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AN AUTOMATED MODULAR MICROSYSTEM FOR ENZYMATIC DIGESTION WITH GUT-ON-A-CHIP APPLICATIONS

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ABSTRACT

Gut-on-a-chip models have gained attention as replacements for other cell-based assays or animal studies in drug development or toxicological studies. These models aim to provide a more accurate representation of the *in vivo* situation in form and function; however, no digestive processes have been included in these systems so far. This work describes a miniaturized digestive system based on artificial digestive juices that digest liquid samples in a series of three microreactors. After optimization of the pH value of juices and mixtures, samples leading to fluorescent products were digested to demonstrate enzyme functionality and to determine kinetic parameters.

KEYWORDS: Gut-on-a-chip, Digestion, Organ-on-a-chip, Enzyme kinetics

INTRODUCTION

Several gut-on-a-chip models have been developed over the past few years, mimicking the barrier function of the human intestinal wall by growing intestinal cells on a porous membrane [1]. These models recreate the anatomy of the gastrointestinal (GI) tract for applications in drug or toxicology testing. However, the digestive functions of the GI tract have not been taken into account in these systems, and test samples still require larger-scale, batch-wise *in vitro* digestion by a skilled person before introduction to the device. This work describes progress in the development of an automated, miniaturized, digestive system based on artificial digestive juices [2]. Samples are continuously mixed with these juices (*i.e.* saliva, gastric juice and duodenal juice/bile – see Table 1) in physiologically relevant ratios, using three hybrid glass–poly(dimethylsiloxane) (PDMS) micromixers as bioreactors [3]. Enzymes in these mixtures digest compounds present in the test samples in a two-step process, in which the enzyme, E, first reversibly binds to a substrate, S, after which product, P, is formed:

$$E + S \rightleftharpoons E \cdot S \rightarrow E + P \tag{1}$$

The Michaelis-Menten equation shows that the reaction rate, v, depends on the substrate concentration, $[S]$:

$$v = \frac{d[P]}{dt} = \frac{V_{max} \cdot [S]}{K_m + [S]} \tag{2}$$

In this equation, the maximum reaction rate, V_{max}, and Michaelis constant, K_m, are also included. These two parameters can be estimated experimentally by measuring the formation of the reaction product over time at various $[S]$. In this work, these enzymatic parameters were determined on-chip using labeled substrates, which produce highly fluorescent reaction products after hydrolysis by digestive enzymes.

Table 1: Optimization of the pH value of artificial digestive juices.

Juice or Mixture	Ratio	Main species	pH in original composition [2]		pH in optimized composition	
			Calc.	Meas.	Calc.	Meas.
Sample	1	Pure H_2O	7.00	6.96	7.00	6.96
Saliva	4	$H_2PO_4^-$, OH^-	6.71	6.45	7.00	6.75
Mixture in the Mouth	*5*	*All of the above*	6.71	6.47	7.00	6.76
Gastric Juice	8	HCl, $H_2PO_4^-$	1.02	1.16	2.46	2.57
Mixture in the Stomach	*13*	*All of the above*	1.25	1.38	3.00	3.04
Duodenal Juice	8	HCO_3^-, HCl	7.58	8.82	7.12	8.32
Bile	4	HCO_3^-, HCl	7.76	9.03	7.35	8.81
Mixture in the Intestine	*25*	*All of the above*	2.12	2.53	7.00	7.31

EXPERIMENTAL

The composition of artificial digestive juices [2] was optimized to obtain the physiological pH value for enzyme function in the respective compartments (Table 1). Three hybrid glass–PDMS micromixers, containing grooves to generate chaotic flow profiles, were coupled in series to act as bioreactors [3,4], mixing the sample with digestive juices containing different enzymes (Figure 1, left). Substrates with quenched fluorophores were digested in each stage of this system, producing highly fluorescent products. Pictures were taken at the beginning and the end of each channel (50 mm length), representing a 40 s residence time in the channel. The difference in fluorescence between these two points in the channel is directly proportional to [*P*], and can be used to determine the reaction rate in the initial 40 s.

