

Virosome Technology

Pevion Biotech's virosome technology at a glance

- High-quality antibody responses
- Excellent safety track record
- Unique tolerability-immunogenicity equilibrium
- Suited for vulnerable populations
- Robust, flexible, semi-synthetic GMP manufacturing process, established at commercial scale

OVERVIEW

Vaccine development has continuously shifted away from live attenuated or inactivated whole organisms. Both approaches feature good efficacy but the risk/benefit ratio leaves room for improvement since their highly complex compositions inherently result in safety concerns. Subunit vaccines represent the next generation of vaccines, and provide equal efficacy with a superior safety profile. Subunit vaccines contain by definition only those fragments of the pathogen which are relevant for the induction of protective immunity. Therefore subunit vaccines offer a major growth opportunity in the vaccine business. The key to successful subunit vaccines is a suitable and safe adjuvant system, since small, isolated pathogen fragments by themselves are generally weak immunogens.

Pevion Biotech's virosome technology is a clinically and market approved carrier and adjuvant system designed specifically for subunit vaccines. Virosome-based vaccines have been approved by regulatory authorities in more than 40 countries, and of the three licensed vaccines more than 50 million doses have been distributed so far. This commercial validation provides the foundation to overcome the two major limitations in subunit vaccine development: in the first place, the availability of an adjuvant system with a highly favorable safety track record, which novel competing technologies cannot provide and second, the availability of an established, robust manufacturing process on a commercial scale, which poses a high entry barrier for many other approaches due to the complex nature of vaccine formulations as compared to small molecule drugs.

Pevion Biotech's virosome carrier and adjuvant technology represents a unique combination of technical versatility and clinically proven safety and immunogenicity. In contrast to the vast majority of carrier or adjuvant technologies in development, virosomes have a well-tuned equilibrium of efficacy and tolerability by means of their mode of action as a virus-like particle (VLP).

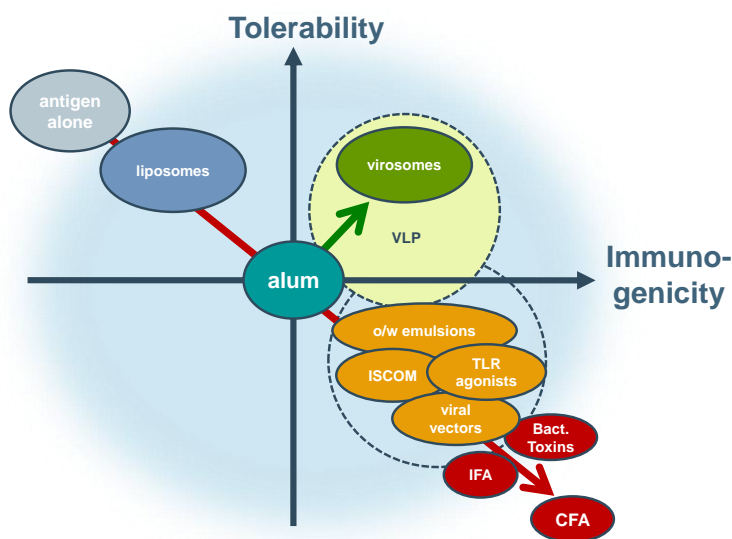
The proprietary manufacturing process clearly distinguishes Pevion Biotech's virosome technology from other VLP-based adjuvant systems. The *in vitro* assembly method of virosomes is unique and allows a precise and reproducible shaping of a VLP from defined components, which is a prerequisite for industrial scale manufacturing in accordance with good manufacturing practice (GMP) guidelines. The fully controlled *in vitro* process yields a highly pure final product, which translates directly into safety advantages.

NEED

State-of-the-art subunit vaccines have a tailored design and more and more use minimal, defined antigens, i.e. single proteins, small peptides, or carbohydrates. At the same time, stripping off all other components of a pathogen also means losing portions that are in fact needed to co-stimulate the immune system. Isolated subunits alone are rarely sufficient to evoke a complete immune response. Therefore, co-stimulatory adjuvants need to be added back in. Moreover, such isolated subunits need to be formulated with a carrier that protects them from untimely degradation and which allows efficient delivery and presentation to the right cells of the immune system. The challenge for new generation vaccines is to deliver subunit antigens to the right place and to properly trigger a meaningful immune response, all without overdoing stimulation and thus provoking adverse effects. These needs are even greater in population groups which do not respond sufficiently due to an

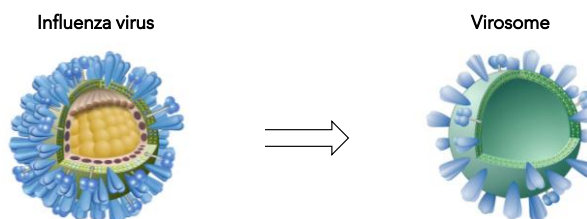
impaired immune system. Examples are the elderly (immunosenescence), infants (immature immune system), or immunosuppressed individuals (e.g. chronically diseased, AIDS or transplantation patients). At the same time, these populations are particularly vulnerable to adverse reactions.

