Design of polymeric nanoparticles selectively directed to the blood-brain barrier for depression treatment

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Major depressive disorder (MDD) has been considered by the World Health Organization as the third cause of the burden of disease worldwide in 2008, expected to become the first cause by 2030. This implies a worsening of life quality in a huge part of the population, since it produces a persistent low mood, lack or decreased interest in enjoyable activities, lack of energy, concentration, appetite, sleep…, turning into a big burden for society, being one of the leading causes of disability worldwide. It is believed that MDD is a multifactorial disease, recently associated with neuroregulatory systems dysregulations, causing disturbances in neurotransmitter systems, such as the ones involving serotonin, norepinephrine and dopamine. [1]

The primary problem of treating brain diseases is the lack of accessibility caused by the presence of the blood-brain barrier (BBB), a structure composed by endothelial cells that interact with pericytes, astrocytes, neurons and microglia. This barrier is highly regulated, with only some molecules being able to cross it, either due to their small size, or their interaction with a receptor.[2] For this reason, nanomaterials provide properties such as drug loading capacity, passive or active targeting, biodegradability and biocompatibility, which help the delivery of multiple substances into the brain. In this case, poly-(beta-aminoester) (pBAE) polymers are used. After a modification of their ends with oligopeptides (**Figure 1**), they acquire positive charge that allows them to electrostatically encapsulate genetic material, interact with cells and be able to escape the endosomes.[3]

In this project we work with pBAE nanoparticles, modified with four amino acid peptides consisting of one cysteine followed by three lysines, histidines or arginines (CKKK, CHHH, CRRR); which give the polymer a positive charge. A second modification being done is the addition of targeting peptides, that direct the system towards receptors overexpressed in the BBB. The peptides being used are SEQ12, a twenty amino acid long peptide, targeted towards the low-density lipoprotein receptor; and T7, a seven amino acid long peptide, targeted towards the transferrin receptor [4]. A second version of pBAE was synthesized by the introduction of a zwitterionic moiety on the side chain, firstly by a Steglich esterification with a chain transfer agent, followed by the addition of a sulfobetaine chain that is simultaneously positively and negatively charged (**Figure 2**). This zwitterionic modification is used to give the nanoparticle stealth characteristics, and thus after combining it with the polymers containing the targeting moieties, having a directed delivery system. [5]

After polymer modifications, different combinations of them are made either to improve their interactions with cells, or their endosomal escape, or their targeting abilities. The combinations are formed into nanoparticles by complexing with the genetic material of choice (DNA, mRNA, miRNA…). These formulations are characterized by Dynamic Light Scattering and proven to be correct when they have a size around 200 nm and a polydispersity index lower than 0.3. In this project we used a mixture of the polymer containing lysine (K), histidine (H), SEQ12, T7 and zwitterionic (Kz) in different proportions, generating the following formulations: KH, KHSEQ12, KHT7, KzH, KzHSEQ12, KzHT7 (60/40, 55/40/5, 55/40/5, 30/70, 25/70/5, 25/70/5 respectively).

To test the affinity of the different formulations for the BBB, we selected different cell lines, that in vitro, express the receptors we chose to target, such as BEAS-2B, CaCo-2, HeLa and BBMVECs. We confirmed the expression of the receptor on these cell lines by Western Blot, at different passages. Another experiment we carried out was studying the transfection efficiency and uptake of our desired formulations on these cell lines, also testing and proving their lack of cytotoxicity.

First results showed that after 4 h all the nanoparticles have been internalized by BBMVECs. On the comparative studies between cell lines, the transfection efficiency is higher for the nanoparticles that do not have the zwitterionic moiety, and in those that do contain it, when they also have the targeting peptides, their transfection increases. This is close to what we expected, since the addition of the zwitterionic moiety, generates stealth nanoparticles that should not interact with cells, but when we introduce targeting peptides to these formulations, they have a receptor-mediated interaction with cells, and not only by charge.

