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Mini-Review

Key Problems in the Translation of Extracellular Vesicles Derived from Eukaryotic Prokaryotic Cells

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Abstract

Both eukaryotic and prokaryotic cells secrete extracellular vesicles (EVs). To meet the requirements of clinical application, the facile EVs were also prepared by various physical, chemical and biological methods to mimic the natural ones. Although some EVs have been at the phases of clinical trials, there are lots of challenges facing the clinical application of EVs. Herein, the most outstanding problems concerning the three stages of commercialization of EVs, including production scalability, quality control and immunotoxicity, were comprehensively discussed. In addition, some strategies toward solving these issues are also proposed.

Introduction

According to concept of ISEV (The international Society for Extracellular Vesicles), "extracellular vesicles (EVs) are defined as the particles naturally released from cells that are comprised of a lipid bilayer

membrane [1]. Acting as important mediators between cells that regulate both physiological and pathological conditions in the living bodies, EVs are nanosized spherical compartments and contain lipids, proteins and various nucleic acids of their source cells [2,3]. Based on their biogenesis and sizes, EVs are generally categorized into three types, including exosomes, microvesicles and apoptotic bodies. Exosomes are generated from endosomal compartments in the cells. The size of exosomes is around 30 to 100nm in diameter. Microvesicles are directly produced through outward budding and fission of the cell plasma membrane, and their sizes have a wide range between 50-2000nm. When cells are going to die or subject to apoptosis, the cell membrane is disrupted to form apoptotic bodies with the size of 50 to 5000nm [4,5].

Besides EVs derived from eukaryotes, prokaryotes also secrete EVs. It has been reported that Gram-positive and Gram-negative bacteria can both generate EVs [6-8]. Gram-negative bacteria spontaneously release EVs via shedding from outer membrane, so-called outer membrane vesicles (OMVs). Since Gram-positive bacteria do not have "outer membrane" when compared to Gram-negative bacteria, Gram-positive bacteria release OMVs from the inner membrane and the released membrane vesicles go through the cell wall and form so-called OMVs. The size of EVs from Gram-positive bacteria was reported to be ~20-100nm in diameter, which was similar to EVs derived from Gram-negative bacteria [8].

Inspired by the generation of naturally secreted EVs, many researchers are also seeking to prepare biomimetic EVs by physical [9,10], and chemical [11] methods in recent years. In general, the artificial EVs exhibit similar properties to the natural ones. As a supplementary to the natural EVs, biomimetic EVs possess some advantages in some aspects, such as purity, yield, targeting ability, etc.

There is no doubt that the purpose of all above efforts is to translate EVs and utilized their good properties to serve the humankind's healthcare. Though some application of EVs have been tried in animals, there are only few cases for their clinical use [12-14]. In this review, the most outstanding challenges in each stage of commercialization of EVs will be reviewed and the potential solving direction will also be discussed.

Challenges in Application

Although some clinical trials on the applications of EVs have been reported, there are still some challenges existing in the three necessary stages of commercialization, namely production, quality control and application (Figure 1). Here, three most remarkable problems of each steps will be specified.

