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Microfluidic interactions between red blood cells and drug carriers by image analysis techniques

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ABSTRACT

Blood is a complex biological fluid composed of deformable cells and platelets suspended in plasma, a protein-rich liquid. The peculiar nature of blood needs to be considered when designing a drug delivery strategy based on systemically administered carriers. Here, we report on an in vitro fluid dynamic investigation of the influence of the microcapillary flow of red blood cells (RBCs) on micron-sized carriers by high-speed imaging methods. The experiments were carried out in a 50 μm diameter glass capillary that mimicked the hydrodynamic conditions of human microcirculation. Spherical μ-particles (μ-Ps), with sizes ranging between 0.5 and 3 μm, were tested. Images of the flowing RBCs and μ-Ps were acquired by a high- speed/high-magnification microscopy. The transport and distribution of rigid particles in a suspension of RBCs under shear flow were investigated by analyzing: (i) the velocity profile of both μ-Ps and RBCs in the capillary; (ii) the radial distribution of μ-Ps in the presence of RBCs; (iii) the migration of μ-Ps towards the vessel wall due to their hydrodynamic interactions with RBCs. This study suggests that the therapeutic efficacy of μ-Ps could be ultimately affected by their interactions with the flowing RBCs in the vasculature.

1. Introduction

Human blood is a complex non-Newtonian biological fluid consisting in a suspension of cells in plasma, a protein-rich Newtonian fluid. Blood cells are constituted mainly (~99%) by red blood cells (RBCs), the remaining being white blood cells and platelets. One of the most remarkable properties of RBCs is their high deformability, which allows the flow even through microcapillaries of diameter smaller than their size [1–3]. The peculiar properties of RBCs need to be considered when designing a drug delivery strategy based on systemically administered carriers and the delivery efficiency has to be evaluated by analyzing the distribution of micro-carriers within blood vessels radius [4]. Recently, computational modeling has been used to demonstrate that, while nano-particles (diameter about 100 nm) present a uniform radial distribution and limited near-wall accumulation when flowing with RBCs, micro-particles (diameter about 1 μm) tend to accumulate near the tube wall [5,6], as happens for platelets when they concentrate in the cell-free layer near the vessel wall, in a mechanism known as margination [7]. The latter is a combination of three phenomena: (i) the migration of RBC towards the vessel centerline due to their deformability, leaving a cell-free layer near the vessel wall [4,5,8]; (ii) the concentration of platelets in the cell-free layer near the wall, due to cell rigidity, that allows a rigid-body flipping motion near the wall [4]; (iii) the cross-flow migration of platelets towards the vessel wall due to their hydrodynamic interactions with RBCs [4,7]. In vitro experiments evidenced that the presence of deformable RBCs is fundamental for the near wall concentration of platelets [7,9,10].

Here, we report on an in vitro flow-based imaging method to investigate the fluid dynamic influence of RBCs on micron sized drug carriers migration in the cell-free layer near the vessel wall, taking inspiration by platelets margination phenomenon. The effect of wall shear rate on micro-particle margination has also been investigated. A suspension of poly D,L-lactic-*co*-glycolic acid (PLGA) microparticles (μ-Ps) and RBCs has been used. PLGA is a copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA). Although many numerical works on particles radial distribution in tube flow [4,11–13] are present in the literature, to our knowledge only few experimental works [6,13] potremmo citare JCR focused on this issue. The aim of

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the present study is to provide a quantitative fluid-dynamic analysis of the influence of RBCs flow on micro-drug carriers migration, to model in vitro the mechanisms that regulates the transport of injectable carriers in microcirculation and to help the design of microparticles with physical and chemical features optimized for vascular delivery.

2. Materials and methods

2.1. Blood sample

Fresh venous blood samples were drawn from healthy donors and diluted to a concentration of 10% by volume with ACD anticoagulant (0.6% citric acid, 1.1% anhydrous dextrose, 2.3% sodium citrate, 96% water) and bovine serum albumin BSA. All the samples were used within 4 h from collection. The concentration of 10% has been selected in order to have the typical hematocrit Hct value (i.e., the percentage of cell volume with respect to total blood volume) found in microcirculation, where Hct ranges from 10 to 26% [14]. In fact, the higher values of Hct (around 45%) measured in healthy blood tests are associated with large vessels, whereas in going from macro- to micro-vasculature Hct decreases due to entrance effects and the inhomogeneous radial distribution of RBCs, which tend to concentrate along the centerline (the so called Fahraeus effect [15]).

2.2. PLGA μ-particles

Classical methods for the preparation of PLGA particles are based on bottom-up techniques such as phase separation, spray drying or solvent extraction/evaporation processes. In the present work, PLGA microspheres (3–0.5 μm) have been prepared by double emulsion method, which is the most used one to encapsulate hydrophilic payload such as proteins, peptides and nucleotides [16]. The double emulsion method involves the formation of a primary water-in-oil emulsion (W1/O), which is then dispersed into a secondary aqueous solution (W2) to form a (W1/O/W2) emulsion. After the evaporation/extraction of the solvents, porous microspheres are formed with size depending on different parameter such as polymer concentration, evaporation velocity, and type of surfactant [17].

