

EDITORIAL

## The International Society for Extracellular Vesicles launches the first massive open online course on extracellular vesicles

Cecilia Lässer<sup>1\*</sup>, Clotilde Théry<sup>2</sup>, Edit I. Buzás<sup>3</sup>, Suresh Mathivanan<sup>4</sup>,  
Weian Zhao<sup>5,6,7</sup>, Yong Song Gho<sup>8</sup> and Jan Lötvall<sup>1</sup>

<sup>1</sup>Krefting Research Centre, Institution of Medicine at Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; <sup>2</sup>Institut Curie, PSL Research University, INSERM U932, Paris, France; <sup>3</sup>Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary; <sup>4</sup>Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Victoria, Australia; <sup>5</sup>Sue and Bill Gross Stem Cell Research Center, Chao Family Comprehensive Cancer Center, Edwards Lifesciences Center for Advanced Cardiovascular Technology, Department of Biomedical Engineering, University of California, Irvine, Irvine, CA, USA; <sup>6</sup>Department of Biological Chemistry, University of California, Irvine, Irvine, CA, USA; <sup>7</sup>Department of Pharmaceutical Sciences, University of California, Irvine, Irvine, CA, USA; <sup>8</sup>Department of Life Sciences, POSTECH, Pohang, South Korea

The International Society for Extracellular Vesicles (ISEV) has organised its first educational online course for students and beginners in the field of extracellular vesicles (EVs). This course, “Basics of Extracellular Vesicles,” uses recorded lectures from experts in the field and will be open for an unlimited number of participants. The course is divided into 5 modules and can be accessed at [www.coursera.org/learn/extracellular-vesicles](http://www.coursera.org/learn/extracellular-vesicles). The first module is an introduction to the field covering the nomenclature and history of EVs. Module 2 focuses on the biogenesis and uptake mechanisms of EVs, as well as their RNA, protein and lipid cargo. Module 3 covers the collection and processing of cell culture media and body fluids such as blood, breast milk, cerebrospinal fluid and urine prior to isolation of EVs. Modules 4 and 5 present different isolation methods and characterisation techniques utilised in the EV field. Here, differential ultracentrifugation, size-exclusion chromatography, density gradient centrifugation, kit-based precipitation, electron microscopy, cryo-electron microscopy, flow cytometry, atomic-force microscopy and nanoparticle-tracking analysis are covered. This first massive open online course (MOOC) on EVs was launched on 15 August 2016 at the platform “Coursera” and is free of charge.

Keywords: *exosomes; microvesicles; extracellular vesicles; education; massive open online course; International Society for Extracellular Vesicles; Coursera*

\*Correspondence to: Cecilia Lässer, Krefting Research Centre, Institution of Medicine at Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, Email: [cecilia.lasser@gu.se](mailto:cecilia.lasser@gu.se)

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Cells release several different types of vesicles, collectively called extracellular vesicles (EVs) that can take part in cell-to-cell communication. One of the first observations suggesting the presence of EVs was made as early as in the 1940s, when it was discovered that platelet-free serum contained a clotting factor (1), later demonstrated to be 20–50 nm sized, lipid-containing particles (2). Furthermore, in the 1970s and 1980s, studies showed that (a) ~50 nm vesicles could be identified in serum, (b) 30–500 nm vesicles could be found in prostatic fluid and seminal plasma and (c) maturing reticulocytes could release ~50- to 100-nm-sized vesicles formed in the

endocytic pathway, which were described as virus-like particles, prostasomes and exosomes, respectively (3–8).

Since this early work on EVs, several new vesicles have been identified and assigned various names including microvesicles, microparticles, ectosomes and oncosomes. EVs have now been described to be released by all cells investigated and their presence in several body fluids has been demonstrated. During the last 20 years, the interest for the biological role of these vesicles has increased exponentially (9). Therefore, a workshop, International Workshop on Exosomes (IWE), was held in Paris in 2011, and during this meeting, it was decided that the International

Society for Extracellular Vesicles (ISEV) should be established ([www.isev.org/](http://www.isev.org/)). Since then, the society has organised numerous meetings and workshops to allow for researchers in the field to interact. As a next step in developing the EV field, ISEV has decided to produce a series of educational massive open online courses (MOOCs). An MOOC is an online course where recorded lectures and presentations are used. It is open access via the World Wide Web and can be accessed by an unlimited number of participants.

The first ISEV-produced MOOC, “Basics of Extracellular Vesicles,” was launched on 15 August 2016 at the platform “Coursera” ([www.coursera.org/learn/extracellular-vesicles](http://www.coursera.org/learn/extracellular-vesicles)) in collaboration with the University of California Irvine (USA), University of Gothenburg (Sweden) and Pohang University of Science and Technology (South Korea).

