Microfluidic platform to restore the angiogenic balance in preeclampsia

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1. Introduction Preeclampsia is a hypertensive disorder of pregnancy linked to placenta insufficiency, and associated to extreme prematurity. It is currently considered that preeclampsia is connected with maternal endothelial dysfunction due to the excess or the lack of angiogenic factors such as sFlt-1 (soluble Fms-like tyrosine kinase 1) and PlGF (placental growth factor) into the maternal circulation [1]. Clinical studies have shown the involvement of the sFlt-1/PlGF ratio in the syndrome of preeclampsia and its correlation with the severity of the disease [2]. Restoring the angiogenic balance is a solution to delay the birth of an extremely preterm infant and increase from 10% to at least 30% his or her chances of survival without sequelae.

In this context, we present here a microfluidic extra corporal technology as a platform to screen strategies to remove factors that play a causative role in the pathophysiology of preeclampsia. Our team developed a system of micro-fluidized bed, based on the equilibrium between two forces: a drag force created by the fluid that flows inside the micro-chamber of the system and a magnetic force that maintains the particles in the micro-chamber under the hydrodynamic flow [3]. This very special configuration enhances transfer between a fluid and the surface of the beads, with low back pressure and reduced risks of clogging. By grafting specific ligands on the surface of the beads, we can use this asset to capture a target present in the sample flowing through the bed. This system has already shown its efficiency in many fields of application [4]. We present here an innovative approach to restore the angiogenic balance by a competitive bioassay that allows capturing the protein sFlt-1 in excess while releasing its ligand PlGF in default.

2. Methods PDMS chips were made by pouring polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) on brass molds produced by micro-milling. The bonding was performed through oxygen plasma. Surface treatment was performed using PDMA-AGE 0.5% (w/v), with one hour incubation. Magnetic field is created by a NdFeB 1.47 T permanent magnet positioned at 1.5 mm from the chip. Control of the flow was performed using Fluigent systems such as MAESFLO controller and flow unit. Beads used were coated with VEGF (Vascular Endothelial Growth Factor) at 35% using streptavidin-biotin interaction. Experiments were performed on culture supernatants of human trophoblastic cells and pools of maternal plasma. Concentrations of growth factors were measured by immunoassays at the hormonology laboratory at Cochin hospital.

3. Results To shift the equilibrium of sFlt-1-PlGF binding, we coated the surface of magnetic beads with a competitive ligand (VEGF). The microfluidic system has been developed to optimize the interaction of sFlt-1 with the functionalized magnetic beads. We demonstrate its ability to capture it specifically (up to 46% ± 1 of capture) at rates similar to those obtained on batch (52% ±5) in both matrices of culture supernatant and pool of maternal plasma. Compared to classical apheresis columns [5], the nonspecific absorption is completely controlled (<3%). Using our competitive biomimetic binding approach, the ligation of sFlt-1 increases the bioavailability of PlGF. We were able to show a decrease of 83% of the ratio sFlt-1/PlGF from original samples at preeclamptic growth factors concentrations, leading to a final sample with a healthy ratio (lower than the clinical limit).

Conclusion We demonstrate an alternative way to restore the angiogenic balance thanks to a competitive approach, in order to delay the birth of an extremely preterm infant. This approach has been adapted from culture supernatant to more complex matrices, such as pool of maternal plasma.

References: [1] Sibiude J and al. *Placental growth factor for the prediction of adverse outcomes in patients with suspected preeclampsia or intrauterine growth restriction*. PLoS One,7, 11 (2012)
[2] Levine RJ *Circulating angiogenic factors and the risk of preeclampsia*. N Engl J Med. 350:672-683 (2004)
[3] Iago Pereiro and al. *Magnetic fluidized bed for solid phase extraction in microfluidic systems* Lab Chip, 17, 1603-1615 (2017)
[4] Iago Pereiro and al. *A new microfluidic approach for the one-step capture, amplification and label-free quantification of bacteria from raw samples* Chem. Sci., 8, 1329-1336 (2017)
[5] Thadhani R and al. *Pilot study of extracorporeal removal of soluble fms-like tyrosine kinase 1 in preeclampsia* Circulation. 23;124(8):940-50 (2011)