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EDITORIAL

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

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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. 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*

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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

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Society for Extracellular Vesicles (ISEV) should be established (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) 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 heterogonous 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.

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Table I. Summary of lectures included in the course.

Lecture #	Title of lecture	Lecturer	Affiliation	Length of lecture
Week 1: Introduction to the course and the field of EVs				
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	*Introduction to EVs*			*9 questions*
Week 2: Biogenesis, cargo and uptake of EVs				
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	*Biogenesis, cargo and uptake of EVs*			*15 questions*
Week 3: Collection and processing of cell culture media and body fluids prior to isolation of EVs				
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	*Collection and processing of cell culture media and body fluids prior to isolation of EVs*			*18 questions*
Week 4: Methods for isolating EVs				
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	*Methods for isolating extracellular vesicles*			*11 questions*
Week 5: Techniques for characterisation and quantification of EVs				
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	*Techniques for characterisation and quantification of EVs*			*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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