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Nanomedicine to fight amyloid aggregation in neurodegenerative diseases

La nanomedicina para combatir la agregación amiloide en enfermedades neurodegenerativas

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La vida no es la que uno vivió, sino la que uno recuerda, y cómo la recuerda para contarla.

Gabriel García Márquez

Antes de todo, me gustaría agradecerle a mi tutora, Ana Belén Caballero, la ayuda que me ha dado a lo largo de este trabajo. Sus consejos y sugerencias han sido una gran guía, desde las explicaciones para que entendiera mejor los conceptos hasta las ideas para mejorar el trabajo y la forma de expresarme. He encontrado el tema muy interesante y, por esa razón, también agradezco el haber tenido la oportunidad de haber podido aprender sobre ello. Quiero dedicar una mención especial a aquellos profesores que consiguen hacer más amenos los estudios y despiertan el interés de los estudiantes. Al COVID-19, sin embargo, no le puedo agradecer nada ya que no ha hecho más que causar muchas dificultades.

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REPORT

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1. SUMMARY

This work is about nanomodulation in amyloid protein aggregation, which is associated with various neurodegenerative diseases, such as Alzheimer's disease. Although the exact cause why these pathologies occur is still unknown, in the latest years, nanomaterials have been studied and it was concluded that they might target protein aggregation and accumulation effectively.

Following the amyloid protein and conformational diseases relationship, this work explains a new approach to fight neurodegenerative pathologies. In order to do so, it is divided in three sections. First of all, there is a review of what is nanomedicine, its development and its current state of knowledge. Then the characteristics and properties of nanomaterials are exposed, as well as their possible interactions with proteins since they present a promising potential. At last, amyloid protein folding and its interactions are studied in more detail.

Once these sections are reviewed, it is discussed the main question of this work, which is whether amyloid aggregation can be modulated or not through synthetic chaperones based on nanoparticles.

Keywords: nanomedicine, nanotechnology, nanoparticle, protein, amyloid, interaction neurodegenerative disease, conformational, Alzheimer, nanomodulation, chaperone

2. RESUMEN

Este trabajo está centrado en la nanomodulación de la agregación de la proteína amiloide, la cual está relacionada con varias enfermedades neurodegenerativas, como la enfermedad de Alzheimer. A pesar de desconocer el mecanismo exacto por el que se dan, en los últimos años se ha comprobado que los nanomateriales pueden ser efectivos contra la agregación proteica y su acumulación.

Debido a la relación entre la proteína amiloide y las enfermedades conformacionales, este trabajo expone un nuevo enfoque en la lucha contra las enfermedades neurodegenerativas. Para ello, se ha llevado a cabo una revisión empezando por explicar qué es la nanomedicina, en qué consiste y su estado actual. Además, se exponen las características y propiedades de las nanopartículas, así como también los distintitos aspectos a tener en cuenta para su uso y las diferentes interacciones que pueden darse con las proteínas. De esta manera, se muestra el gran potencial que abre este campo en el tratamiento y la diagnosis de las enfermedad conformacionales. Por último, se estudia en mayor profundidad el plegamiento de la proteína amiloide y sus interacciones.

Una vez revisada toda la información, se discute la cuestión principal de este trabajo, que trata sobre la posibilidad de modular la agregación amiloide a través de la síntesis de chaperonas artificiales basadas en nanopartículas.

Palabras clave: nanomedicina, nanotecnología, nanopartícula, proteína, amiloide, interacción, enfermedad neurodegenerativa, conformacional, Alzheimer, nanomodulación, chaperona

3. INTRODUCTION & OBJECTIVES

In the last decades, medicine has developed in an incredible way thanks to research that has widened our knowledge and technological progress. This has improved our health and lifestyle. Nevertheless, there are still some unanswered questions remaining. One of the most common diseases which has a huge impact on worldwide population is Alzheimer's disease. Even though it affects many people, currently there is no cure for it. This is a first sign to realise how hard it is to treat this pathology. This work explains one of the latest developments in medicine, which consist in nanoparticles and how can this establish a new reality for medicine, the impact of Alzheimer's disease on population and presents a possible new approach for its treatment.