### Course content

The course is divided into 5 modules, where the leading experts in the field provide online lectures within their area of expertise (Table I). During the first module of the course, the field of EVs is introduced. EVs are heterogenous in their biogenesis, cargo, function and distribution. Therefore, topics that are covered during the introduction week are the nomenclature for the different subpopulations of EVs as well as an introduction to the diversity of organisms releasing EVs and the tissues and body fluids where EVs can be found. Furthermore, one of the pioneers, Professor Emeritus Philip Stahl, shares the story about how he and his colleagues discovered exosomes in the early 1980s (4).

The second module focuses on the biogenesis and release of EVs and how this differs between the EV subpopulations: exosomes and microvesicles. Additionally, the different uptake mechanisms of EVs when they are encountered by a recipient cell are covered in depth (10). As EVs have been shown to contain functional RNAs, proteins and lipids, this module also covers the different types of molecules present in EVs as well as a brief overview on what the potential functions of these molecules are. Furthermore, the techniques that are commonly used to detect these molecules and to analyse the cargo of EVs will be highlighted.

In the third module, the focus is on the collection and processing of cell culture media and body fluids prior to isolation of EVs. Here, considerations and guidelines that are important during the collection of the EV-containing material and when isolating EVs from these fluids are discussed (11,12). This module will help the students to reflect over the many different choices, such as anticoagulants, collection time points and protein inhibitors, which are important for the outcome when working with a particular body fluid compared with conditioned media or other body fluids. This module also illustrates some

examples of studies on EVs from body fluids such as blood, urine, breast milk and cerebrospinal fluid and why it is of interest to analyse EVs from these bodily fluids.

The fourth module highlights the most commonly used methods for isolating EVs. Here, the basic concepts and some guidelines for methods such as differential ultracentrifugation, density gradient centrifugation, size-exclusion chromatography and kit-based precipitation are covered. Furthermore, this module covers how the techniques are used in the field of EVs as well as their limitations and benefits. The importance of evaluating the heterogeneity, purity and characteristics of the isolated vesicles regardless of isolation method is also highlighted (13).

The fifth module covers some of the different techniques that can be used to characterise EVs. Here, the basic concepts for techniques such as electron microscopy, cryo-transmission electron microscopy (cryo-TEM), flow cytometry, atomic-force microscopy (AFM) and nanoparticle-tracking analysis (NTA) are covered. Furthermore, this module covers how the techniques are used in the field of EVs as well as their limitations and benefits.

### Who is the course for?

This course is recommended for anyone interested in the field of EVs including biology and medical students and PhD students without previous experience in the field as well as clinicians, cell and molecular biologists and researchers who want to broaden their understanding of the field and deepen their knowledge about particular techniques.

### Course format

The course contains 5 modules, where each module contains 4–7 recorded lectures (6–35 min/lecture). Each module contains in total 1–2.5 h of recorded materials, and all lectures are in English. Each of the 5 modules is followed by a quiz in the format of multiple choice questions. Each of the 5 quizzes is worth 20% of the grade. The passing threshold for each quiz is 70%.

### Learning outcomes

After completing the course, the student should be able to:

- discuss the nomenclature and subgroups of EVs,
- describe the release and uptake mechanisms of EVs,
- describe the RNA, protein and lipid content of EVs,
- explain the considerations that are important during the collection and isolation of EVs from different body fluids,
- describe the basic concepts about the most common isolation and characterisation techniques and how these techniques are used in the EV field and
- state the benefits and limitations of the most common isolation and characterisation techniques for EVs.

Table 1. Summary of lectures included in the course.

