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Microfluidic Digestive Systems for Drug Analysis

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Chapter 1
General Introduction

The work described in this thesis has been multidisciplinary from the very beginning, incorporating topics at the interface of physics, chemistry, biology, and related areas such as the pharmaceutical and nutritional sciences. Multidisciplinarity is both a blessing and a curse: it ensures that the necessary elements of the adjacent scientific fields are combined into one 'new' scientific field, bringing the best of these worlds together and leading to new insights by synergy. On the other hand, it may also lead some to think that this new field is derivative in itself, thereby requiring solid arguments of validity for practical use in the non-academic world – with the added disadvantage that one cannot have expertise in each and every single one of the constituent fields of research. The work described in this thesis addresses its relevance and validity to the applied life sciences throughout. It is presented as a valuable contribution to the life sciences and related industries, as well as a more fundamental study of the processes occurring at the microscale. The work in this thesis comprises the fields of microfluidics in general, and more specifically the newly emerged field of organs-on-chips. The focus lies on the emulation of processes occurring in the human gastrointestinal tract, and the monitoring thereof. It therefore required a combination of some biology (touching upon the fields of pharmacy and toxicology), some physics, some chemistry, and some engineering, with an emphasis on the analytical aspects. This chapter aims to give a brief introduction into relevant aspects of these respective fields, which in no way does justice to either one of them but aims to help understand the principles and choices underlying the organ-on-a-chip work in this thesis. The aims and scope of this thesis are included at the end of this chapter.