To date, only five adjuvants for human use have been approved in Europe, and three in the US. Alum has been widely used for decades and remains the benchmark for novel adjuvants with regard to safety and potency, even though its properties are far from optimal. The vast majority of adjuvants show a negative correlation of immunogenicity and tolerability, depicted in the figure below. Many of the newly discovered adjuvants are indeed very potent, but at the same time bear the risk of increased reactogenicity, of severe acute reactions, or even of causing autoimmunity. Others may be safe, but lack efficacy. The one class of adjuvants which escapes this negative correlation are the virus-like particles (VLP), including the virosomes. The key to this escape is direct access to immune cells and their specific, balanced activation without triggering nonspecific inflammation.



VIROSOME BASICS

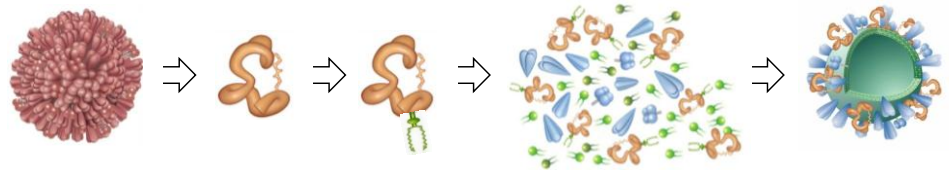
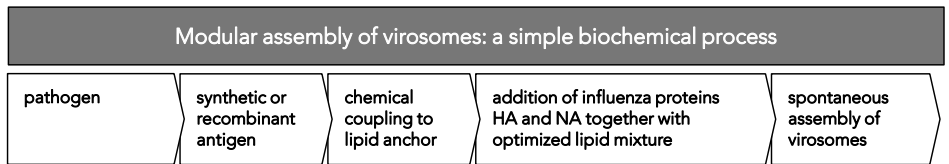
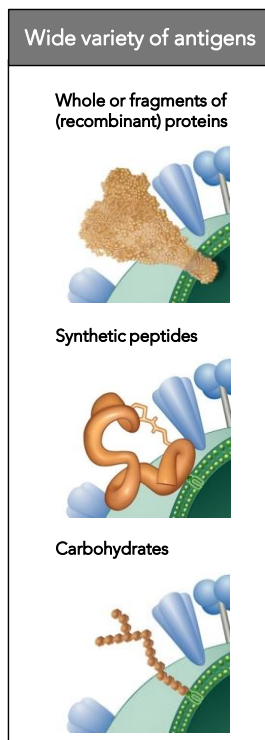
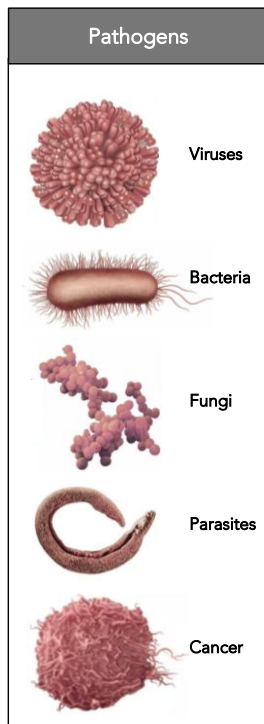
Virosomes are spherical, unilamellar vesicles with a mean diameter of 150 nm. Essentially, virosomes represent empty influenza virus envelopes composed of phospholipids and influenza virus envelope proteins, but they are devoid of internal proteins and genetic material of the parental virus.



Virosomes are unable to replicate but retain many properties of the parental virus with respect to the interaction with host cells. The viral proteins hemagglutinin (HA) and neuraminidase (NA), embedded in the spherical membrane, not only confer structural stability and homogeneity to the virosome particles, they also are the key to their immune-stimulating properties.