Table 1
Values of zeta potential of the microparticles.

Sample name	Size (μm)	Zeta potential (mV)
PLGA (50:50) 5% w/w	0.5–3	−27.2 ± 0.65
PLGA (50:50) 20% w/w	0.5–3	−25.6 ± 0.75

2.2.1. Synthesis

PLGA microspheres (PLGA ester terminated (lactide/glycolide = 50:50) viscosity range 0.95–1.20 dl from LACTEL (Pelham, Alabama, USA)) were prepared by a modified S/O/W emulsion method as mentioned in our previous studies [16]. Briefly, PLGA (50:50) was dissolved in dichloromethane (DCM) to form 20% or 5% w/v PLGA/DCM solution. The organic phase was mixed with of PVA, 2.5% w/v by vortex mixing and sonication (3000 rpm) for 3 min. The mixture was gradually dropped into a solution of PVA1% w/v. The resulting suspension was stirred with a magnetic stir bar for 6 h and the DCM was eliminated by evaporation (Fig. 1A). The suspension was washed 3 times by centrifugation at 5000 rpm for 10 min. The microspheres were filtered by MF-Millipore^{TM} Membrane Filters at 3 μm and then freeze-dried and stored as well at −80 °C. The resulting mean diameter of PLGA composite μ-Ps was 2.1 μm (SD ± 1.3). Multiple syntheses of μ-Ps have been performed to demonstrated repeatable distributions. Scanning electron microscopy (SEM) images of μ-Ps showed a regular spherical morphology (Fig. 1B). In Table 1 the zeta potential value of the particles used in this study is shown.

2.3. μ-Ps and RBCs/μ-Ps suspensions

Two different suspensions have been used: a μ-P suspension, used as control, made by poly (lactide-co-glycolide) PLGA μ-Ps (Fig. 1) suspended in ACD and BSA (0.004% weight), and a μ-P/RBC suspension consisting of μ-Ps (0.004% weight) in a RBC suspension

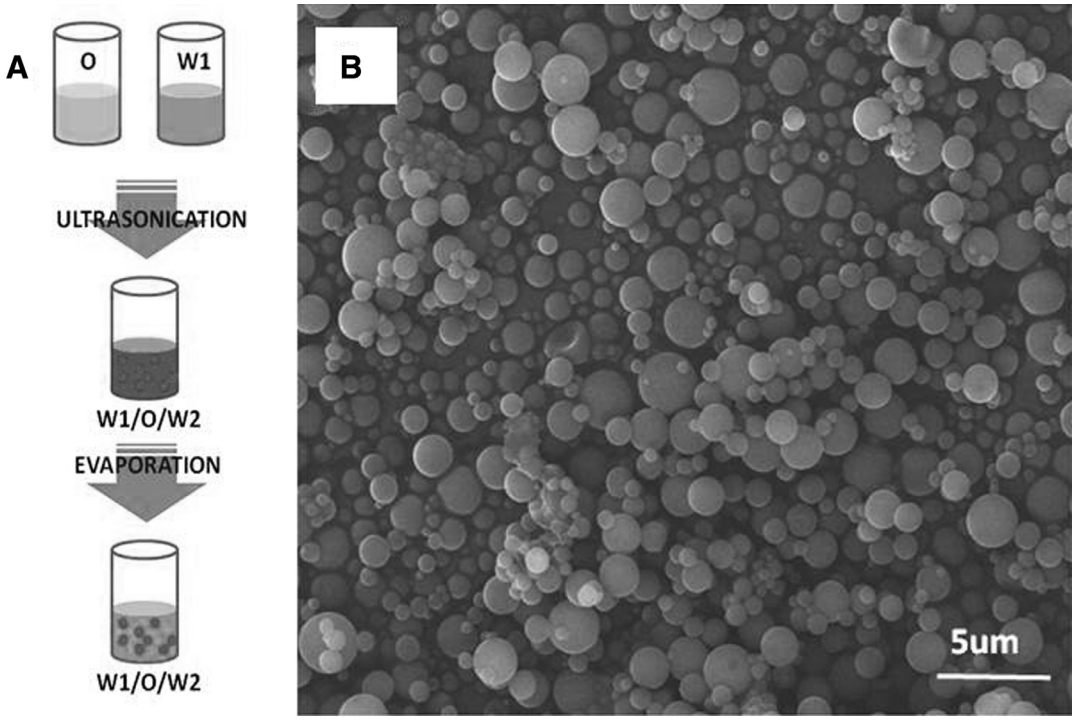

Fig. 1. Synthesis and characterization of PLGA microparticles. (A) Scheme of the double emulsion synthesis. (B) Scanning electron micrographs of PLGA microparticles.

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