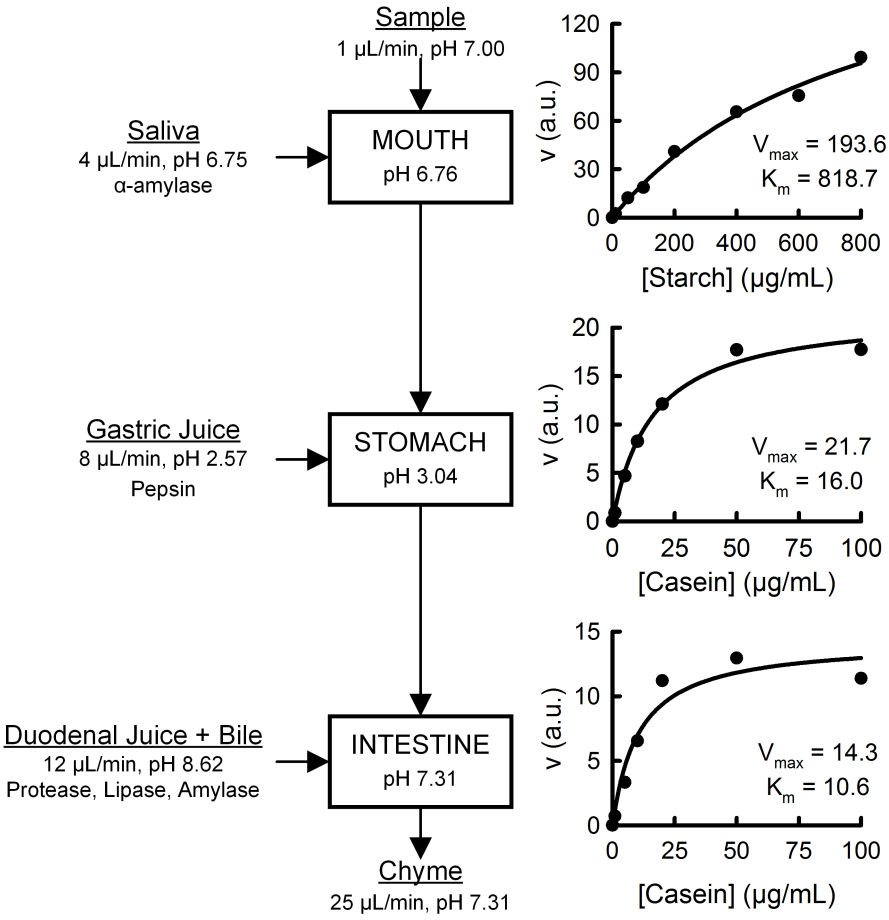

Figure 1: Diagram of the three-stage enzymatic digestive system. Chaotic micro-mixers digest samples using artificial digestive juices containing enzymes. The Michaelis-Menten graphs on the right display the reaction rate, v, in each of these digestive stages versus the concentration of the substrate used.

RESULTS AND DISCUSSION

Starch was digested as a model compound in the first compartment (mouth), and casein (a protein naturally occurring in milk) was digested by proteases in the stomach and intestine, at different values for [*S*]. The Michaelis-Menten plots (Figure 1, right) show a typical shape, with a clear saturation at higher [*S*]. Parameters V_{max} and K_m were estimated mathematically and are displayed in the plots.

CONCLUSION

We demonstrate an automated, modular microsystem for enzymatic digestion, in which the different enzymes occurring in the human GI tract digest samples of model compounds. The output of this system – or chyme – may be transferred to a gut-on-a-chip barrier model of the human intestine.

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REFERENCES

[1] Huh *et al*. Nat Protoc 2013;8(11):2135-57.
[2] Walczak *et al*. Nanotoxicology 2013;7(7):1198-210.
[3] Ianovska *et al*. RSC Adv 2017;7(15):9090-9.
[4] Stroock *et al*. Science 2002;295(5555):647-51.

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