mixing is facilitated by contractions in the stomach wall turning the food into a semifluid mixture called chyme.
- Controlled emptying and transferring of the chyme into the small intestine for further processing.

Anatomy of the stomach and the cell wall

The GI cellular wall comprises several layers including longitudinal and circular smooth muscle layers responsible for motor

functions and the innermost mucosa containing several cell types depending on the section of the system (stomach vs duodenum). Mucous cells (also described as goblet cells or single-cell mucous glands) are found in the entire GI tract and secrete mucus to lubricate and protect the epithelial surface against digestive enzymes. The stomach mucosa also comprises a large numbers of acid- and pepsinogen-secreting glands (oxyntic glands).

The mean thickness of the stomach walls is around 2.0 mm. [5]

Mechanical properties of the stomach

The mechanical properties of the cellular wall of the stomach are highly specific and depend on the site and it is greatly influenced by food, environmental factors and age.

In general the wall of the stomach is stronger and stiffer longitudinally than circumferentially, the maximum loads the tissue can withstand are 1691±340 mN cm^{-1} and 1175±181 mN cm^{-1} respectively. [4] It has been also demonstrated that the tissue has nonlinear viscoelastic properties.[4]

The axes of anisotropy in the stomach walls are oriented along the direction of the longitudinal and circular smooth muscle fibres. It does not resist compression forces and possesses zero flexural rigidity.[4]

The tensile strength of the stomach walls, transversal specimens was found to be of around 0.5 ± 0.16 MPa, and for longitudinal specimens around 0.6 ± 0.21 MPa. [5]

Biomechanics in the stomach - Transport and shearing of food

Motor propulsive activity in the stomach originates in the upper part of the organ. Three types of mechanical waves can be observed. The first two types are small isolated contraction waves and peristaltic waves that slowly move from the point of origin down towards the pyloric splinter. These types of contractions produce slight and deep indentations in the wall and serve as mixing, crushing and pumping mechanisms for the gastric contents. The third type of wave is non propagating in nature and is a result of the tonic simultaneous contraction of all muscle layers that are normally superimposed on small and peristaltic contractions.[6]

The peristaltic movements in the stomach are similar to the ones produced by a peristaltic pump.[7] Peristaltic movements originate from the stomach wall and spread toward the antrum, mixing and forcing the antral contents towards the pylorus.[8]

The stomach transforms the food contents into a multiphase slurry (aqueous solutions, fats and solids) called chyme. The particle size of the food emptying through the pylorus is less than 1 to 2 mm during the fed state. [9] The viscosities of the gastric contents have a range 0.01 – 2 Pa s with a density similar to water. [10, 11]

FUNCTIONS OF THE SMALL INTESTINE

The small intestine is important in degrading, absorbing and transporting (active and passive) the digested food nutrients to the circulatory system for catabolic and anabolic reactions in providing energy, in cellular resynthesis etc. When food is ingested signalling molecules (CCK, etc.) immediately initiate the secretion of digestive enzymes from the pancreas and bile salts from the gallbladder into the upper part of the duodenum.

The main functions are:

- to hydrolyse proteins into peptides and amino acids by the pancreatic proteolytic enzymes (trypsin, chymotrypsin, eleastase etc)
- to hydrolyse lipids/triglycerol into monoglycerol and free fatty acids by the pancreatic lipase/colipase and bile salts
- to hydrolyse starch by the pancreatic α-amylase

- to recirculate the bile salts to the liver
- to transport undigestible part of the food (carbohydrates - fibers) to the large intestine

Anatomy of the small intestine

The duodenum is the first section of the small intestine. The small intestine is a long cylindrical tube that extends from the stomach to the caecum of the colon. In the abdomen most of the intestine is loosely suspended by the mesentery and it is looped upon itself.[7]

The diameter of the duodenum is typically 25-35 mm.

The mean thickness of the small intestinal wall is around 1 mm. [5]

The duodenum can be regarded as a soft cylindrical shell formed of identical overlapping myogenic functional units (loci).

The intestinal wall is a biological composite formed of four layers: mucosa, submucosa, muscular and serosa. The mucosa is the innermost layer and its primary function is to digest and absorb nutrients.

The muscle coat is made of two smooth muscle layers, a thick (inner) layer of circumferentially oriented smooth muscle cells and a thin (outer) layer of longitudinally oriented muscle elements.