This work reviews the amyloid cascade hypothesis of Alzheimer's disease as well as some of the possible strategies to its treatment based on nanoparticles. Thus, following that information, it will be analysed and discussed several topics:

- General concept of nanomedicine and nanotechnology, the advantages as well as the inconveniences that they present.
- Relationship between amyloid aggregation and neurodegenerative diseases, taking the main focus on Alzheimer's disease.
- Nano-modulation in amyloid aggregation. Explaining how proteins and nanoparticles interact and a study of chaperones.

Once all the data is gathered, it will be possible to answer the real question of this work, which is if nanoparticles are an acceptable method to fight Alzheimer's disease by modulating amyloid protein aggregation.

4. NANOMATERIALS FOR THERAPEUTIC APPLICATIONS

4.1. NANOTECHNOLOGY AND NANOMEDICINE

Nanotechnology refers to the control of matter below 100 nanometres. Nanotechnology first appeared in 1959, in a lecture given by the American physicist Doctor Richard Feynman. He explained how in the future it would be possible to control matter, in a molecular or atomic size, in order to create structures.

In 1977, it was invented an atomic layer deposition which allowed for depositing a layer with a thickness equal to a single atom. In the beginning of the 1980s, the scanning tunnelling microscope was invented. Both instruments are still in use; however, when Doctor Drexler, an American engineer, published his book "Engines of creation: the coming era of nanotechnology" the vision of materials and instrumentation changed. [1] Nanotechnology can be classified in nanomaterials and nanomachines. It is important to highlight that neither of them excludes the other, in fact in most cases they are related since a lot of nanomaterials are used in nanomachines.

There are several definitions to describe what a nanomaterial is. [2] [3] Nevertheless, the two most common definitions are:

- Material having one or more external dimensions in the nanoscale or which is nanostructured.
- Materials in the size range of 1-100 nanometres.

There is another definition adopted by the European Commission that considers the material's surface area to 60 m^2/cm^3. [4] [3] This lack of agreement in the scientific community can be problematic since it can be a handicap in its regulation. But limiting it to just a definition might imply failing to consider important aspects. However, although this may not be a consensus, nanomaterials are not something new. Some of them have already been discovered, such as fullerenes or carbon nanotubes. Moreover, there are six well-known methods to produce

nanomaterials: chemical vapour deposition, ball mining, plasma arcing, sol gel, electrodeposition and natural nanoparticles. [5]

Nanoparticles are one of the main elements in this study. They are considered as a subcategory of nanomaterial, being a more specific material within nanoscale. Nanoparticles present various factors that have to be taken into account when it comes to their synthesis. Some of them are geometry, size and surface. **(Fig.1)** It will be explained later on, how its characteristics play a crucial role on their future applications and properties.

The discovery of nanotechnology and its implications led to a large new number of possibilities, technologically speaking. For example, it is present in batteries, sensors, magnets, etcetera. It was seen that combined with other scientific fields, such as biology, it could be revolutionary. This was the origin of nanomedicine.

Beginning of nanomedicine

The first appearance of the term *nanomedicine* was in the late 1990s. It was defined as the medical application of nanotechnology according to the knowledge of the body. The whole nanomedicine field is still incredibly new and innovative, there is still much to research and understand, although there are some applications in the market for burns or cancer treatments.

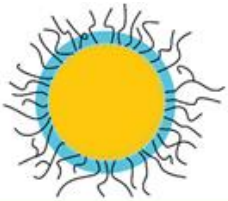
Anyway, the real goal to be achieved with nanomedicine consists in the research of diagnosis and possible treatments for different diseases. The great milestone is to arrive to a personalized and tailored treatment to everyone. This would be one giant leap for medicine.

4.2. COMPARISON BETWEEN NANOMATERIALS AND TRADITIONAL (MOLECULAR) DRUGS

The combination of nanomaterials in the human body is still a field that requires a lot of research due to its newness. It is highly important to know how they would react and how to control them. Because of that, design and engineering are such a relevant issue in its development. Compared to traditional drugs, nanomedicine is much more complex due to the difficulty of manipulating nano scale materials.