MODULAR ASSEMBLY

A virosome particle is a suitable carrier and adjuvant for a wide variety of antigens of interest, such as synthetic peptides, recombinant proteins, bacterial toxins, or carbohydrates. For optimal induction of antibodies against the antigen of interest, multiple copies of the antigen have to be displayed on the surface, tightly associated with the virosome structure. How this is achieved with highest efficiency depends on the biochemical properties of the antigen of interest.



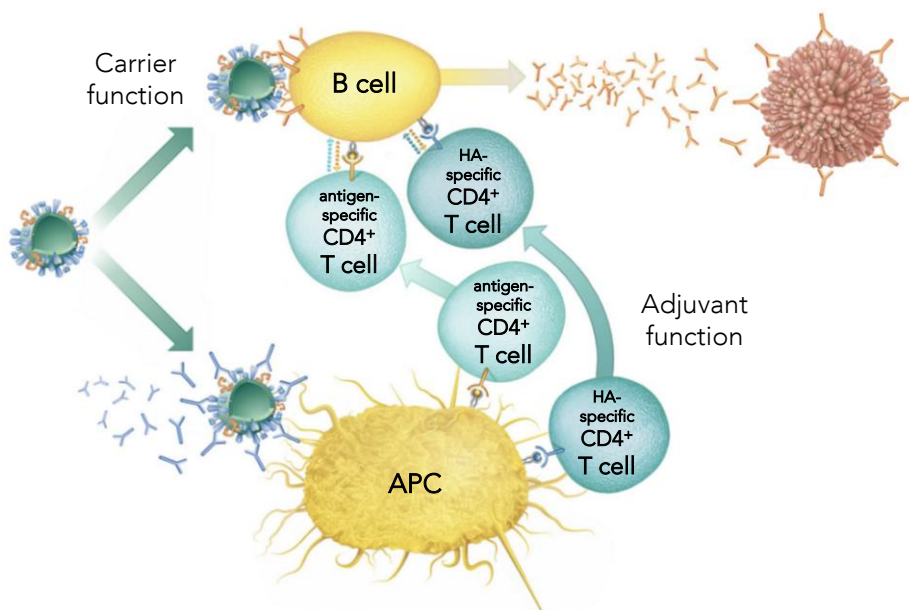
If the antigen of interest features a lipophilic domain (e.g. transmembrane proteins), it integrates directly into the virosome membrane without further modification. In most cases, however, the antigen has to be linked to a lipid molecule in order to anchor it in the virosome membrane. Pevion Biotech has developed extensive know-how in these conjugation methods, and has several approaches at its disposal. The source of the antigen (viruses, bacteria, fungi, parasites or cancer cells) is not relevant for the formulation, but synthetic or recombinant antigens are preferred for their higher purity and potential to focus the immune response on relevant epitopes.

The controlled assembly process allows a fine tuning of the concentrations and ratios between the individual components (antigen of interest, lipids, virus proteins) to achieve the desired product properties with respect to size, stability, and immunogenicity. Virosomes therefore represent a true platform technology with very broad applicability.

Virosome-based vaccines are a homogenous nanoparticle suspension. The final product is fully amenable to direct and precise physical and biochemical analysis. This is in sharp contrast to vaccines with alum or oil-based adjuvants, both of which interfere with many analytical methods. As a consequence, clear and precise product specifications for virosome-based vaccines can be defined early on, which translate into a more reliable and focused process development, thus providing a convincing database to the attention of regulatory authorities.

MECHANISM OF ACTION

Virosomes act both as a carrier and as an adjuvant, with multiple functions during the induction of an immune response. The carrier function comprises the positive effects of embedding the antigen into a higher structure, the virosome particle. The adjuvant function relates to immune-stimulating properties of the virosomes and their components on the immune system. Most importantly, virosomes succeed in stimulating specific immunity without causing nonspecific inflammation.



CARRIER FUNCTION

The integration of the antigen into the higher structure of the virosome particle stabilizes the antigen, preserves the native status of B cell epitopes, and protects the antigen from degradation. The antigen displayed on the virosomal surface mimics the original pathogen or target cell, and thereby favors the generation of antibodies relevant for protection. Moreover, the presentation of the antigen as a repetitive surface structure enhances its recognition by antibody-producing B cells. Finally, the size and surface structure of the virosome particles make them an attractive target for uptake and processing by immune cells, which is a crucial step in the initiation of an immune response.