The serosa is composed of a thin sheet of epithelial cells and connective tissue.[12]

Mechanical properties of the small intestine

The submucosa consists mainly of connective tissue and serves a purely mechanical function.[12]

The stable organization of the walls of the intestine allows the intestine to undergo reversible changes in length and diameter, offering remarkable properties of stiffness and elasticity. The tissue possesses nonlinear viscoelastic properties which are uniform along the cylindrical shell. This shell is supported by intraluminal pressure.[12]

The tensile strength of the bowel walls, transversal specimens was found to be of around 0.6 ± 0.17 MPa, and for longitudinal specimens around 0.18 ± 0.3 MPa.[5]

The mechanical strength of the intestinal wall is conditioned by the submucosa and muscular layers, while the serosa and mucosa shows no significant strength. Mechanical stability of the gut wall, its strength and ability to resist intensive deformations of long durations, is conditioned by the submucosa.[5] Consequently, a good preservation of these layers is important for the well-functioning of an intestine inserted into the *in-vitro* model.

Biomechanics in the small intestine - Transport and shearing of food

The motility in the small intestine facilitates the digestion and absorption of food. This motility is responsible for the flows patterns and it can be divided into segmentation contractions and propulsive contractions.[13]

It is known that the fluid mechanics in the small intestine affects the absorption of nutrients by affecting the delivery and movements of molecules to the wall of the intestine. The fluid mechanics affect surface renewal and enzyme substrate interactions. The fluid mechanics are a consequence of both the fluid properties and the pumping mechanisms.[7]

The characteristic Reynolds number of the overall flow in the small intestine are in the order of 10^{-3} – 10 with viscosities of 0.001 Pa s for water and up to 6 Pa s for digesta.[14] A flow velocity of 0.01 m min^{-1} is given by Guyton[13] in the small intestine and a diameter of about 0.03 m given by Ganong.[15] The low Reynolds numbers would not provide a good mixing in a cylindrical pipe. However the flows in the small intestine are generated by segmentation and peristalsis and it is thought that segmentation is significant in mixing.[7]

Peristaltic movements propel the digesta and segmentation motion mainly mixes the digesta. Peristalsis waves move at 0.5 to 2 cm s^{-1} and are normally weak contractions which die out after 5 cm. [13]

Dynamic modelling of the stomach, rheological aspects

The proposed dynamic *in-vitro* system covers the digestion process in the stomach. The system is described as follows in reference to Fig. 2: The stomach is represented by the items (1) to (7). Item (1) is a syringe or syringes that represents the dosing mechanism for the gastric fluids (e.g. gastric liposomes, electrolytes, enzymes, etc.). The syringe(s) can be controlled by a syringe pump. Item (2) is a mixer viscometer that aims to measure the viscosity of the gastric fluids while the digestion process occurs. The viscometer (2) will also act as a stirring mechanism to provide a good gastric mixing. The mixer viscometer (2) provides a controllable and measurable amount of shear rates (i.e. an average shear rate) in the fluids during the digestive period. Item (3) represents a titrator which can be used to add different amounts of HCl depending on the buffering capacity of the food. Item (4) is a peristaltic pump that replicates the shearing occurring during peristalsis in the stomach. The peristaltic pump (4) is connected to an acid resistant hose (5) and it can pump continuously the gastric mixture in direction (F1). Before adding new fluids, the pump should run in opposite direction (F2) to allow emptying the hose for an accurate dosing and mixing of the fluids. Item (6) is a jacket heater (e.g. Peltier) which provides 37 °C to the fluid. Item (7) is the cup of the viscometer which works as the mixing chamber. Item (8) represents the connectivity of the viscometer (2) and the peristaltic pump (4) to a PC which allow the remote control and monitoring of the digestion process.

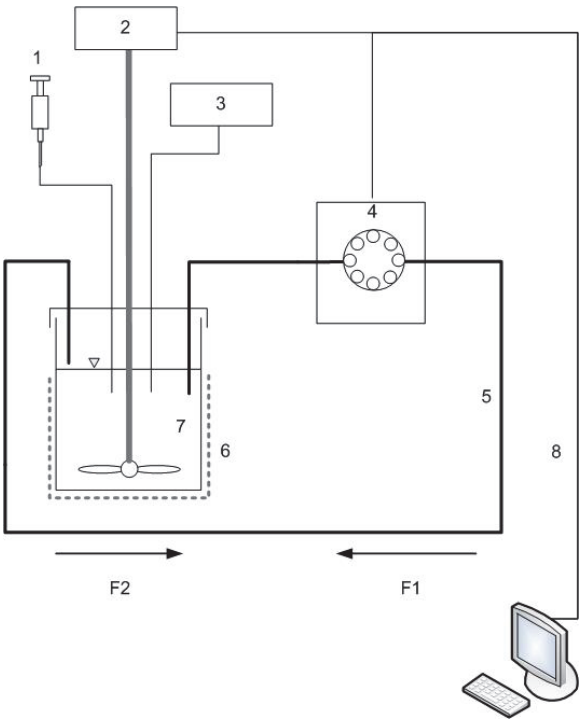

Figure 2. Proposed dynamic *in-vitro* model during digestion in the stomach – Process diagram.