| Lecture #  | Title of lecture   | Lecturer             | Affiliation  | Length of lecture |
|--|--|----------------------|--|-------------------|
| <b>Week 1: Introduction to the course and the field of EVs</b>   |  |                      |  |                   |
| Lecture 1  | Introduction to the field of EVs   | Jan Lötvall          | University of Gothenburg, Sweden                               | 13 min            |
| Lecture 2  | Introduction to the course   | Cecilia Lässer       | University of Gothenburg, Sweden                               | 11 min            |
| Lecture 3  | The origin of EVs throughout the phylogenetic tree   | Yong Song Gho        | Pohang University of Science and Technology, Republic of Korea | 11 min            |
| Lecture 4  | History of exosomes and EVs  | Philip Stahl         | Washington University in St. Louis, United States              | 32 min            |
| Quiz 1   | <i>Introduction to EVs</i>   |                      |  | 9 questions       |
| <b>Week 2: Biogenesis, cargo and uptake of EVs</b>   |  |                      |  |                   |
| Lecture 5  | Biogenesis and release of EVs  | Suresh Mathivanan    | La Trobe University, Australia                                 | 18 min            |
| Lecture 6  | Mechanisms of EV uptake – Part 1   | David Carter         | Oxford Brookes University, UK                                  | 22 min            |
| Lecture 7  | Mechanisms of EV uptake – Part 2   | David Carter         | Oxford Brookes University, UK                                  | 23 min            |
| Lecture 8  | The protein content of EVs   | Suresh Mathivanan    | La Trobe University, Australia                                 | 23 min            |
| Lecture 9  | The RNA content of EVs   | Andrew F. Hill       | La Trobe University, Australia                                 | 35 min            |
| Lecture 10   | The lipid content of EVs   | Edit I. Buzas        | Semmelweis University, Hungary                                 | 18 min            |
| Quiz 2   | <i>Biogenesis, cargo and uptake of EVs</i>   |                      |  | 15 questions      |
| <b>Week 3: Collection and processing of cell culture media and body fluids prior to isolation of EVs</b> |  |                      |  |                   |
| Lecture 11   | Cell culture media   | Cecilia Lässer       | University of Gothenburg, Sweden                               | 13 min            |
| Lecture 12   | Blood plasma and serum   | Kenneth W. Witwer    | Johns Hopkins University, United States                        | 15 min            |
| Lecture 13   | Breast milk  | Esther Nolte-’t Hoen | Utrecht University, The Netherlands                            | 8 min             |
| Lecture 14   | Urine  | Lesley Cheng         | La Trobe University, Australia                                 | 25 min            |
| Lecture 15   | Cerebrospinal fluid  | Julie A. Saugstad    | Oregon Health & Science University, United States              | 15 min            |
| Quiz 3   | <i>Collection and processing of cell culture media and body fluids prior to isolation of EVs</i> |                      |  | 18 questions      |
| <b>Week 4: Methods for isolating EVs</b>   |  |                      |  |                   |
| Lecture 16   | Differential ultracentrifugation – Part 1  | Cecilia Lässer       | University of Gothenburg, Sweden                               | 15 min            |
| Lecture 17   | Differential ultracentrifugation – Part 2  | Cecilia Lässer       | University of Gothenburg, Sweden                               | 16 min            |
| Lecture 18   | Density gradient   | Su Chul Jang         | University of Gothenburg, Sweden                               | 12 min            |
| Lecture 19   | Size exclusion chromatography  | Rienk Nieuwland      | Academic Medical Center, The Netherlands                       | 14 min            |
| Lecture 20   | Kit-based precipitation  | An Hendrix           | Ghent University, Belgium                                      | 12 min            |
| Lecture 21   | Summary of isolation methods for EVs   | Cecilia Lässer       | University of Gothenburg, Sweden                               | 6 min             |
| Quiz 4   | <i>Methods for isolating extracellular vesicles</i>  |                      |  | 11 questions      |
| <b>Week 5: Techniques for characterisation and quantification of EVs</b>                                 |  |                      |  |                   |
| Lecture 22   | Electron microscopy – Part 1   | Johanna Höög         | University of Gothenburg, Sweden                               | 9 min             |
| Lecture 23   | Electron microscopy – Part 2   | Johanna Höög         | University of Gothenburg, Sweden                               | 14 min            |
| Lecture 24   | Electron microscopy – Part 3   | Johanna Höög         | University of Gothenburg, Sweden                               | 6 min             |
| Lecture 25   | Cryo-TEM   | Alain Brisson        | University of Bordeaux, France                                 | 14 min            |
| Lecture 26   | Atomic-force microscopy  | Shivani Sharma       | University of California, United States                        | 19 min            |
| Lecture 27   | Flow cytometry   | Marca H. Wauben      | Utrecht University, The Netherlands                            | 25 min            |
| Lecture 28   | Nanoparticle tracking analysis   | Chris Gardiner       | University College London, UK                                  | 17 min            |
| Quiz 5   | <i>Techniques for characterisation and quantification of EVs</i>                                 |                      |  | 18 questions      |

Cryo-TEM, cryo-transmission electron microscopy; EVs, extracellular vesicles.

The initial response to the course has been overall positive with high ratings, and one student commented the course as:

This course was really well organized and paced but packed full of a lot of really good information from great sources and leaders in the field. I really didn't

know anything about exosomes before I started this course and now I feel like I can even teach the people in my own lab a few tricks.

We are pleased to see this initial feedback to the course and ISEV will now initiate the work of producing more

online courses on other related topics such as the biological functions of EVs in health and disease.

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## Conflict of interest and funding

This course was funded by the International Society for Extracellular Vesicles and supported by grants for pedagogic development from the Sahlgrenska Academy, University of Gothenburg. YSG is the inventor of patents for using EVs as therapeutics, diagnostics and vaccines and is the founder of Aeon Medix and Rosetta Exosome and own stock in the company. JL is the co-owner of patents for using exosomes as therapeutics and is currently an employee of Codiak BioSciences, Inc. in parallel with his position at University of Gothenburg. Other authors declare no conflicts of interest.

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