Enhancing Delivery of Peptides with Exosomes

Reconstituting with exosomes leverages their biological capabilities to enhance the efficacy of peptides by improving the peptides solubility and their delivery to cells.

When peptides have low solubility in water, they may not dissolve properly, leading to uneven distribution and dispersion, reducing their effectiveness. Further, poorly water soluble peptides can also degrade faster, thus limiting their potential.

By combining peptides with exosomes, their ability to mix with water improves significantly. This ensures that **exosomes-loaded peptides are more evenly distributed in the body, increasing their molecular stability, and their effectiveness** when reaching the cells. In addition, loading peptides in exosomes can maximize their delivery and entrance into cells, thus improving their benefits.

Exosomes, which are small extracellular vesicles, facilitate the targeted delivery and entrance of bioactive molecules into specific cells, thereby facilitating the effectiveness of peptides in cells. This method capitalizes on the natural cellular communication pathways intrinsic to exosomes, promoting tissue regeneration, controlling inflammation, and improving overall cellular function.

Maximize Benefits with Exosome-Reconstituted Peptides

- **Enhanced Cellular Delivery:** Exosomes increase the likelihood of peptides reaching their target cells, improving the efficiency.
- **Improved Solubility and Distribution in body:** Human tissues are mainly composed of water, thus solubility of partial hydrophobic peptides is enhanced, allowing for easier movement within the body and more uniform distribution in our solution.
- **Augmented Peptide Entry:** Exosome association helps the cellular entry of peptides, ensuring they reach intracellular targets more effectively.
- **Increased molecular Stability and Half-Life:** Hydrophobic peptides exhibit higher stability and extended half-life when inserted into exosomes. Exosomes also shelter the peptides, protecting them from rapid degradation by extracellular enzymes, therefore enhancing their duration in the blood and other body tissues.
- **Regenerative Benefits:** The benefits of exosomes are well-documented, supporting tissue repair and regeneration by delivering peptides to damaged or inflamed tissues.

Scientific Basis for Improved Solubility

Water, known as the universal solvent, determines how well different compounds mix. Some compounds mix well with water (hydrophilic), while hydrophobic ones have aversion to water, becoming insoluble in water.

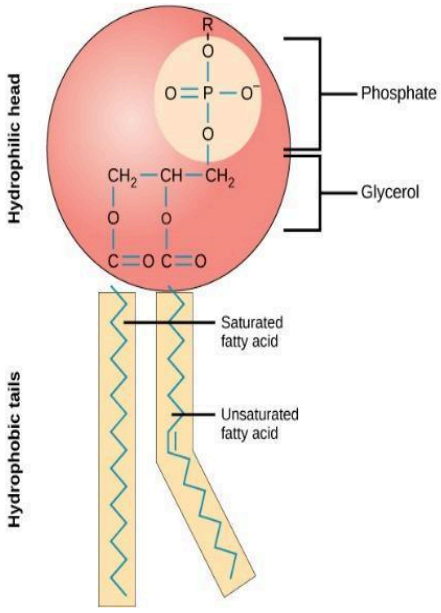
Solubility of any compound is determined by its capacity to interact with the solvent molecules, therefore this physical property is crucial when mixing compounds in aqueous solutions.

Hydrophilic Molecules (water soluble)

Hydrophilic molecules, such as glucose, can be easily dissolved in water due to their molecular structure capable of establishing chemical interactions (called hydrogen bond) with the water atoms (Hydrogen and Oxygen).

Hydrophobic molecules (water in-soluble)

On the other hand, hydrophobic molecules, such as oil, are unable to dissolve in pure water because their spatial structure can not perform these aforementioned hydrogen bonds. To escape the water, the hydrophobic molecules will directly interact with each other, physically isolating themselves from the water molecules (for example, oil in water will form small droplets or a single layer non miscible to water). This problem can be easily resolved if hydrophobic molecules are added to water in the presence of compounds which can contact both hydrophobic and hydrophilic moieties.

