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Mini Review

BIOTECHNOLOGICAL AND THERAPEUTIC IMPORTANCE OF BACTERIAL VESICLES AND THEIR INHIBITORS

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Abstract

Bacterial cells release nanosized bag-like structures in the environment. These vesicles help bacteria in cell-to-cell communication within the community, interaction with the host cells, secretion of enzymes and toxins, self-defence, adaptation to environmental stress (e.g high salt, high temperature) and acquisition of nutrients. Accumulating evidences also indicate their involvement in antibiotic-resistance of bacteria. Their efficacy as a tool for drug-delivery, vaccine adjuvants and bioreactors has been demonstrated. Inhibitors of vesicle formation appear to be useful in clinical management of bacterial infections. Importance of vesicle research is emphasized in the Indian context.

Keywords : Bacterial vesicles; Outer Membrane Vesicles; OMVs; Communication; Virulence factors; Antibiotic-resistance; Drug delivery; Vaccine adjuvants; Inhibitors

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Bacteria release vesicles: Bacteria are unicellular organisms that occur everywhere. Some of them are pathogenic while the majority of them are benign. In fact, the harmless bacteria help us sustain our life in various ways. Some of these organisms are used in making various types of medicines, food materials and other useful chemicals. Some others are used for degrading waste products (bioremediation). Although individual bacterial cells appear to be independent entities, they live as the members of a community. In order to live in a society, we need to communicate with our neighbours. Bacterial cells also communicate among themselves using various tools. Outer Membrane Vesicles (OMVs) are one of the means of communication. They are small (diameter: 20-300 nm) bag-like structures released predominantly by gram-negative bacteria in the outer environment. Vesicle formation is also observed in some gram-positive bacteria (Jagannadham and Chattopadhyay, 2015).

Contribution of two Indian scientists: Though release of vesicles by the colon bacterium *Escherichia coli* was observed for the first time by D. G. Bishop and Elizabeth Work at the Twyford Laboratories, London in 1965, it was two Indian scientists viz, S.N Chatterjee and J.Das, who published more concrete evidence of vesicle formation for the first time in 1966 and 1967. In course of their electron microscopic studies on the cholera pathogen *Vibrio cholerae* at the School of Tropical Medicine in Kolkata, they observed some blebs on the outer membrane of the organism. In the face of scepticism expressed by other scientists on their observation, they continued their studies and established beyond any doubt that vesicle formation occurs in the logarithmic phase of the bacterial growth. They also proved that it is an active process and not the result of cell lysis or any structural deformity of the cells. Subsequently, vesicle formation was demonstrated in some other

bacteria, even in the tissues infected by some pathogenic bacteria (Jagannadham and Chattopadhyay, 2015).

Functional importance of bacteria vesicles: Extensive investigations during the past few decades have revealed that vesicle production is an integral part of survival of gram-negative bacteria. So far, it has not been possible to isolate a variant of any gram-negative bacterium that does not produce the OMVs. Their role in communication between individual cells in a bacterial community is already mentioned. They foster bacterial virulence by carrying virulence factors (like enzymes) the target which might be located far away from the site of infection. Also because of their small size, the vesicles could easily reach the host tissues and deliver toxins and other virulence factors. The vesicles carry hydrophobic proteins. Transport of these molecules in the aqueous environment of human body might be problematic otherwise. Vesicles also help bacteria in secreting more than one protein at a time. Vesicles protect the producer from the attack of bacteriophages (viruses that infect bacteria). When bacteria are challenged with some unfavourable conditions (e.g, high salt, high temperature), some proteins are denatured within the cells. Vesicles package these junks and transport them to the outer environment thus relieving the cell of the toxic burden. The vesicles help the producer organism to acquire nutrients from the surroundings. This is how pathogenic bacteria obtain iron in an iron-deficient environment encountered inside the human body (Kulkarni and Jagannadham, 2014). Vesicles also protect bacteria from antibiotics in various ways. In some cases, they carry antibiotic-inactivating enzyme. They are also found to carry the gene that encodes antibiotic-inactivating enzyme. Following incubation with such vesicles, antibiotic-sensitive cells of a bacterium were found to turn antibiotic-resistant by a group of researchers.

In some cases they appear to package the antibiotic and remove it from the cell. In some other cases, they enclose the antibiotic-inactivating enzyme and shield it from inactivation by anti-enzyme antibody, produced in our body (Chattopadhyay and Jagannadham, 2015). Vesicles produced by an organism were found to bind two membrane-active antibiotics at our laboratory. The drugs could not reach the target cells as a consequence (Kulkarni*et al.,* 2015).

