



Reflection

Reflections on Plenary Lecture “Extracellular Vesicles from Blood Cells and in Blood; The Avenue of Future Therapies” by Saara Laitinen at 12th Socratic Lectures

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Abstract:

Extracellular vesicles have been the scope of the Socratic Lectures since 2008 keeping the interested parties and the students informed on recent developments in the field. The first plenary lecture on extracellular vesicles at the recent symposium was by a distinguished researcher Saara Laitinen. In this contribution, the lecturer is shortly presented, contents of the lecture as reflected by the students are given along with images of extracellular vesicles from blood, and references selected by the lecturer.

Keywords: Extracellular vesicles; Extracellular particles; Microparticles; Microvesicles; Blood extracellular particles; Plasma extracellular particles

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1. Introduction

The plenary lecture Extracellular vesicles from blood cells and in blood; The avenue of future therapies was presented at the symposium 12th Socratic Lectures that took place January 11, 2025 online. It was organized by the Centre of Lifelong Learning of the Faculty of Health Sciences, University of Ljubljana as a part of Z-STEAM activities (Science, Technology, Engineering, Arts and Mathematics). Z-STEAM activities are centred on the students and Socratic Lectures are a part of the curriculum that features top scientists from different fields for students of all levels. Also, Socratic Lectures are constitutive parts of the projects FarmEVs, Nanostructurome, J3-60063, and the program P3-0388. We are indebted to excellent scientists for including students into the discourse and thus enable them to take a part in one of the research segments. The students have attended the symposium and recorded the lectures. They have combined their impressions and the informations from the recording. Images of blood cells and extracellular vesicles (EVs) (Figures 1 and 2) are added for illustration.

2. Saara Laitinen: the presenter of the plenary lecture Extracellular vesicles from blood cells and in blood; The avenue of future therapies

Saara Laitinen took her Master of Science degree from Plant Physiology and her PhD at the University of Helsinki, Department of Biosciences, Finland. Her career started at the University of Helsinki and developed at distinguished institutions L'Institut Pasteur de Lille, France, Research Institute Minerva, Biomedicum, Helsinki and Department of Molecular Medicine, National Public Health Institute, Helsinki. Currently, she is a Research and Development manager at Finland Red Cross Blood Services. She is a principal investigator and responsible person for strategic research topic: Blood Cells and Products. She is responsible for the laboratory facilities at the Research and Development Department providing and maintaining the high-quality platform for all four strategic research focus projects currently running in the Finnish Red Cross Blood Service.

Laitinen has over fifteen years of research experience on EVs and is an internationally recognised blood EV researcher participating in the work of the International Organisation Committee for Blood EV Workshop of ISEV (International Society of Extracellular Vesicles). Her interest in EVs started from the mesenchymal stromal cells (MSC). Her current research focus is on the immune regulation of blood cells, platelets, and red blood cells and the role of the membrane lipids in the functionality of the cells and the extracellular vesicles.

Laitinen was a principal investigator of numerous projects, publications and invention declarations. Her work hitherto received more than 20,000 citations in Google Scholar base. She supervised 5 PhD and 6 Master students and was a student mentor in the University of Eastern Finland. She was an invited lecturer at several national and international conferences, organiser and lecturer in ISEV educational workshops <https://www.isev.org/blood-ev-workshop>, <https://www.isev.org/blooddev2024>, invited chair in several ISEV and other conferences, founder of Finnish Society of Extracellular and its president 2020-2023. She was awarded Honour from International Society for Extracellular Vesicles as principal organiser of the world first Blood EV Workshop in Helsinki Finland in 2022. She was a member of the editorial board of Journal of Extracellular Vesicles during 2016-2019 and a reviewer in several peer reviewed journals i.e. Stem Cell Research and Development, Cytotherapy, Journal of Extracellular Biology and Biochimica Biophysica Acta.