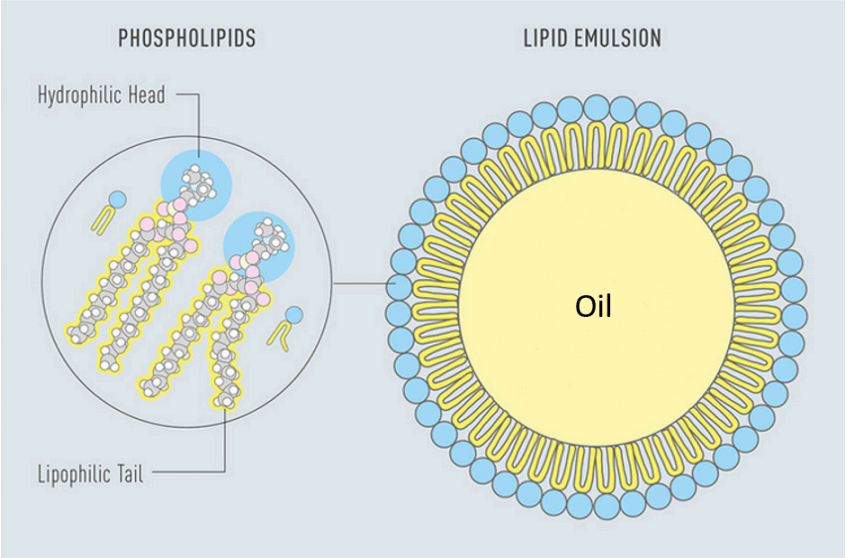


Improves Solubility, Phospholipids act as a bridge between water and Hydrophobic molecules

The best known and used compounds for this purpose are the phospholipids. Phospholipids are a class of lipids composed of 2 distinct domains: one side composed by a hydrophilic “head” (with affinity to water) while the other side is formed by 2 chain (“tails”) of lipids (fatty acid) which are hydrophobic and lipophilic by nature, as illustrated in figure 1.[1]

[1] Figure 1: A schematic illustration showing the phospholipid structure, with its hydrophilic part (hydrophilic head) and hydrophobic tail

For example, when oils and phospholipids are vigorously mixed in pure water, nanomicelles called lipid emulsion will be created in the solution, with the oil phase directly interacting with the hydrophobic / lipophilic tails, whereas the hydrophilic heads of phospholipids will contact the water molecules, as seen in figure 2.[2]



Solubility increases distribution in the body

In other words, phospholipids are excellent emulsifiers for permitting hydrophobic components to be dissolved in water.

Finally, lipid emulsions naturally occur in nature, such as milk, in where some hydrophobic micronutrients (eg. vitamins A, K, E, D) are passively associated with the milk fat[3]. For this reason, several vitamins are removed when fat is skimmed from the whole milk, thus requiring some vitamin fortification with addition of small quantities of oil containing an emulsifier[4]. Emulsions are not toxic and used in the preparation of several foods (eg. ice creams, mayonnaise)[5]. In this scenario, recent researches indicate that exosomes can be very useful to improve the solubilization of hydrophobic molecules in aqueous solution. In addition, exosomes are able to directly deliver hydrophobic molecules to cells, as discussed in the following section.

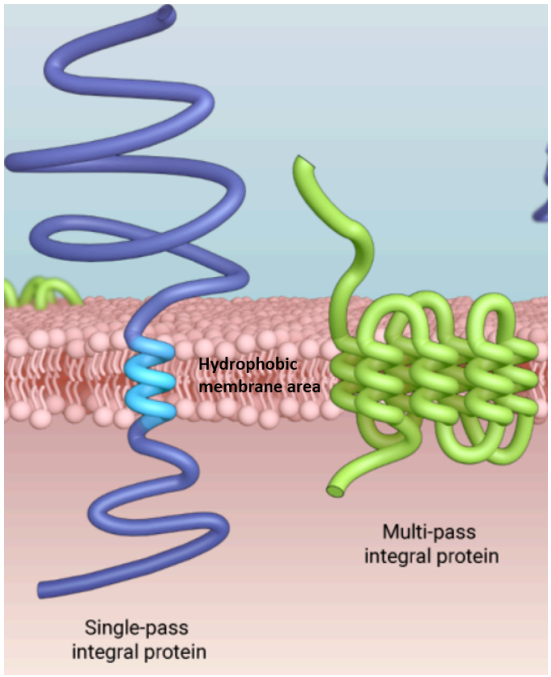
[2] Figure 2: An illustration showing phospholipid molecules (on left) composing the lipid emulsion. The phospholipid heads (in light blue) are hydrophilic and contact the water. The hydrophobic molecules, such as oil (shown in pale yellow), directly interact with the phospholipid tails. This micelle allows hydrophobic molecules to be soluble in water.
[3] Effect of emulsifiers and fortification methods on light stability of vitamin A in milk
[4] Effect of emulsifiers and fortification methods on light stability of vitamin A in milk
[5] Effect of emulsifiers and fortification methods on light stability of vitamin A in milk

