Experimental Study of Particle Accumulation in a CAM-based MC Testbed

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***Abstract*—Molecular communication (MC) research is increasingly focusing on applications within the human body, such as health monitoring and drug delivery, which require testing in realistic, living environments. Therefore, the development of *in vivo* MC testbeds is crucial. Recently, we introduced the Chorioallantoic membrane (CAM) model as the first *in vivo* MC testbed. The CAM is a highly vascularized membrane that forms in fertilized chicken eggs. It allows to study the distribution of molecules inside a closed-loop vascular system and their accumulation in specific regions. In this abstract, we experimentally investigate the accumulation of fluorescent molecules in the liver of the chicken embryo and provide a first simple approximate analytical model for the accumulation.**

I. Introduction

One of the major challenges of MC research is to bridge the gap between theoretical concepts and their practical realization [1]. In the medical sector, MC primarily targets in-body applications, requiring the validation of concepts and technologies in realistic *in vivo* environments. However, many promising MC technologies are highly invasive and disruptive, which makes testing on animals or humans extremely challenging and, in many cases, nearly impossible.

Despite significant progress in experimental MC in recent years, the impact of realistic living *in vivo* environments has not yet been thoroughly explored. Recently, we have proposed the CAM model (see Fig. 1) as the first versatile and realistic *in vivo* MC testbed [2]. The CAM, which forms in fertilized chicken eggs, is a highly vascularized extraembryonic membrane that functions as the respiratory organ during the embryo's development. Serving as a simple and accessible *in vivo* model of a cardiovascular system, including blood circulation and organs of the chick embryo, the CAM model is ideally suited as a next-generation MC testbed.

In [2], we investigated the short-term (a few minutes) distribution of the fluorescent molecule indocyanine green (ICG) inside the CAM model and showed that the ICG concentration very quickly reaches a steady state inside the CAM's vascular system. The CAM model is also suitable for studying particle accumulation, e.g., in human tumor tissue engrafted on the CAM, or in the organs of the chick embryo [3]. Such accumulation studies are of particular importance for the development of in-body communication schemes and for the design of targeted drug delivery systems. Therefore, in this abstract, we focus on the long-term (approximately one hour) behavior of the ICG propagation with focus on their accumulation in the chick embryo's liver. In particular, we experimentally investigate the long-term accumulation of the fluorescent molecule ICG in the embryo's liver, and provide a first approximate analytical model to describe the ICG accumulation.

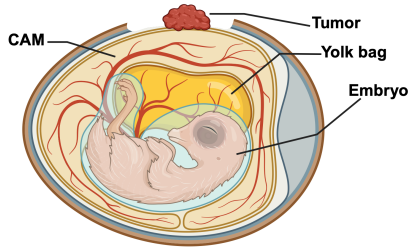

Fig. 1: Illustration of the CAM model consisting of the chicken embryo, the yolk bag, and the CAM. Furthermore, a tumor is engrafted on the CAM (Created with BioRender.com).

II. ICG Distribution and Accumulation

Figure 2 shows the ICG fluorescence intensity I at different time instances after injection of ICG molecules at $t = 0$[1]. As shown in [2], the ICG quickly distributes and reaches a steady state. However, observing the long-term behavior, it can be noticed that the ICG fluorescence intensity gradually increases in the embryo's region, particularly in the liver, while the fluorescence intensity in surrounding vessels gradually decreases as the ICG moves from the vessels to the liver. This is due to ICGs selective uptake by hepatocytes in the liver, where it is efficiently excreted into bile unchanged, without re-entering circulation or undergoing enterohepatic recirculation.

III. MC System Model

Figure 3 shows the MC system topology for studying the ICG accumulation in the liver. In general, depending on the Day of Embryonic Development (DED), the yolk bag and organs of the embryo can be considered as Receivers (RXs), where particles accumulate (see [2, Fig. 4]). The closed-loop vascular system serves as the propagation channel. As we focus on the long-term particle accumulation in the liver, we consider the liver as the RX of interest, see Fig. 3.

Based on the observations in Fig. 2 and [2, Fig. 6], the ICG propagation inside the CAM model can be divided into three phases, i.e., *transient phase*, *steady phase*, and *accumulation phase*. The distribution model derived in [2] covers the *transient phase* and the *steady phase*. In this abstract, we focus on modeling the *accumulation phase*. To this end, we approximate the ICG intensity $I(\mathbf{x}_{\mathcal{R}}, t)$ over time inside a

[1]We note that we assume that the measured ICG fluorescence is proportional to the ICG concentration [4].

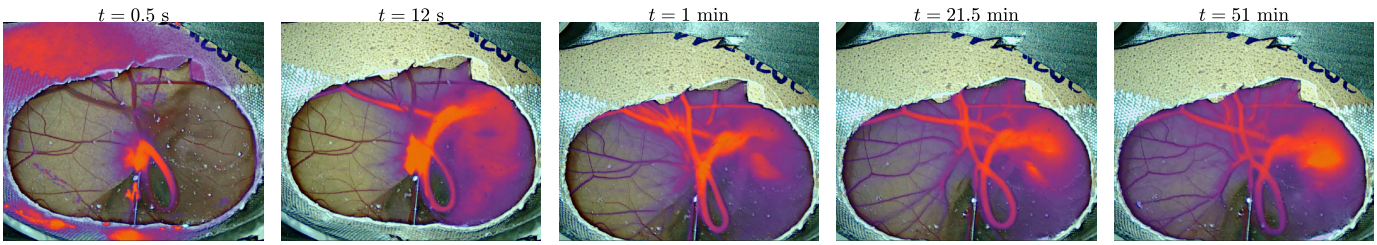

Fig. 2: Photos of the ICG fluorescence intensity distribution and accumulation in the CAM model over time for a long-term experiment.