Use of the vesicles as vehicle for drug delivery: Vesicles could be used as a vehicle for drug delivery. Aminoglycoside antibiotics (gentamicin, kanamycin, neomycin) are broad-spectrum antibacterial agents. But they are ineffective against some pathogens viz, *Shigella* spp., *Listeria* spp., and *Salmonella* spp. during intracellular growth of the organisms since mammalian cell membrane is impermeable to these drugs. It was observed by two researchers at the University of Guelph (Ontario, Canada) that vesicle formation by the bacterium *Shigella flexneri* (which causes diarrhoea in man) increased when it was treated with gentamicin and the vesicles were found to contain the antibiotic. When cultured human cells were incubated with gentamicin-induced vesicles containing the antibiotic, the vesicles were found to penetrate into the cells, deliver the antibiotic and kill the intracellular pathogens (Kadurugamuwa and Beveridge, 1998). This is a model example of using vesicles as a tool for the delivery of drugs which are otherwise unable to reach their site of action. Scientists are also looking into the possibility of delivering therapeutic proteins into the target cells using vesicles. Attempts have also been made from time to time to overproduce the protein of interest in the periplasm (the space between the inner and outer membrane of bacteria), so that during formation of the vesicles, the desired protein would be packaged into them and could be delivered to the target

cells. But this strategy is beset with some obvious limitations because the chance of a particular protein being packaged into the vesicles is not dependent only on its abundance. It appears more strategic to anchor the desired protein directly to the vesicles. It will also be a challenging task to get rid of the empty vesicles which will co-occur with the vesicles loaded with the desired protein.

The kinesin spindle protein (KSP) plays a crucial role in cell division. Hence inhibitors of KSP can arrest mitosis. Various KSP-inhibitors have been tested so far as potential anticancer agents. RNA-interference is a process that involves inhibition of gene expression using small double-stranded RNA. They are called small interfering RNA (si-RNA). Regression of tumour could be achieved in an animal model using some engineered vesicles with reduced toxicity to deliver si-RNA, specifically targeted to KSP (Gujrati *et al.,* 2014).

Vesicle-based vaccines: Bacterial OMVs carry and deliver proteins. This property can be exploited in the development of new vaccines. The vaccines are preventive medicines which carry whole cells or their products. They trigger immune response in the recipient leading to the production of some defence proteins called antibodies, which are preserved as a memory. When he is challenged with the infection, the antigen (foreign protein released by the pathogen) is recognized by the memory leading to the production of the relevant antibody in high amount. A substance that is added to a vaccine formulation to stimulate the production of antibodies is called an adjuvant. The OMVs contain immune stimulators like lipopolysaccharides (LPS), proteins and DNA. Hence they are good adjuvants. Vesicles derived from the pathogens have been used for a long time in the development of vaccines against the respective organisms. In general, the OMV-based vaccines

developed from gram–negative bacteria use detergent extraction to minimize the amount of LPS, which is toxic to the host cells. Several other strategies are being developed to produce OMVs from mutant strains containing detoxified LPS. Various methods are used for the production of engineered vesicles and the efficacy of the preparation in generating the specific antibody is observed. A vesicle-based vaccine against the causative organism of meningitis was approved for clinical use in Europe some time back. The prospect of the use of wild type OMV vaccines against MenB (a disease caused by group B strain of meningococcal bacteria) had been studied since the 1970s (Petousis-Harris, 2018). Pertussis (whooping cough) is a contagious bacterial infection characterized by severe coughing fits. It affects 16000 people worldwide every year and claimed 61000 lives in 2013. Earlier, killed cells of the causative organism (*Bordetella pertussis*) were used as a component of DTP (diptheria-tetanus-pertussis) vaccine. It was associated with some undesirable side- effects. In 1980, an acellular vaccine was developed. However, resurgence of pertussis infection in the twenty first century called for further development. An OMV-based vaccine was found to be safe and it also induced immunity against pertussis in mouse model (Asensio *et al.,* 2011).

Therapeutic potential of the inhibitors of vesicle formation: It is evident from the foregoing discussion that the vesicles have a potential role in antibiotic-resistance of bacteria (Chattopadhyay and Jagannadham, 2015;Kulkarni *et al.,* 2015). They also promote pathogenesis. Hence inhibitors of vesicle formation are likely to be helpful in clinical management of bacterial infections. A couple of years back, a team of researchers led by Professor Nobuhiko Nomura at the University of Tsukuba (Japan) reported the repressive effect of indole and some of its derivatives on

vesicle production by *Pseudomonas aeruginosa*. However, potential of these compounds as therapeutic agents needs to be established through extensive investigations (Tashiro *et al.,* 2010).

Other uses of the vesicles: The efficacy of a multi-enzyme catalysed process is dependent on the assembly of the enzymes. For example, out of the 8 enzymes involved in the Krebs Cycle (an intracellular process consisting of a series of chemical reactions to generate energy), 6 are organized in a supramolecualr complex. The organization facilitates the enzyme-substrate reaction by increasing the local concentration of the enzyme and the substrate. It also helps in channelling the intermediates between the consecutive enzymes and by avoiding interference from other reactions. It has been possible to achieve a 23-fold increase in glucose production by hydrolysis of cellulose using three enzymes assembled on bacterial vesicles, compared to the production that could be achieved using free enzymes (Park *et al.,* 2014).

Concluding remarks: Hence, it is obvious that bacterial vesicles have immense potential to be used for various biotechnological and therapeutic purposes. So far, very few Indian laboratories have undertaken studies on these aspects. Large-scale investigations appear to be the need of the hour to exploit these potentials. National symposia at different places of the country are likely to keep scientists abreast of the recent developments in vesicle research and also to generate new ideas.

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