3. Extracellular vesicles from blood cells and in blood; The avenue of future therapies by Saara Laitinen

2.1. Blood as a liquid tissue

Blood delivers and removes cargo to and from cells, maintains homeostasis, responds to stress and injuries, and is involved in immune and coagulation modulation. It includes cells, gas, hormones, nutrients, and has a vital importance for others tissues and organs. It can be considered as a fluidic tissue and there are similarities and differences between blood and other tissues and organs. Initially considered a simple fluid, blood is now recognized for its complex composition, including red blood cells, platelets, plasma, and components that are not fully understood. Technological advances have shifted the focus of

treatment from whole blood to specific components, thereby improving therapeutic applications. Certain components are yet disregarded or misunderstood, some (e.g. plasma – in practice - blood depleted of erythrocytes (**Figure 1A**)) may even be destroyed during processing.

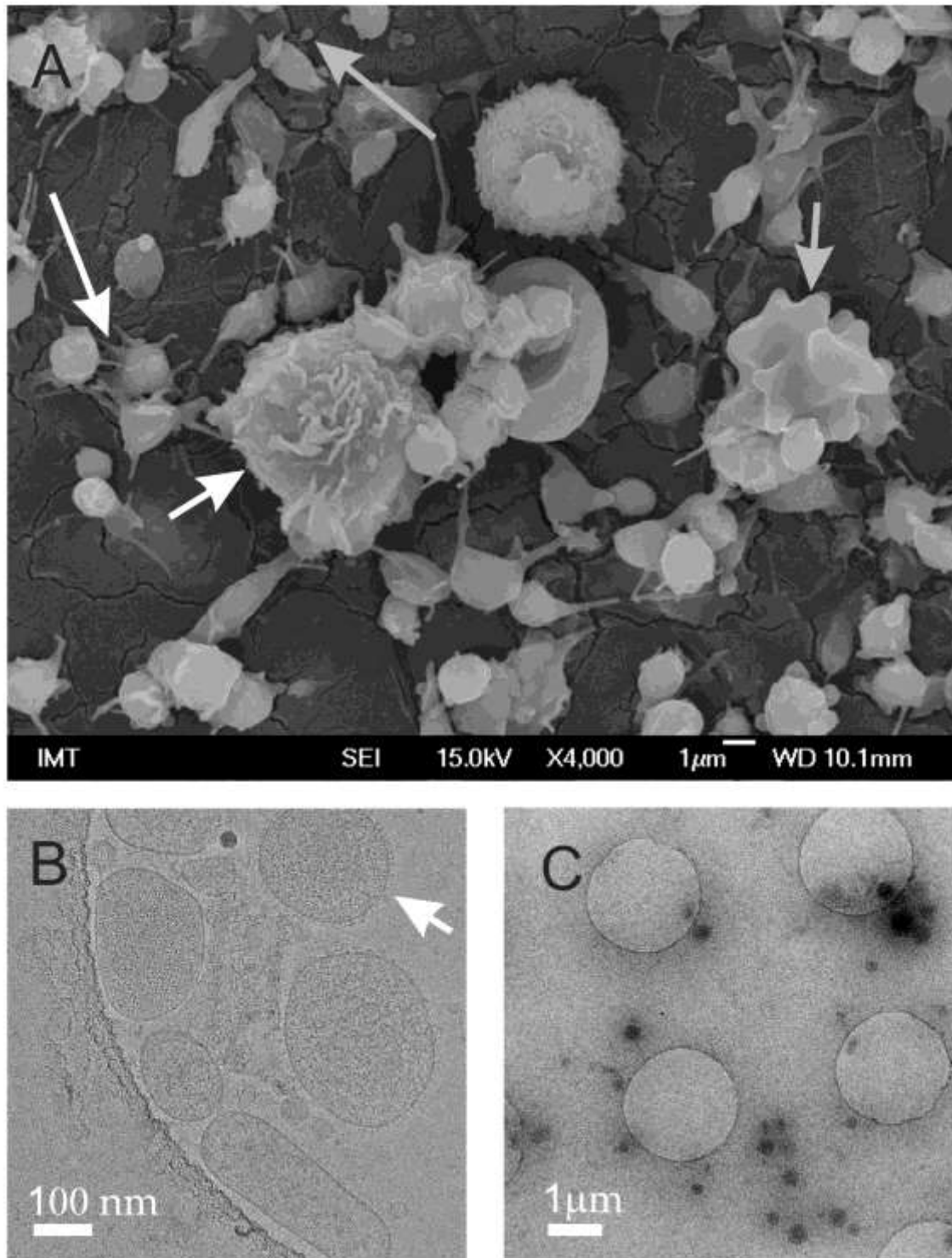


Figure 1. A: Human plasma after 15 hours of fasting (From Božič et al., 2020). White arrow points to a leukocyte, gray arrow points to an erythrocyte, long white arrow points to a platelet and long grey arrow points to an EV. B,C: EVs in isolate from plasma. White arrow points to the corona. A: from Božič et al., 2020), B, C: from Romolo et al., 2022.

The biology of platelets is complex, and they are known to play a role in various physiological and pathological processes, including blood clotting and immune responses. There is a continued relevance of blood products in medicine, despite a decrease in blood-demanding surgical procedures. Blood and plasma are being used for modified antibody-based cancer treatments.

3.2. *Extracellular vesicles (EVs) in blood*

EVs are small membrane-enclosed particles released by cells (Jeppesen et al., 2022). They play a crucial role in intercellular communication. EVs in blood derive from blood cells, endothelial cells, but also other types of cells (Nieuwland & Siljander, 2024; Thangaraju et al., 2020). They are shed off from all types of cells to become free to move locally, and if they enter blood, they can travel with it and reach distal cells. Thus, blood EVs are a heterogeneous population of vesicles originating from different cell types, including red blood cells and platelets. The first