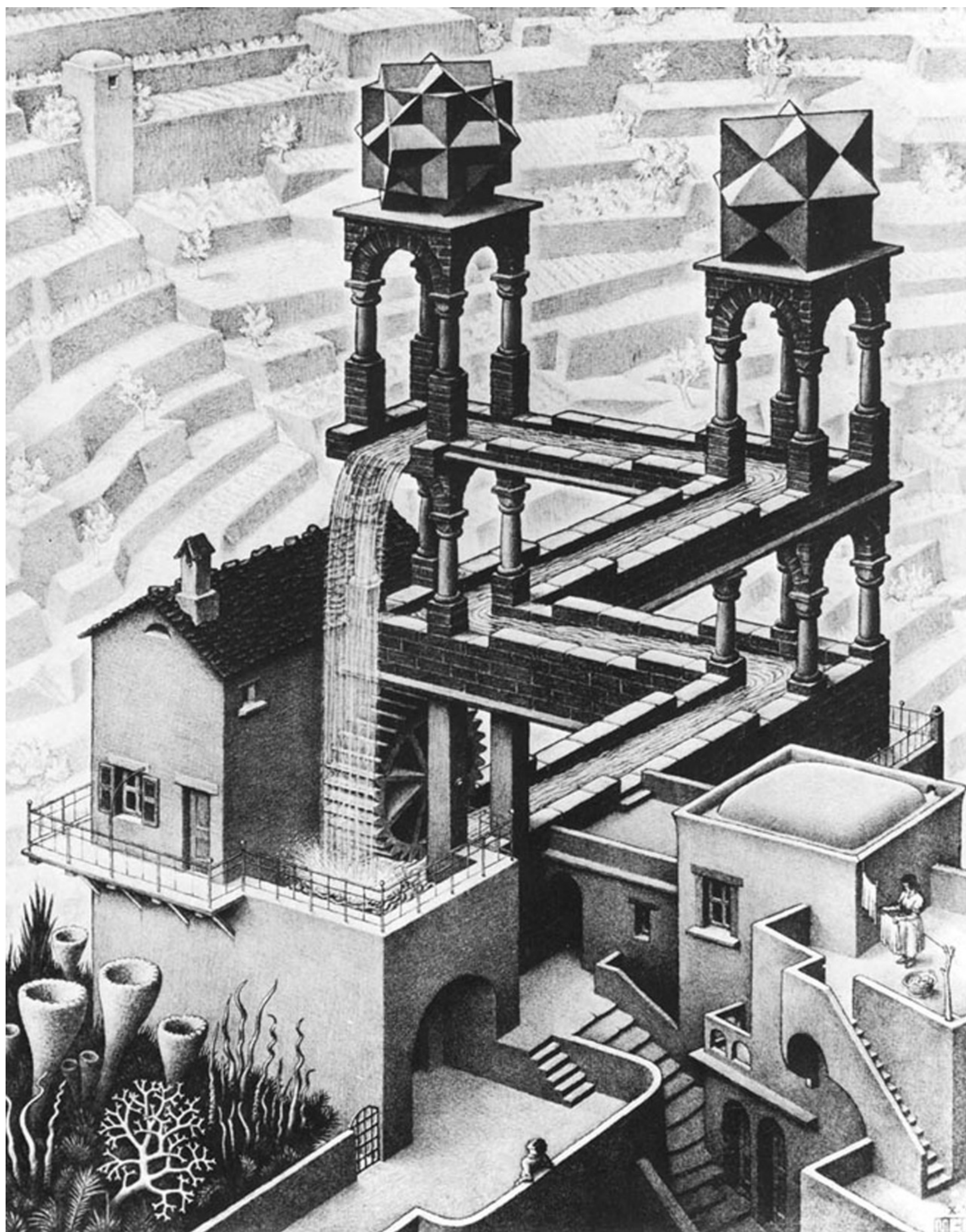

Figure 1-1 ‘Waterfall’, M.C. Escher, 1961. The Hagen-Poiseuille equation states that a pressure difference is necessary for flow.

1.1. Some Biology

Minute volumes down to 10^{-18} L can be manipulated using microfluidic technologies, with several applications including miniaturized total analysis systems.[1,2] Organ-on-a-chip technology is a relatively new addition to the field of microfluidics, in which models of human tissues or organs are created on the microscale.[3] Interconnected microchannels were first described as useful tools for miniaturized chemical analysis systems, and these systems have been used for a plethora of chemical applications before finding applications as miniaturized organ models.[1–3] Various organs have been recreated as organs-on-chips, mostly starting with empty microfabricated channels into which biological components such as cells are grown. This has been termed the 'bottom-up' organ-on-a-chip approach, as opposed to the 'top-down' approach where pieces of living tissue are kept alive and studied *ex vivo*.[4] One of the most studied organs is the gut-on-a-chip – a miniaturized model of the human gastrointestinal (GI) tract, in order to study both physiological and pathophysiological processes.[5] In the pharmaceutical field, it is envisioned that these gut-on-a-chip models will become reliable enough to serve as models of the GI tract during drug development. This would have several advantages: a reduction in the need for animal trials, which would require less animal testing and have the added benefit that the potential new drug is tested directly onto human tissue, instead of animal tissue, as it is known that there are many interspecies differences. Besides this, the use of organs-on-chips may also lead to a lower consumption of valuable materials and faster processing times, saving time and money in the long and costly process of drug development.[6,7] The gut-on-a-chip systems that are described in this thesis focus on the aspects of the GI tract that are relevant to the pharmaceutical, toxicological, and nutritional fields: (1) Enzymatic digestion of compounds by digestive juices; and (2) Absorption of compounds and their possible metabolites by the intestinal epithelium. To a lesser extent it includes some interactions between drugs, food, digestive juices, and epithelium; and possible intracellular metabolism of ingested compounds in intestinal cells was not included in this work. Gut-on-a-chip devices can be considered miniaturized version of the conventional transwells, in which two compartments are separated by a porous membrane. Intestinal cells are cultured on top of the porous membrane, creating a barrier separation into two mL-sized compartments: the apical compartment on top, which represents the gut lumen, and the basolateral compartment on the bottom, representing the body. Early gut-on-a-chip devices incorporated the same basic elements into a miniaturized, flow-through system.[8,9] Two features are changed by doing so: transport processes are different, because a constant concentration gradient can be applied

across the barrier in flowing models (see below); and the flow itself induces changes in the cell morphology and functioning, including the supposedly faster differentiation of intestinal Caco-2 cells into other (non-enterocyte) intestinal cell types and the formation of three-dimensional villus-like structures.[10,11] It is not yet fully understood why this happens under flow, but it has been hypothesized that *in vivo*-like shear stress levels contribute to these morphological and metabolic changes. Shear stress levels may vary greatly in the GI tract, but they have been estimated at 0.02 – 0.8 dyn/cm^2 *in vivo*.[12,13]