Dynamic modelling of the small intestine, rheological aspects

The proposed *in-vitro* dynamic system for the digestion and subsequent absorption processes occurring in the small intestine is represented in Fig. 3.

The small intestine is represented by the items (1) to (11). Item (1) is a syringe or syringes used to add duodenal fluids (e.g. duodenal enzymes, bile salts, simulated duodenal fluid - solution of salts, etc.). The viscometer (2) is used to provide a continuous mixing of the fluids while monitoring the viscosity of the digested contents, the titrator (3) is used to monitor pH and gradually increase pH to mimic duodenal conditions by either sodium hydroxide (NaOH) or sodium bicarbonate ($NaHCO_3$). The peristaltic pump (4) is connected to an acid resistant hose (5) to pump continuously the intestinal fluids in direction (F1). The pump can run in opposite direction (F2) to empty the hose before adding the intestinal fluids. Item (8)

represents a porous media like membrane (e.g. dialysis tubing) or the intestine from a recently slaughtered organism (e.g. mouse, fish, pig, etc.). The porous media (8) can be located inside of a container or inside a larger diameter hose (9) where a fluid (19) having similar osmotic pressure as the body can be pumped through a peristaltic pump (11) in direction (F3) in an annular gap. A computer can be connected to the viscometer (2) and to the peristaltic pump (4 and 11) to measure and control the system.

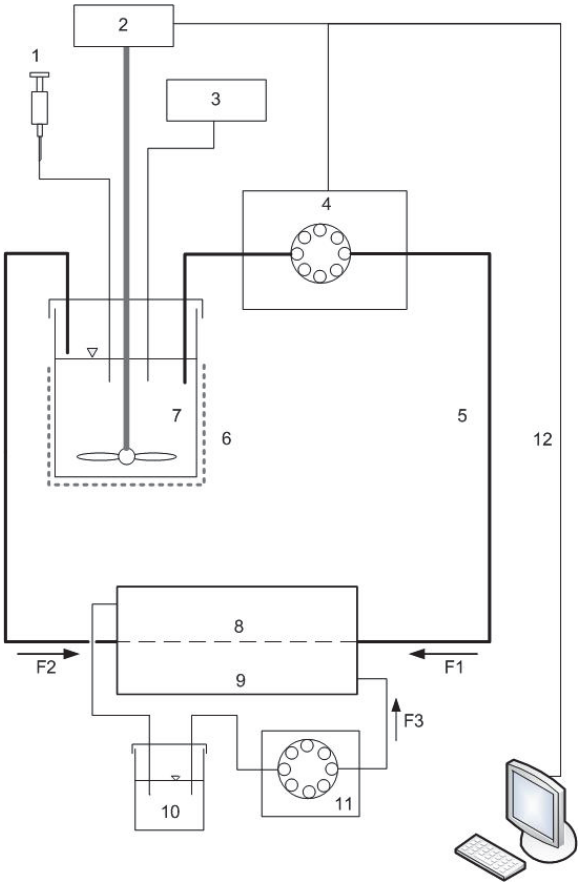

Figure 3. Proposed dynamic *in-vitro* model during absorption in the small intestine – Process diagram.

Proposed dynamic in-vitro model. Rheological aspects

Fig. 4 is a combination of Fig. 2 and Fig. 3. As it has been described previously[2] rheological aspects and the fluid dynamics in the system are of great importance in the digestive system.

By estimating the shear rates in the system, one could quantify the severity of the mechanical treatment in the system. The biomechanics in the stomach and small intestine are replicated though the mechanical action of the mixer viscometer and the peristaltic pump.

Without estimating the shear rates and the fluid mechanics inside the *in-vitro* system, the system would become a black box concerning physical action, leading to a trial and error at best when comparing with *in-vivo* systems. By estimating the shear rates in the *in-vitro* system, one could distinguish the effects that the fluid mechanics have over the digestive process.

As it has been explained previously, the proposed *in-vitro* system consists of three main parts; the mixing vessel, the peristaltic pump and the flow channels which are built of silicone hose or circular membrane.