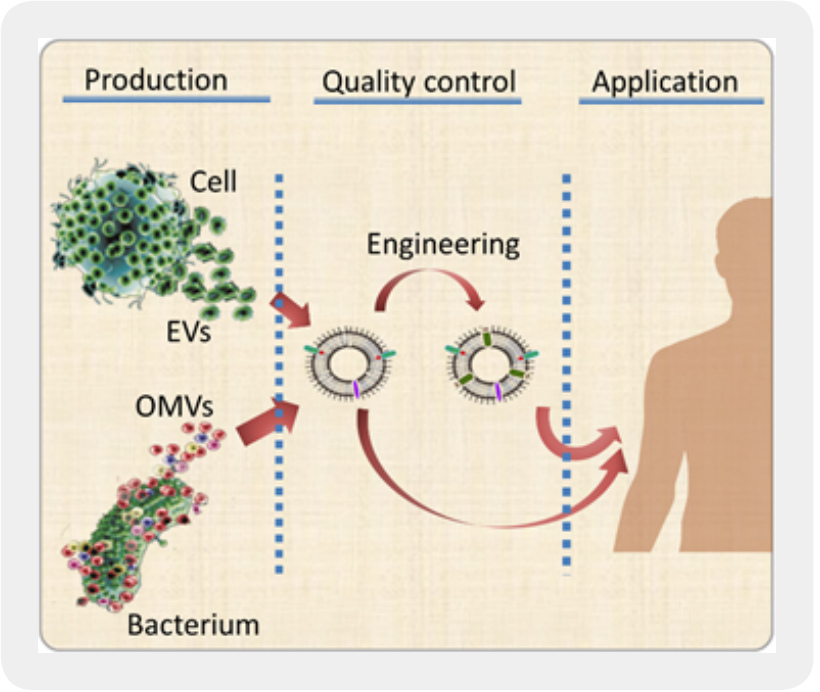

Figure 1: *The different stages of application of EVs and OMVs. EVs are secreted by cells and OMVs are generated by bacteria. The resulted EVs or OMVs may be applied directly and can be utilized after engineering. EVs, extracellular vesicles. OMVs, outer membrane vesicles.*

Manufacturing Issue-Scalability

First of all, it is necessary and urgent to develop a reproducible and scalable approaches to generate clinic-grade EVs [15]. Some strategies have been applied to improve the generation of natural EV, for example culture conditions, such as hypoxia [16], increased calcium concentrations in medium [17,18] and serum starvation [19] may increase the production of EVs. In addition, the continuous culture system has shown the high yield of EVs by 40 times compared to traditional culture flask settings [20]. To scale up the manufacturing process of EVs, a new method have been developed based on nitrogen cavitation to generate biomimetic nanovesicles, and the result showed that the production of EVs was 16-fold higher than naturally secreted EVs [21]. In addition, Gho *et al.* [22,23] also utilized extrusion and sonication to achieve the very high production of EVs by over 100 folds compared to natural secreting way. Although it looks like that the self-assembly of phospholipid and membrane proteins can form EVs-like nanoparticles [24] and their scalability is possible, this self-assembly process cannot guarantee the surface orientation of membrane proteins and isolated membrane proteins may not maintain conformations after they leave the cell membrane. To meet the basic requirements of biopharmaceuticals (such as repeatability, efficiency and costs), the bioreactor system [20] may hold the most promising perspectives in manufacture of EVs. Although the generating method is a little bit cost- and labor-consuming, the method can produce EVs efficiently in a continuous way. However, the success of this method is dependent on the physiological conditions, including aging, of cell culture.

Quality Control-Uniformity

Besides the necessary bioactive and targeting ingredients, there are also a lot of components in EVs, including DNA, RNA, proteins, carbohydrates, phospholipids, etc. These components can be affected by a lot of factors, including culture conditions, physiological status (as mentioned above), and manufacturing methods. However, there was rare reports regarding the difference between EVs generated from different methods. We once compared the components of naturally secreted EVs and artificial EVs by nitrogen cavitation, and some differences were found [21]. However, as a biopharmaceutical, or its carrier, the uniformity is critical for translation and an ideal method for this purpose is still lacking. The types and contents of each protein on EVs may be characterized by proteomics way, while types and contents of each phospholipid may be analyzed by phospholipids fatty acid spectrum.

Application Stage-Immunotoxicity

The last concern about the clinical application of EVs is the possible immunotoxicity induced by EVs. In the previous studies, we found the EVs generated from nitrogen cavitation can activate HUVECs, while the function can be mitigated by the loaded anti-inflammatory drug. The following studies also demonstrate that the immunotoxicity may be caused by the exposed phosphatidylserine, originally locating on the inner surface of cell membrane, due to the external forces [21]. To avoid the impairment from immunotoxicity, anti-inflammatory therapy may be necessary and helpful in some cases.

Conclusion

In the past decades, the research on EVs indicates that EVs are transforming the traditional nanoparticle-based drug delivery because EVs have the unique features. EVs have the excellent biocompatibility, long circulation time and low immunogenicity, and most importantly, they maintain their parent cell features that make them excellent drug carriers. Different to the conventional drug carrier, some EVs themselves perform as bioactive drug for disease therapy. Some special tissues, such as brain, can also be targeted by EVs, and no special targeting ligands are required. The above features confer EVs some outstanding advantages to be a drug carrier or disease treating agent.

In summary, this review summarized the current most eminent challenges facing each step of commercialization of EVs. With advances in nanotechnology and immunology and genetic engineering, industrialization of EVs may become more and more realistic and eventually realize, and EVs will eventually turn to be a powerful tool for therapy of many human diseases.

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