step in isolation of EVs from blood is preparation of plasma by sedimentation of erythrocytes, most commonly by centrifugation. However, the present protocols of plasma preparation in general disregard the effect of blood processing on the identity, number density, morphology and content of EVs, and these processes are currently being investigated (Ilvonen et al., 2024). The composition of blood EVs is not constant and can vary depending on the cell of origin. The cell-specific origin of EVs is crucial in understanding their behaviour and potential therapeutic applications. **Figure 2** shows EVs isolated from suspension of aged erythrocytes.

In addition to EVs, there are other types of particles released by cells, known as non-vesicular extracellular particles. These particles vary in abundance and protein concentration. They can be lipid-rich or protein-rich and have distinct compositions and functions. EVs reflect the material of the mother cell. As an example information on the origin can be obtained from the analysis of RAB proteins, revealing how cellular stimuli influence EV composition (Kilpinen et al., 2013). Lipoproteins are abundant in plasma and they can interact with EVs. These particles (initially considered contaminants) may have significant functions, in particular in forming of the vesicle corona (**Figure 1B**). When EVs are released into the bloodstream, they acquire the corona composed of proteins and other components (Tóth et al., 2021; Buzas, 2022).

3.3. *EVs as diagnostic vectors*

EVs are considered as potential diagnostic vectors. Research on the interaction of EVs with immune cells, such as monocytes and macrophages, is complex and depends on various factors, including the type of EVs and the time of incubation. These interactions may vary depending on the polarization state of the cell. However, difficulty in identifying specific EVs within a diverse population of particles remains a challenging task often described as finding a "needle in a haystack". Characterization methods are being developed (Koponen et al., 2020). The challenge arises from the diversity of extracellular vesicles and other particles. To overcome this, it is necessary to develop effective targeting moieties and marker combinations. Nevertheless, EVs hold promise as tissue- or cancer-specific diagnostics, provided that appropriate sensitivity thresholds and diagnostic techniques are developed.

