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SENSING OF OXYGEN CONCENTRATION IN A MICROFLUIDIC DEVICE MIMICKING LIVER 3D MICROARCHITECTURE

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ABSTRACT

We designed a microfluidic structure which closely reproduces liver microarchitecture, constraining primary rat hepatocytes at a high density and in three dimensions (3D), and in which a gradient of oxygen can be generated. The device includes an oxygen sensitive membrane that could map the oxygen consumption of hepatocytes.

KEYWORDS: Microfluidics, Liver, 3D microtissues, Organ-on-a-chip, Oxygen gradient, Oxygen sensor

INTRODUCTION

Compared to classical two-dimensional cell culture, microfluidic devices or/and 3D culture conditions were evidenced to increase the period of time during which primary hepatocytes retain their functions [1]. Moreover, microfluidic techniques offer the opportunity to mimic the *in vivo* hepatocyte zonation, by subjecting hepatocytes to oxygen gradients [1-2]. Such oxygen gradients that can be estimated by numerical simulations, were recently experimentally assessed using an oxygen sensitive fluorescent membrane [3]. We proposed to include the oxygen sensitive membrane within a miniaturized fluidic device mimicking several hepatic cords in series, and inducing a gradient of oxygen on those. Moreover each of those hepatic cord units was inducing 3D organization of hepatocytes, due to the 72 μm height of culture chambers in which they can aggregate.

EXPERIMENTAL

The microfluidic structure is composed of hepatocyte chambers and medium-conveying channels, separated from each other by small channels (5x5x40 μm^3) (*Figure 1a, structure inspired from* [4], with higher chambers to induce the 3D organization of cells). These elements respectively mimic hepatocyte cords, liver sinusoids, and the space of Disse. Compared to only one chamber devices proposed in [4], twenty hepatocyte chambers are arranged in series in our design, in a way to induce an oxygen gradient. Indeed, because of the consumption of oxygen in every chamber, an oxygen gradient can be induced between the first and last chamber reached by the medium. Simulation of the oxygen mapping was performed using finite element analysis. An oxygen sensitive membrane [3] was incorporated at the bottom of the microfluidic structure and the polydimethylsiloxane (PDMS) surface was made impermeable to gas. The device outlets were then closed to prevent gas from entering. The evolution of cell morphology and oxygen concentration was followed by imaging the device in bright field and at 650 nm for 20h.

RESULTS AND DISCUSSION

A gradient of oxygen in the chambers could be evidenced, depending on the flow rate conditions (*Figure 1b*). With a low flow rate (50 nl/min), a variation of 30% in oxygen concentration between the first and the last chambers can be observed, compared to 50% *in vivo* between periportal and pericentral hepatocytes [2]. By carefully choosing the flow rate, it is thus possible to reproduce *in vivo* oxygen conditions. The oxygen gradient was then characterized experimentally. The cells were loaded in the successive chambers (*Figure 2a*) and the membrane was shown to be sensitive enough to detect the oxygen consumption of primary rat hepatocytes after 20h on the device (*Figure 2d*). Such approach, here used successfully to detect the oxygen consumption in

liver micro-architecture, is very promising for the characterization of oxygen concentration on a chip, for the reproduction of liver zonation purpose.

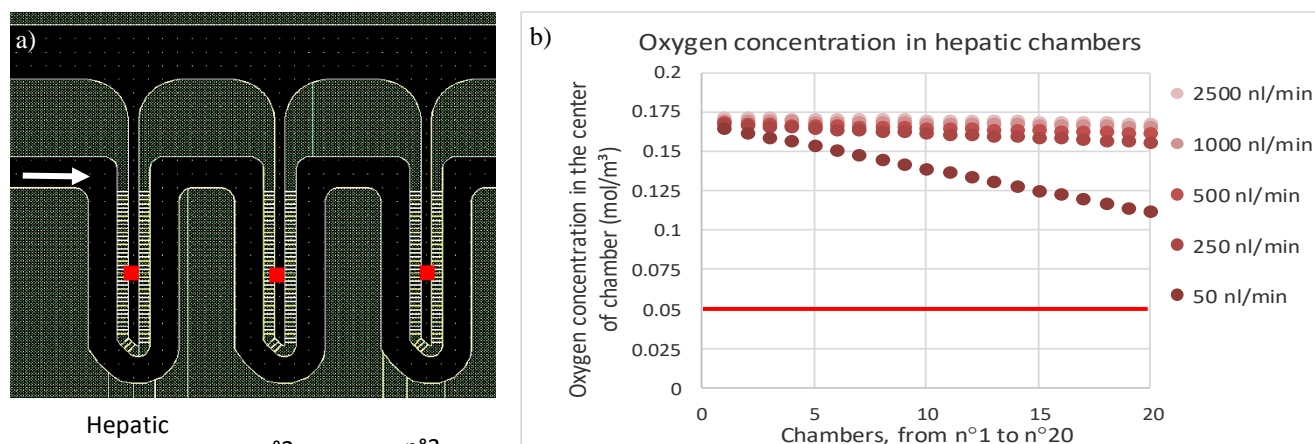


Figure 1: a) zoom on the microfluidic device structure, composed of 20 hepatic chambers in series, b) computational simulation of the oxygen concentration in the middle of every hepatocyte chamber, from chamber n°1, which is the first one reached by the medium, to chamber n°20, the last one reached by the medium, for different flow rates. Red line: hypoxic limit.

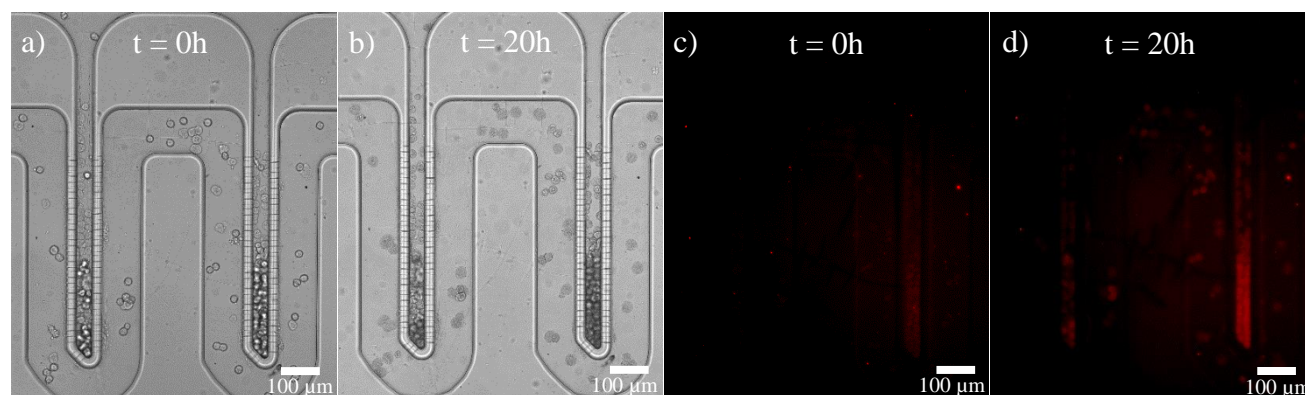


Figure 2: culture of primary rat hepatocytes in the microfluidic device containing the oxygen sensing membrane, in static conditions, and without gas exchange with the outside environment. In bright field at t = 0h a) and at t = 20h b), and at 650 nm at t = 0h c) and t = 20h d).

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