1.2. Some Physics

When scaling down to the microscale, physical parameters scale down at different ratios, and this may lead to entirely different phenomena when compared to larger-scale situations. One important parameter is the surface to volume ratio.[14] The surface area of an object (*e.g.* a sphere) is determined by a quadratic equation, whereas its volume is determined by a cubic equation:

$$\frac{surface\ area}{volume} = \frac{4\pi r^2}{\frac{4}{3}\pi r^3} = \frac{3}{r} \tag{1-1}$$

This equation is true for spheres, a three-dimensional object with characteristic dimension *r*. Generalization of this equation to any regular three-dimensional object with characteristic length, *l*, (*i.e.* the radius of a sphere, the side of a cube, etc.) states the surface-to-volume ratio as follows:

$$\frac{surface}{volume} \propto l^{-1} \tag{1-2}$$

Since the surface-to-volume ratio is inversely proportional to the characteristic length, scaling down to the microscale leads to an increase of the surface area relative to the volume. This lies at the basis of a number of physical and chemical phenomena that will be described in more detail. Flows of liquid in microfluidic systems are generally laminar, resulting in very predictable, defined flow patterns. This makes the design of microchannels and modeling of flows therein easier. The most common way to induce flow is to apply pressure to it, causing a flow from high- to low-pressure segments. Figure 1-1 shows a paradoxical flow system by Dutch graphic artist M.C. Escher, where gravity is the implied pressure source – but as this system is displayed as a circuit, no separate gravity-induced high- or low-pressure segments can exist. Careful design of flow circuits is thus essential. The liquid flows described in this work are pressure-driven flows, which can be described by the Hagen-Poiseuille equation[14]:

$$Q = \frac{\pi r^4}{8\eta L}\Delta p \qquad (1\text{-}3)$$

This equation describes the volumetric flow rate, Q, through a circular duct with radius, r (different versions are available for other duct cross-sections), as a function of the applied pressure difference, Δp. Other factors are the dynamic viscosity, η, and duct length, L. Although not described in more detail, it is important to note that it includes the characteristic length to the power of four. This means that when this equation is rearranged to express the required pressure for a certain flow rate, relatively high pressures are required to cause flows inside micrometer-sized channels.

The laminar-flow behavior in microfluidic channels leads to impaired mixing of species as no mixing by simply stirring is possible. Mixing processes therefore solely depend on diffusion of species, as described by Fick's first law of diffusion[15]:

$$Flux \equiv J = -D\frac{dc}{dx} \qquad (1\text{-}4)$$

In this equation, the flux of species, J ($mol/m^2/s$), is dependent on the diffusion coefficient, D (m^2/s), and the concentration gradient dc/dx (mol/m^3). The distance, L (m), traveled by a freely diffusing particle in a time, t (s), is described by the Einstein-Smoluchowski equation[15]:

$$L = \sqrt{2Dt} \qquad (1\text{-}5)$$

The diffusion coefficient is specific for a combination of compound, solvent, and temperature, so this cannot be changed experimentally. Combining the information in equations 4 and 5, it can be concluded that diffusive mixing can only be enhanced by using a stronger gradient, or a shorter diffusion distance. The so-called 'chaotic' micromixers used in Chapters 3 and 4 utilize this principle for fast mixing on the microscale.

Fick's law of diffusion can also be applied to other transport phenomena, such as the translocation of compounds between two compartments through a membrane (or through a cell layer, as described in Chapter 5). In this case, the transport rate dQ/dt (µmol/s) across a membrane with surface area, A (cm^2), depends on the concentration difference between compartments a and b ($\mu mol/cm^3 \triangleq mM$)[16]:

$$\frac{dQ}{dt} = P_{app} \cdot A \cdot (C_a - C_b) \qquad (1\text{-}6)$$

The diffusion coefficient has been replaced by the apparent permeability, P_{app} (cm/s), which is a compound-specific parameter for membrane penetration. In rearranged form, the same equation may be used to experimentally estimate P_{app}:

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{A(C_a - C_b)} \tag{1-7}$$

In the case of intestinal absorption studies, C_a denotes the concentration in the apical compartment (gut lumen), and C_b that in the basolateral compartment (body), in order to study uptake of molecules such as drugs into the body. In flowing systems like a gut-on-a-chip device, flows of medium continuously replenish the medium on either side of the membrane, leading to a continuously high C_a and a low (or zero) C_b, establishing a stable concentration gradient.