Exosomes improve the delivery of peptides to cells

Exosomes are cell-derived extracellular vesicles (EVs) which work as intercellular messengers due to their ability to carry and transfer cargo molecules to recipient cells[6]. Exosomes can deliver their content to any cell type, and because EVs are naturally produced by cells, they are very biocompatible, tolerable and stable when injected in humans. Besides their natural constitution, an important concept is that exosomes can be artificially loaded with molecules, either packaged inside or associated with their membrane[7].

The most external part of EVs is composed by a phospholipid membrane containing proteins and cholesterol inserted into, in a composition very similar to the plasma membrane of their producer cells[8]. In particular, exosomal membranes, like biological membranes, are composed by 2 layers of phospholipids diametrically oriented for having the "tails" interacting with each other in a hydrophobic moiety.

In the membrane-bound vesicles, the hydrophilic "head" is exposed to the extracellular and intracellular milieu, which are environments mainly composed of water. This characteristic orientation of membrane phospholipids creates a hydrophobic/lipophilic moiety that is very important for the solubility of hydrophobic compounds, like peptides. Contrary to proteins that are large, **peptides are a short sequence of amino (typically 2 to 50) and an example of molecules that can be loaded into exosomes.**



As already explained in the previous topic, total or partial hydrophobic peptides in aqueous solution will passively find their better moiety to be miscible with. In the presence of EVs, this hydrophobic / lipophilic moiety is given by the phospholipid tails, thus the **hydrophobic / lipophilic molecules will passively immerse into these membranes to escape the water (depicted in figure 3[9])[10].**

[6] Engineering exosomes for targeted drug delivery
[7] Engineering exosomes for targeted drug delivery
[8] Engineering exosomes for targeted drug delivery
[9] A conventional membrane with proteins inserted into. Note that the membrane is composed by a phospholipid bilayer oriented to keep their hydrophobic tails contacting themselves. If the peptide or protein has a hydrophobic part, it will passively insert into the biomembrane to directly interact with these phospholipid tails. A protein (single-pass integral protein) containing hydrophilic parts (represented in dark blue) is shown. In this case, its hydrophobic areas (represented in light blue) are inserted into phospholipid tails (hydrophobic membrane area), thus escaping water interactions. Another protein (multi-pass integral protein) has several hydrophobic motifs inside the phospholipid tails.
[10] A Microscopic View of Phospholipid Insertion into Biological Membranes

Hydrophobic Peptides can use Exosomes and Liposomes to improve absorption to cells

To summarize, hydrophobic peptides will insert themselves into membrane-bound vesicles (like exosomes and liposomes) when added in aqueous solutions containing EVs[11].

As the main advantage, peptides-loaded EVs will have their delivery to cells facilitated in several orders of magnitude by 4 reasons:

1. Peptides will ride inserted into the exosomes, using them as nanocarriers;
2. Will enter the cells after membranes fusion;
3. Exosomes-associated peptides have low dispersion in the milieu if compared to their free forms;
4. Molecular stability is frequently increased if the compound is in its right moiety, thus hydrophobic peptides should have a higher stability when associated with exosomes.

Finally, we emphasize that liposomes and exosomes fuse their membrane to the plasma membranes of recipient cells or can be taken up through phagocytosis, micropinocytosis, and endocytosis mediated by lipid raft, caveolin, or clathrin[12]. This process ensures the delivery of their content directly inside the cell (eg. cytoplasm), meaning that cell penetration of any molecule will be better if it is already inserted in exosomal membranes.

Reconstitution XO Solution ***

Injection / Nasal	2mL	5mL	10mL
5% Exosomes	$129	$228	$367
10% Exosomes	$211	$413	$697
20% Exosomes	$376	$784	$1,357

Six months BUD when refrigerated and one year when frozen.

Learn more

[11] Liposomes: structure, composition, types, and clinical applications
[12] Engineering exosomes for targeted drug delivery