ADJUVANT FUNCTION

Influenza virus is constantly circulating in the global human population, with an estimated annual infection rate of 20%. Therefore, the vast majority of people have some degree of natural, pre-existing immunity against influenza. For protection against influenza infection, a strong immunity specific for the infecting virus strain is necessary. In sharp contrast, a low level of immunity against any influenza strain is fully sufficient to support the adjuvant effect of virosomes. The adjuvant function of virosomes relies on the presence of influenza-derived envelope proteins, in particular the predominant hemagglutinin (HA). Basically, the influenza proteins provoke an immunological déjà-vu effect, and thereby enhance the immune response against the antigen of interest. In addition, there is increasing evidence that virosomes directly activate innate immunity, a powerful and essential enhancer of an adaptive immune response against any antigen.

The déjà-vu effect comprises both the humoral and the cellular immunity against influenza.

- Pre-existing antibodies against influenza bind to virosomes and tag them efficiently for rapid uptake and processing by antigen presenting cells (APC). The natural function of antibodies is to bind virus and block infection. Yet, the very same mechanism is beneficial for the virosomes as adjuvant, because antibodies specifically target virosomes to APC, which is essential and ideal for the induction of adaptive immunity.
- Pre-existing influenza-specific helper T cells are activated by those APC displaying the processed fragments of the influenza proteins. Activated helper cells rapidly proliferate and secrete cytokines to support and enhance the induction of effector immune cells, e.g. antibody-producing cells.

Since the antigen of interest and the influenza proteins are tightly associated on the same virosome particle, any APC-processing virosomes will present both the antigen of interest and the influenza proteins simultaneously. As a result, the induction of immunity against the antigen of interest within a given B cell is supported not only by helper cells specific for the antigen of interest, but further enhanced by the co-activated pre-existing influenza-specific helper cells.

EFFICACY

Virosome-based vaccines have a proven efficacy in humans with various classes of antigens:

- Pevion Biotech's bivalent malaria vaccine PEV3 generated robust and long-lasting antibody titers in both adults and children, with a dose of only 10 µg peptide antigen. The antibodies recognized the authentic parasite protein and were functional, as demonstrated by parasite growth inhibition and inhibition of invasion assays.
- Pevion Biotech's trivalent breast cancer vaccine PEV6 induced antibody responses in 7 out of 10 women with breast cancer and aged 55 to 84 years, again with a dose of 10 µg per peptide. The antibodies induced recognized the authentic tumor-associated antigen Her2/Neu and inhibited tumor growth *in vitro*.
- The hepatitis A vaccine Epaxal® by Berna Biotech/Crucell is composed of virosomes with inactivated hepatitis A virus adsorbed to the surface. Epaxal® conferred 100% protection against HAV infection in pre-licensure studies. Epaxal® induces a higher antibody response with less antigen, with a faster onset, and a longer duration of seroprotection in comparison to the alum-adsorbed competitor vaccine Havrix® (GSK).
- The seasonal influenza vaccine Inflexal® V has been registered in Europe and other countries by Berna Biotech/Crucell. Its immunogenicity is similar to split or subunit vaccines in healthy adults. Several studies suggest superior immunogenicity in viable populations (in the elderly, individuals with chronic diseases).

SAFETY AND TOLERABILITY

Over 50 million doses of the registered virosome-based vaccines Epaxal® and Inflexal® V have been distributed to date. Both vaccines have an excellent safety track record. Both vaccines have a clinically proven superior local tolerability over the competing products. The comparison between the hepatitis A vaccines (virosome-based Epaxal® versus alum-adsorbed Havrix®) is particularly striking, with three clinical studies independently showing a three-fold reduction of pain at the injection site (20% vs. 60% of vaccinees).

