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Effect of shear stress in the flow through the sampling needle on concentration of nanovesicles isolated from blood

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ABSTRACT

During harvesting of nanovesicles (NVs) from blood, blood cells and other particles in blood are exposed to mechanical forces which may cause activation of platelets, changes of membrane properties, cell deformation and shedding of membrane fragments. We report on the effect of shear forces imposed upon blood samples during the harvesting process, on the concentration of membrane nanovesicles in isolates from blood. Mathematical models of blood flow through the needle during sampling with vacuumtubes and with free flow were constructed, starting from the Navier–Stokes formalism. Blood was modeled as a Newtonian fluid. Work of the shear stress was calculated. In experiments, nanovesicles were isolated by repeated centrifugation (up to 17,570 × *g*) and washing, and counted by flow cytometry. It was found that the concentration of nanovesicles in the isolates positively corresponded with the work by the shear forces in the flow of the sample through the needle. We have enhanced the effect of the shear forces by shaking the samples prior to isolation with glass beads. Imaging of isolates by scanning electron microscopy revealed closed globular structures of a similar size and shape as those obtained from unshaken plasma by repetitive centrifugation and washing. Furthermore, the sizes and shapes of NVs obtained by shaking erythrocytes corresponded to those isolated from shaken platelet-rich plasma and from unshaken platelet rich plasma, and not to those induced in erythrocytes by exogenously added amphiphiles. These results are in favor of the hypothesis that a significant pool of nanovesicles in blood isolates is created during their harvesting. The identity, shape, size and composition of NVs in isolates strongly depend on the technology of their harvesting.

1. Introduction

Sub-micron membrane-enclosed vesicular fragments of cell interior (nanovesicles — NVs) in body fluids present a promising material for diagnostics and therapy of many diseases (Buzas et al., 2014; Fais et al., 2016; Lener et al., 2015; Yanez-Mo et al., 2015). NVs are released from cells by different mechanisms (Ogorevc et al., 2013; Štukelj et al., 2013) (Fig. 1), but their origin, identity, composition and properties are not yet completely understood. The acknowledged mechanisms are exovesiculation of the plasma membrane (Hägerstrand and Isomaa, 1989; Kralj-Iglič et al., 2000), release of exosomes from internal cell compartments (Pan et al., 1985; Johnstone et al., 1987; Raposo and Stoorvogel, 2013; Thery et al., 2002) and release of larger vesicles that may carry genetic material (Atkin-Smith et al., 2015; Black, 1980)—ascribed to apoptotic bodies. Packing and distribution of membrane constituents creates local membrane curvature which is consistent with lateral sorting of membrane constituents and drives the formation of buds and vesicles (Kralj-Iglič, 2012; Yanez-Mo et al., 2015). These features are affected by pathophysiological processes such as cell activation, hypoxia, irradiation, oxidative injury, exposure to complement proteins and exposure to shear stress

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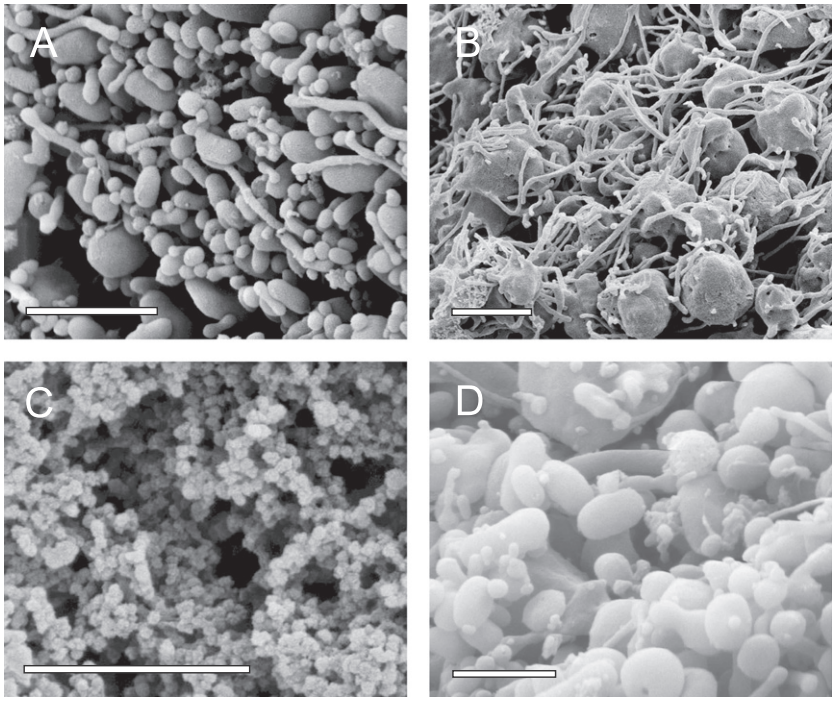

Fig. 1. Scanning electron micrographs of nanovesicles (NVs) and related mechanisms. A: NVs harvested from cerebrospinal liquor, B: Activated platelets from harvesting pellet, C: NVs harvested from pleural effusion, D: NVs isolated from peripheral blood. Bars = 1 μm. Methods for preparation and imaging are described in Mrvar-Brečko et al. (2010).