3.4. *EVs as therapeutic vectors*

Blood-derived EVs have been used therapeutically for many years, particularly in the form of platelet concentrates. These concentrates contain billions of EVs and have been shown to be safe for use in patients. Blood EVs, particularly those derived from red blood cells, have vast potential therapeutic applications depending on the indication, route of administration, and other factors. Potential therapeutic applications of EVs derived from erythrocytes are immune regulation and capillary dilation. Compared to the development of antibody drugs, blood products have historically driven therapeutic advances. Blood-derived EVs provide a natural source of particles that reflect their in vivo environment, offering advantages in terms of safety and functionality. Rapid production of platelet-derived EVs presents opportunities for targeted drug delivery, especially in cancer treatment. It is highly probable that blood and blood cell-derived products will be the next generation of drugs, but the process to accomplish this seems to be protracted.

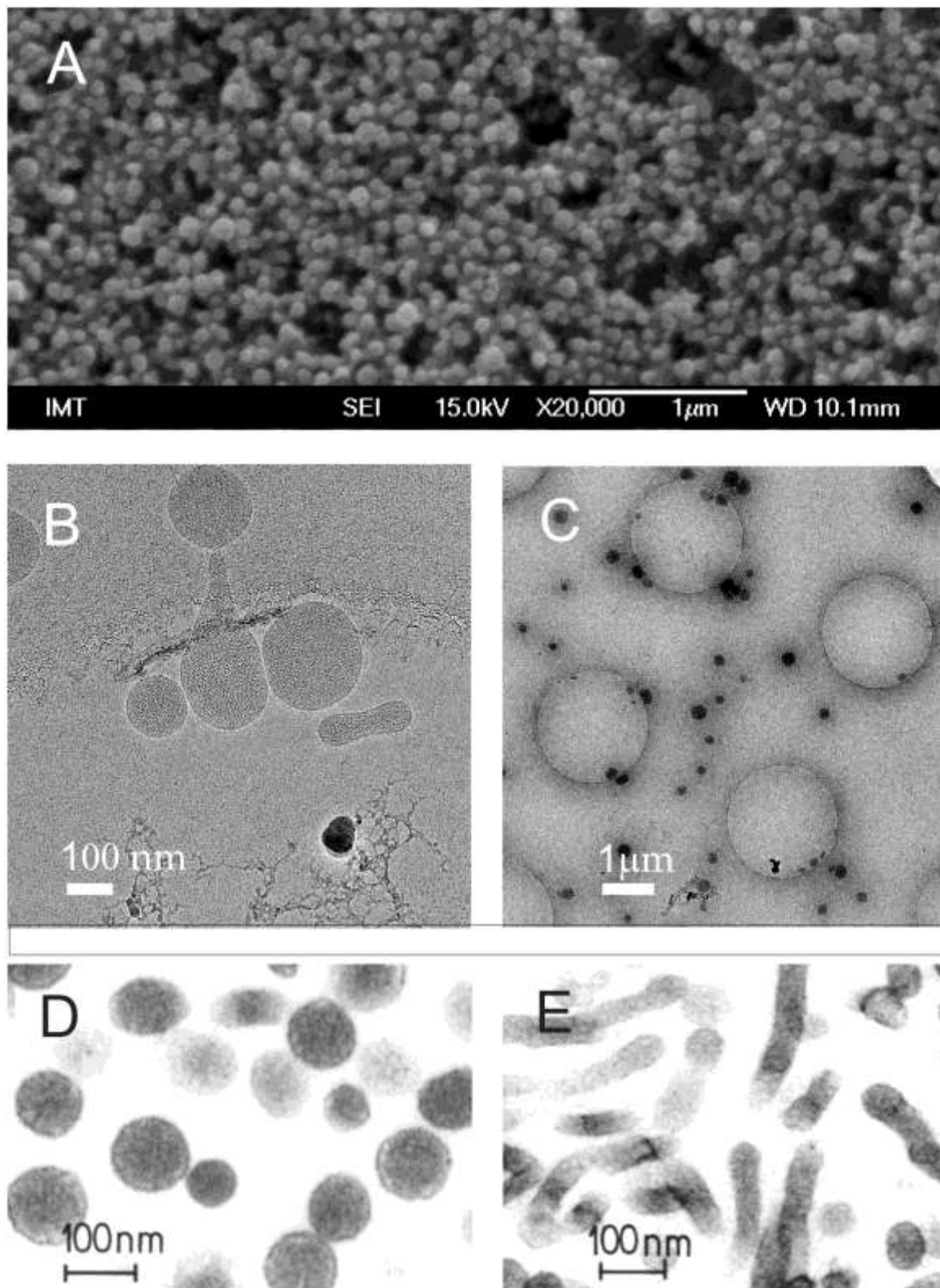


Figure 2. A: Scanning electron micrograph and (B,C): cryogenic transmission electron micrograph of EVs isolated from the suspension of ageing erythrocytes. D and E: Transmission electron micrograph of EVs obtained by isolation from erythrocytes treated with two different detergents, respectively. A: from Jozelj et al. (2022), B and C from Romolo et al. (2022), D and E: from Kralj-Iglič et al. (2020).

There is a lack of universal EV markers, which makes it difficult to distinguish EVs from other particles in the blood. EVs from different blood cells such as erythrocytes and platelets have different properties. Cell-specific isolation of EVs is crucial to increase therapeutic potential.

Blood compatibility is critical for EV-based therapies, as EVs may contain blood group antigens. Research on the interaction of EVs with immune cells, particularly macrophages, is ongoing. These interactions may vary depending on the polarization state of the cell. EVs show potential to target cancer cells, but therapeutic development is still in its early stages. The use of EVs for drug delivery faces obstacles, particularly regarding their ability to penetrate biological barriers such as the blood-brain barrier. Current manufacturing protocols of blood plasma and further plasma medicals (growth factors, immunoglobulins etc) destroy EVs.

3.5. *Challenges in EVs harvesting, characterization and applications*

Although EV-based therapies have great potential, challenges in isolation, purification, drug delivery, and diagnostics need to be addressed before they can be widely used in clinical settings. Also, it is necessary to understand the source of EVs and their composition before developing therapeutic strategies. EV cargo (Palviainen et al., 2020) and membrane composition are highly influenced by the blood environment, which complicates their use in standardized therapeutic applications. Blood-derived EVs present challenges due to the unculturable nature of many cells, but this also confers advantages for risk management and post-activation modification.