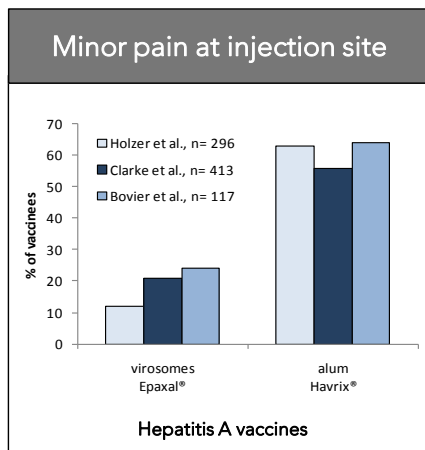
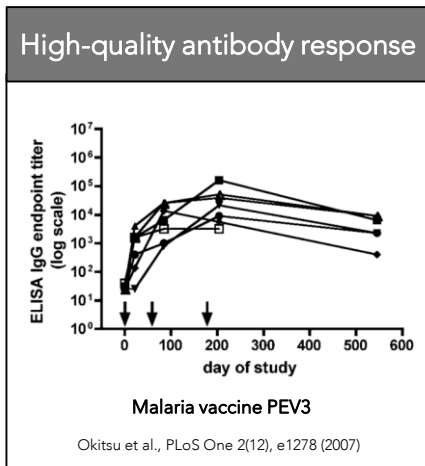
Pevion Biotech's peptide vaccines, including the lyophilized and multivalent vaccines, have been tested in four clinical trials comprising a total of 150 subjects. All safety and tolerability endpoints were met in each study, and the data were comparable to the favorable safety profiles of Epaxal® and Inflexal® V.

Virosome-based vaccines were tested and found to be very well tolerated in vulnerable populations (Inflexal® V in infants, children, elderly, chronically diseased; Epaxal® junior in children; malaria vaccine PEV3 in children; breast cancer vaccine PEV6 in elderly patients). Furthermore, subcutaneous and intracutaneous injection of virosome-based vaccines were tested and found fully acceptable.

MANUFACTURING

Biologicals in general and vaccines in particular are highly complex products. The challenge is to establish a sleek, robust, cost-effective manufacturing process which yields a precisely defined nanoparticle structure composed of multiple components for medical use, the virosome.

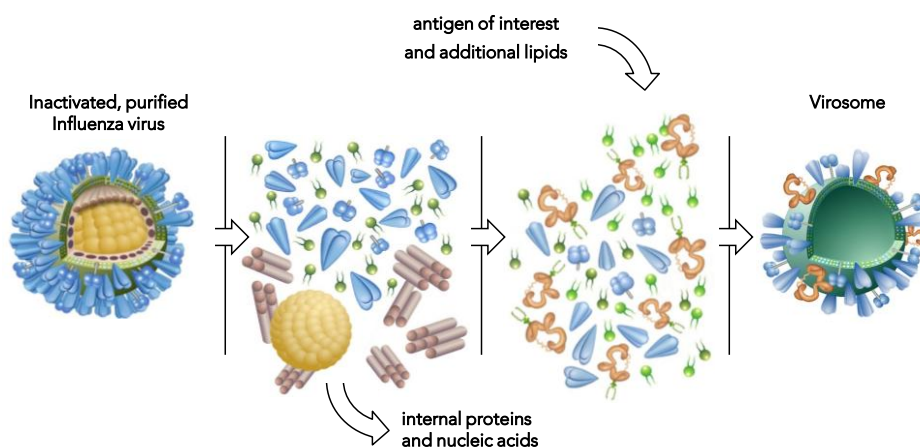
The individual components of the vaccine are produced separately and are subsequently assembled into virosomes. Synthetic or purified lipids, synthetic



peptides, and recombinant proteins are sourced from GMP-certified providers. Since inactivated influenza virus used for virosome manufacturing is the same material as used for the commercial influenza vaccines, it can be purchased from influenza vaccine manufacturers. To date, all virosome-based vaccines tested in humans were produced with influenza virus grown in embryonated chicken eggs. However, virus grown on cell culture has proven equally applicable for formulation of virosomes.

The actual formulation process of virosome-based vaccines, i.e. the *in vitro* assembly of the virosome particles, is a straightforward four step procedure. Essentially, the influenza virus is dissolved by detergent treatment, and the insoluble complexes of inner proteins and nucleic acids are removed, yielding a solution containing the viral envelope proteins and lipids. Subsequently, the lipid components and the antigen of interest are added to the solution. Finally, the detergent is removed by batch chromatography, which causes the hydrophobic components to assemble spontaneously into virosomal particles. Under Pevion Biotech's optimized conditions, these particles are highly homogenous and can be used without further purification.

Semi-synthetic assembly of virosomes: a simple biochemical process



Most importantly, this generic formulation procedure can be applied to virtually any antigen, such as synthetic peptides, recombinant or pathogen-derived proteins, or carbohydrates.

The manufacturing process of virosomes is established on a industrial scale and under full GMP compliance, as confirmed by the continuous production of Epaxal® and Inflexal® V. The *in vitro* assembly of the virosomes is performed at high concentration and thus, in a small volume, thereby allowing for large scale production of up to 500 000 doses per run even in small facilities.

To request further information, please contact.

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