1.3. Some Chemistry

As alluded to above, the model systems of the GI tract described in this thesis not only differ from conventional models because of miniaturization. The other key difference is the introduction of flow: all processes recreating enzymatic digestion in the GI tract were translated from a batch-wise process to a continuous process, which is (arguably) how the human GI tract functions *in vivo*. In a batch process, all reactions take place at the same physical location (e.g. in a reaction vessel such as a test tube), and sequential steps are separated by time. In continuous-flow systems, the reagents for reactions are continuously mixed, and incubated in the volume of pipes or tubing, with reagents for additional steps added at different points downstream.[17] The sequential reaction steps are therefore occurring all the time, but at different locations throughout the flowing system. The distance over which the reaction mixture flows can thus be transposed to reaction time. Multiple timepoints of reaction progress can be assessed at the same time, providing theoretically infinite time-scale resolution. Besides this, a flow-chemical set-up provides better and easier control over process parameters, such as incubation times and temperatures, and creates great potential for automation.[18]

1.4. Some Engineering

All components, including digestion by enzymes, absorption of compounds by intestinal cells, and on-line analysis, were combined into one system to allow the testing of orally administered drugs (Chapter 5). Besides solving the inherent incompatibilities of the system components, more fundamental engineering questions had arisen: when making a model system – any model system – which parameters of the original process must be reproduced in the model, and which

process parameters are not of vital importance, and can be left out to keep the degree of complexity as low as possible? In other words, what is the program of requirements; what are the minimum performance criteria of the new model; and how will the system be benchmarked? A higher degree of complexity would lead to a more realistic (more *in vivo*-like) model, but also to more points where the system could possibly fail. Compromises are therefore always necessary, in order to make a realistic yet reliable model of the processes in the GI tract.

Scaling different components relative to each other is another challenge when bringing the system parts together. Scaling has proven to be a major concern in combining several organs-on-chips into a multi-organ model (sometimes termed body-on-a-chip, or human-on-a-chip).[19,20] Solutions may be to scale the different parts according to their relative *in vivo* weight, blood perfusion rate, or even cell count – however, a compromise between these and several other parameters may be necessary to make the best model system for a particular application. In the work described in this thesis, scaling issues were resolved by taking the *in vivo* volumetric ratios between digestive juices into account, along with their relative (average) *in vivo* residence times. The digestion-on-a-chip model described in Chapter 3 is therefore thought to be a good representation of the adult 'average' digestive processes; the miniaturized infantile digestive tract of Chapter 4 for the 'average' full-term infant.

1.5. Aims and Scope of this Thesis

This thesis describes the design, development, and characterization of a gut-on-a-chip system as a model to study the behavior of medicinal drugs, toxicants, and nutrients in the human gastrointestinal tract, with a focus on the analytical aspects of these processes.

The first part of this thesis encompasses the work on digestive processes and a miniaturized model of the human intestine. The role of gut-on-a-chip models to study the behavior of medicinal drugs and toxicants is reviewed (Chapter 2). Then, model systems focusing on the enzymatic functions of the adult digestive tract (Chapter 3) and the infantile digestive tract (Chapter 4) are described, and their digestive functions were compared by digesting model milk protein, lactoferrin. The digestion-on-a-chip was integrated with a gut-on-a-chip barrier model and mass spectrometric detection to yield one integrated total analysis system for oral bioavailability (Chapter 5). The second part of this thesis encompasses the development of methods, tools, and equipment to support the work with gut-on-a-chip devices. A highly accurate flow control system is described, utilizing pressurized containers and Coriolis-based

mass flow sensors with precise control and integrated feedback (Chapter 6). New methods for the facile fabrication of thin and flexible polymer membranes containing massive arrays of micropores are described, which can be used for the fabrication of organ-on-a-chip devices (Chapter 7). The use of stereolithography-based 3D-printing for the fabrication of microfluidic components for (bio)analytical assays is then reported (Chapter 8). Finally, the work in this thesis is discussed, and some future perspectives are given (Chapter 9).

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