The EV cargo and membrane composition are dynamic entities influenced by the blood environment, creating both complications and opportunities for diagnostics. The high abundance of non-EV particles in blood adds another level of complication. Current separation methods, particularly those size-based, are inadequate in distinguishing EVs from other blood particles, such as lipoproteins, which are significantly more abundant and overlapping with size. As the development of robust, scalable manufacturing processes is essential to move from small-scale research to large-scale clinical use when using cultured cells/isolators, blood can offer competitive option for production of therapeutic EV source. Moving towards therapy application, regulatory authorities require thorough documentation highlighting the existing expertise within blood services to help manage such complexities. Although robustness and quality control issues may be solved, competition with other drug delivery methods remains and challenge EV research to find the focus where they bring additional value.

3.6. *Intertwining EVs with public services*

Blood services possess an extensive history of handling personalized medicine and cell-based therapies, providing a foundation for future advancements. COVID-19 pandemic raised widespread concerns across Europe and globally regarding the security of blood supply. Emerging issues were vaccination strategies and the varying perspectives on the speed of implementation, in the light of historical precedents.

There is a long-standing history of personalized medicine within blood services, exemplified by the understanding of blood groups, tissue typing and their implications for treatment. Moreover, ongoing development of new treatments and services are a key strategic priority. With their experience in addressing regulatory challenges and personalized medicine, blood services are key to the development of cell therapies. It is important to realise that blood-derived EVs have some advantages over EVs derived from various other sources, such as cultured cells or cancerous cells, as blood is readily available in relatively large amounts. Extensive knowledge of blood cell biology, including blood groups, provides a valuable foundation for understanding and working with blood derived EVs.

The Finnish Red Cross Blood Service is centrally responsible for the blood supply throughout Finland, distinct from the more decentralized models in many countries. This centralized structure presents many advantages to implement novel treatments and services nationwide, but it has demanded to develop its logistics to serve entire country.



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