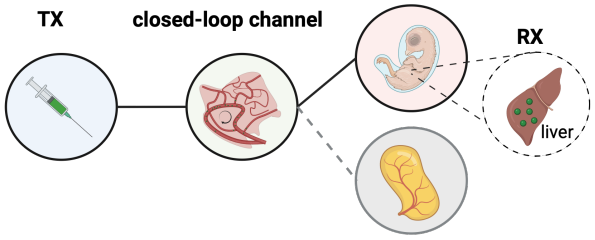

Fig. 3: MC system for the analysis of ICG accumulation in the liver (Created with BioRender.com).

Region of Interest (ROI) $\mathcal{R} \subset \mathcal{A}$, centered at $\mathbf{x}_\mathcal{R}$, where $\mathcal{A}$ is the opened egg area, as follows

$$I(\mathbf{x}_\mathcal{R}, t) \approx \begin{cases} \hat{I}(\mathbf{x}_\mathcal{R}, t) & t \leq t_{\text{acc}}, \\ \tilde{I}(\mathbf{x}_\mathcal{R}, t) & t > t_{\text{acc}}. \end{cases} \tag{1}$$

Here, t_{acc} is the time where the *accumulation phase* starts (see vertical lines in Fig. 4), $\hat{I}$ is the approximation of the ICG intensity corresponding to the *transient phase* and the *steady phase*, and $\tilde{I}$ approximates the ICG intensity *accumulation phase*. The $\hat{I}$ model details can be found in [2]. For the *accumulation phase* of ICG intensity we propose the following simple approximation

$$\tilde{I}(\mathbf{x}_\mathcal{R}, t) = 1 - a \exp(-bt), \tag{2}$$

where the parameters a and b will be obtained by fitting (2) to measurement data in the following.

IV. Experimental Study

For the experiments, we injected ICG molecules via a syringe into the CAM vascular system[2]. We measured the fluorescence intensity $I(\mathbf{x}_\mathcal{R}, t)$ in two eggs over time with an ICG camera and post-processed the data with the Matlab Fluorescence Tracker App.

Figure 4 shows the measured ICG fluorescence intensity I at $\mathbf{x}_\mathcal{R}$ over time (curves with markers). The ROI $\mathcal{R}$ is the liver of the embryo and highlighted on the right hand side of Fig. 4. The solid curves show the approximated fluorescence intensity for the *transient phase* and the *steady phase*, $\hat{I}$ (green), obtained according to [2], and for the *accumulation phase*, $\tilde{I}$ (red), obtained with (2) and the estimated parameter values. In general, the measurements confirm our assumption that the ICG distribution process inside the CAM model can be divided into three phases. Moreover, we can observe from Fig. 4, that the proposed simple model (2) for the accumulation

[2]For a more detailed description of the egg preparation we refer to [2], [3].

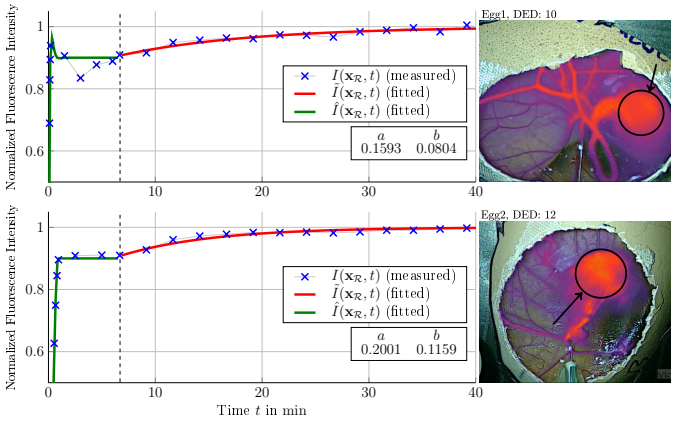

Fig. 4: Experimental and fitting results of ICG distribution in the liver. The vertical dashed line indicates t_{acc}.

(red) is in very good agreement with the measurement data[3]. For both experiments, the estimated parameter values a and b in (2) are also within the same range indicating the validity of the proposed accumulation model.

V. Conclusion and Future Work

In this abstract, we investigated the accumulation of particles in the liver of the embryo within a CAM based MC system. We proposed a simple model for the accumulation of particles in the liver and initially analyzed its performance by a comparison to experimental data. Our results showed that the proposed model is able to approximate the ICG accumulation in the liver. In future work, we want to extend our experimental study on the accumulation in the liver and also in human tumor tissue by engrafting it on the CAM model. Moreover, we want to revise the accumulation model and relate it to physical processes of particle accumulation.

References

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[3]Also the approximation for the *transient phase* and the *steady phase* (green), is in good agreement with measurement data. A detailed discussion and analysis of the model can be found in [2].