Therefore, we can say that out of the different formulations tested, the ones containing targeting peptides have a higher transfection efficiency and that those containing zwitterionic interact way less with the cells. By these results, we managed to formulate nanoparticles that are directed towards the brain and should reduce the secondary effects due to their stealth characteristics.

Next steps will study the most adequate genetic material-based loading to become therapeutic against MDD.

References

[1] L. E. Curley, J. C. Lin, and T. F. Chen, “Major Depressive Disorder,” *Encycl. Pharm. Pract. Clin. Pharm. Vol. 1-3*, vol. 1–3, pp. V3A-672-V3A-685, Apr. 2023.

[2] S. Liebner, R. M. Dijkhuizen, Y. Reiss, K. H. Plate, D. Agalliu, and G. Constantin, “Functional morphology of the blood–brain barrier in health and disease,” *Acta Neuropathol. 2018 1353*, vol. 135, no. 3, pp. 311–336, Feb. 2018.

[3] C. Fornaguera *et al.*, “In Vivo Retargeting of Poly(beta aminoester) (OM-PBAE) Nanoparticles is Influenced by Protein Corona,” 2019.

[4] M. J. Ramalho, J. A. Loureiro, M. A. N. Coelho, and M. C. Pereira, “Transferrin Receptor-Targeted Nanocarriers: Overcoming Barriers to Treat Glioblastoma,” *Pharmaceutics*, vol. 14, no. 2, Feb. 2022.

[5] C. Garcia, “Stealth graft polymers for nanoparticle based mRNA vaccines,” IQS, 2023.

Figures

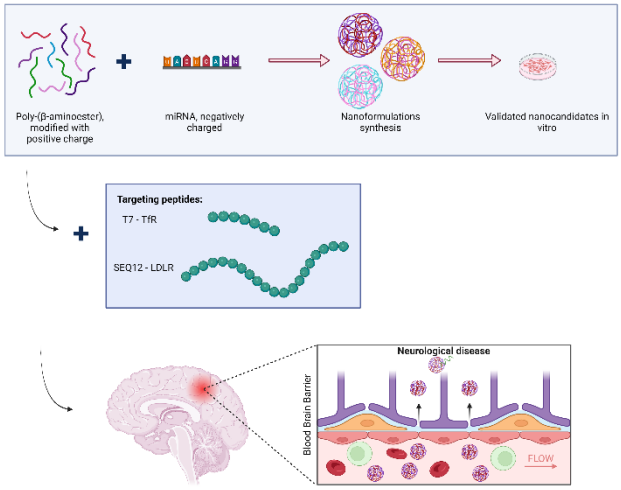

Figure 1. Polymer description of nanoparticle formulation, and peptide structures to direct the delivery system towards the brain.

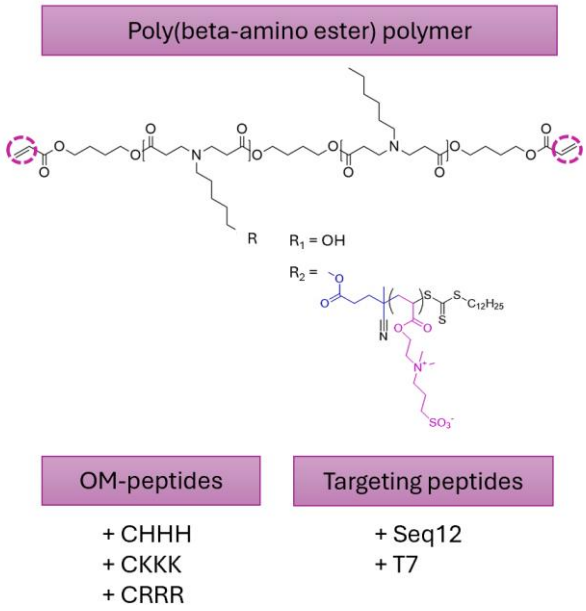

Figure 2. Poly-(beta-aminoester) polymeric main structure, with a 5-amino-1-pentanol chain and a hexylamine chain or a zwitterionic moiety, followed by the different possibilities for positively charged oligopeptides, and targeting peptides.