The viscosity of the fluid passing through the intestine is of special importance. The shear rate will generally vary from zero to some maximum value at any cross-section. The maximum value will normally be located at the wall.

The flow in the mixing vessel is complex. Shear rates depends on position from the centre of the rotating shaft, for this reason mixer viscometers uses an analogue radius approach to obtain a constant which is used to obtain an average shear rate from the angular speeds of the viscometers. [16, 17]

Another alternative to mixer viscometers (2) is the use of a magnetic stirrer which can be placed at the vessel (7). A magnetic stirrer has a moving magnet (mixing element) rotating close to the bottom of the vessel. The magnetic stirrer will touch the bottom, and the small clearances will cause larger shear rates.

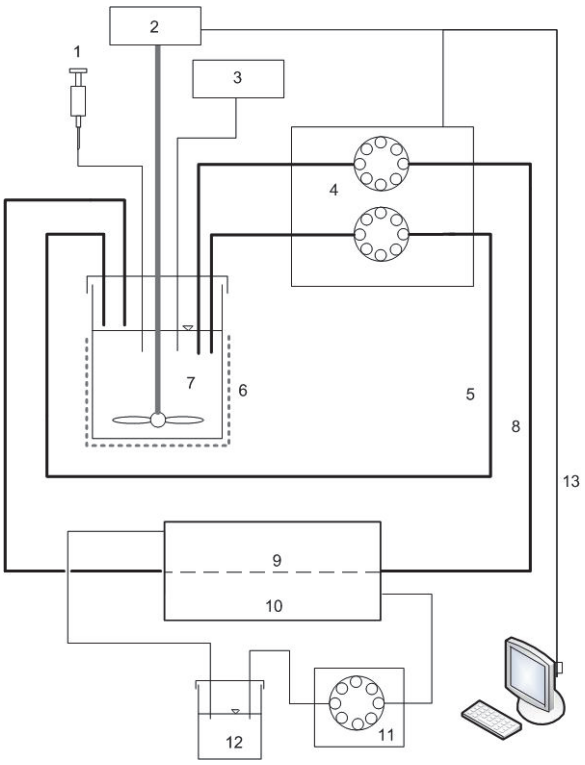

Figure 4. Full configuration of the proposed dynamic *in-vitro* model – Process diagram.

The shear rate distribution in the small mixing vessel can be determined from CFD simulations modelling the small magnetic stirrer element. This has previously been done successfully by modelling in COMSOL Multiphysics[18]. A similar approach can be used to obtain an average shear rate and shear rate distribution for different mixer speeds. Calculations must be run for a range of viscosities.

The fluid will flow from the mixing vessel (7) through flexible cylindrical hoses (5, 8) passing through a peristaltic pump (4). The flow through the peristaltic pump (4) is a type of combined squeeze flow and piston type flow and is probably the most difficult flow situation to describe with regard to shear rates and shear stresses. The flow through the peristaltic pump consists of a compression part where the hose is deformed, then a piston type flow condition where the deformation moves axially along the hose, and finally a decompression phase where the hose again expands to its original circular cross section. Here also CFD modelling may be required to reveal what is happening in this part of the equipment. There are a number of CFD programs like COMSOL Multiphysics, CFX, Fluent or SolidWorks which are able to do this challenging task.

Future work

Under the bio-mechanical point of view, to assess how well the fluid dynamics of the body is replicated into the *in-vitro* system, an *in-vitro* – *in-vivo* comparison will be needed. Also, it would be of interest developing a mathematical model to estimate the intensity or severity of the shearing environment at different running conditions. For example, obtaining an average shear rate from the *in-vitro* system.

Another useful estimation would be the local values of shear rates, for example the maximum and average shear rates in the mixer viscometer, at the peristaltic pump and to obtain dimensionless numbers like Reynolds number at the membrane. It would be also of interest to determine dimensionless numbers related to diffusion mass transfer like Schmidt number (Sc), the Sherwood number (Sh) and the Bodenstein number (Bo); Bo = Re Sc.

CONCLUSIONS

The proposed dynamic *in-vitro* model of the stomach and small intestine includes the main aspects of an *in-vivo* system like the biomechanics, the rheology of the fluids and the capabilities for the overall mass transport. It is intended that the new system will make the user able to estimate the average and maximum shear rates to quantify the severity of the simulated biomechanics. The system will also allow simulating the mass transport. However, from the latter many challenges will remain to be solved like to simulate the active transport happening in the gastrointestinal tract.

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