(Ratajczak et al., 2006). It was found that NV concentration is increased in isolates from blood of patients with various diseases, e.g. autoimmune diseases, cardiovascular diseases, type II diabetes mellitus, neurodegenerative diseases (Boulanger et al., 2006) and in some types of cancer (Janša et al., 2008).

To fulfill great expectations for their numerous potential uses (Buzas et al., 2014; Lee et al., 2012; Lener et al., 2015) NVs must be harvested from samples that contain cells and extracellular environment, (Bard et al., 2004; Diamant et al., 2002; Junkar et al., 2009; Lobb et al., 2015; Momen-Heravi et al., 2013; Mrvar-Brečko et al., 2010; Nordin et al., 2015; Pascual et al., 1994; Saenz-Cuesta et al., 2015) (Fig. 1) and assessed by some relevant method (van der Pol et al., 2010, 2013), such as flow cytometry (Lacroix et al., 2010, 2012), transmission and scanning electron microscopy (Mrvar-Brečko et al., 2010), cryo electron microscopy (Linares et al., 2015), atomic force microscopy (Junkar et al., 2009), mass spectrometry (Pocsfalvi et al., 2016), Raman tweezers microspectroscopy (Tatischeff et al., 2012), dynamic light scattering, nanoparticle tracking analysis based on Brownian motion, and different techniques of molecular content determination (Witwer et al., 2013). There are different methods of NV harvesting such as filtration, chromatography, microfluidics, polymer-based extraction (Witwer et al., 2013) and immunoadsorption/bead capture (Momen-Heravi et al., 2013), however, a simple, low cost and therefore commonly applied method for harvesting NVs from blood is centrifugation and washing of samples (Diamant et al., 2002).

Despite evident perspectives, NV-based methods have not yet been introduced into clinical practice. Harvesting and keeping of NVs represent a bottleneck to a clinically relevant quantitative method of assessment of NVs, as methods and protocols have not yet overcome problems such as poor repeatability and accuracy, complexity in the interpretation of assessment methods (Momen-Heravi et al., 2013; Shah et al., 2008), as well as insufficient understanding of mechanisms of NVs formation in the organism and during the harvesting.

Imaging techniques applied on isolates from blood by repetitive centrifugation (up to 20,000 × g) and washing showed that the harvested material contains small (sub-micron sized) entities that are heterogeneous with respect to size and shape (Junkar et al., 2009; Mrvar-Brečko et al., 2010; Šuštar et al., 2011b). Many vesicles were rather large (diameter between 250 and 400 nm) (Šuštar et al., 2011b). From 50 to 90% of vesicles in isolates carried molecules characteristic for platelets (Šuštar et al., 2011a). These vesicles could not have been created by the budding of the platelet membrane, as the platelets themselves have only about 2 μm in the greatest extension. The observed effects of external parameters on the size and shape of NVs in isolates (Lacroix et al., 2012; Šuštar et al., 2011b; Yuana et al., 2011) led us to the hypothesis that the particles in the isolates are derived from cells and fragments in which the detachment of particular parts is imposed by shear forces during the harvesting process (Šuštar et al., 2011b).

In studies of the effects of detergents on the erythrocyte membrane, membrane vesiculation was induced by adding into the suspension amphiphile molecules (Hägerstrand and Isomaa, 1989, 1992; Hägerstrand et al., 2001). The shape of exovesicles shed from erythrocytes was either spherical or tubular (Dubnickova et al., 2000; Kralj-Iglič et al., 2000) while their size was very small, with at least one extension having 100 nm or less (Dubnickova et al., 2000; Hägerstrand and Isomaa, 1992; Kralj-Iglič et al., 2000; Štukelj et al., 2013). Imaging of the budding erythrocyte membrane confirmed that the buds and the NVs had similar dimensions and shapes (Štukelj et al., 2013). It therefore seems logical to indicate that in those experiments, NVs found in isolates were the entities that were pinched off from the membrane in the process of membrane budding. As all cells are enclosed by the membrane, it could be expected that similar processes would take place in cells of other types, albeit they would be constrained by the cytoskeleton. Thin (nano-sized) tubular buds can be observed in activated platelets (Fig. 1B) while shape changes and microexovesiculation take place also in platelets (Hägerstrand et al., 1996). Also it was suggested that during deformation and re-assembly, the membrane may pick up molecules or their clusters (inclusions) from the outer solution (Kralj-Iglič, 2015), as the membrane curvature may locally compose to fit the intrinsic