Nanomaterials present some characteristics that function well in external applications to the body. However, these properties are unspecific once they interact with organic molecules. Thus, if these nanomaterials want to be introduced into a biological system, there are several factors that must be considered such as surface, porosity, and shape among many others, because even a little change in these factors can completely modify its properties. **(Fig.1)**

As far as these materials are concerned, nanoparticles are the ones which seem more promising for medical applications. Nanoparticles (NPs) are normally constituted by three distinguished parts. Its compartments are: the core where drugs can be inserted; the shell, which can have different drugs form the ones in the core, and finally the surface. **(Fig.2)** Since nanoparticles can transport drugs, they are useful in a therapeutic manner. For this reason, and also because our health and safety are at stake, they must be assessed thoroughly. Unfortunately, there is still no standardized characterization for nanoparticles. [6]



Physical properties	Chemical composition	Targeting ligand
•Size •Geometry •Surface charge (zeta potential) •Elasticity •Stiffness •Porosity •Surface curvature, roughness and hydrophobicity •Electronic, magnetic and optical	•Liposomes (natural and synthetic lipids) •Micelles (amphiphilic biomaterials) •Dendrimers (branch biomaterials) •Polymeric NPs (degradable or non-degradable) •Protein NPs (natural or synthetic) •Viral NPs (engineered virus anes and viral proteins) •Exosomes and natural membrane NPs •Metal and metal-derived NPs •Carbon nanomaterials (carbon allotropes) •Hybrid NPs (combination of two or more)	•Small molecules •Antibodies and fragments •Aptamers and nucleic acids •Proteins and peptides •Sugars

Figure 1. Nanoparticle's properties and structure

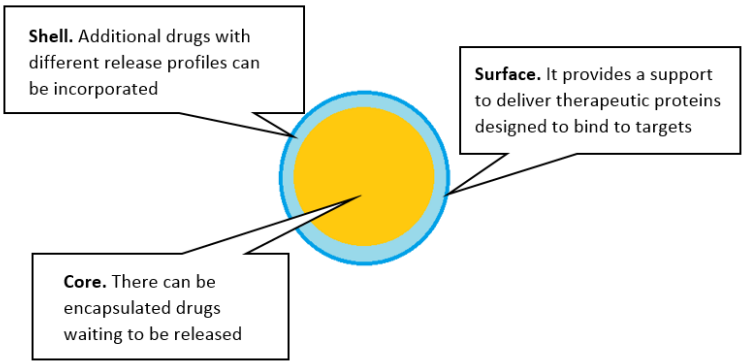


Figure 2. Representation of the different parts of a nanoparticle

There are several institutions in charge of the approval of these products, like the Food and Drug Administration (FDA) or the European Medicines Agency (EMA).

Disadvantages

1) Synthesis and characterization. Nanomedicine is based in complexes each one of which has a specific function. [7] Therefore, it requires sophisticated instruments and tests to evaluate, quantify and characterize the interactions between components. Besides, they must present physicochemical and pharma kinetics parameters extremely stable, accurate and precise to be suitable for biological behaviour. Due to the vast range of properties, to characterize these products becomes a problem. However, a polydispersity parameter can be used, which is really important, since the slightest change from one NP to another can imply different properties. [7] The polydispersity parameter is applied to detect heterogeneity of particles to know if there would be changes in secondary properties such as compatibility, drug date delivery or toxicity. Hence, reproducibility is a huge issue and it is extremely difficult to achieve, since human factor makes it impossible to have uniformity in the synthesis between batches. [8]

2) Biological barriers. Those substances with a major size are inserted through intravenous administration but normally, small ones are delivered orally. When it comes to the design of nanoparticles, it has to be considered that human body presents mechanisms to protect us from external agents that may be noxious for us. One of our most important developments is the blood brain barrier (BBB) that impedes treatment of central nervous system diseases. This is because it restricts the transport of 99% of large or hydrophilic molecules.

3) Regulation challenges. The pre-clinical to clinical stage that all these materials must pass is extremely rigorous. For every new component that may appear in the material, there must be a rationale behind it. [9] Besides, it must be proved that any element that constitutes the material has a specific function. Moreover, in order to be clinical approved, these NPs cannot be inferior in any matter to what it already exists. This means that multifunctionality might not be the best idea since their control can be difficult to assess. [9] These regulations apart from being time consuming are expensive too, but the most noticeable issue is that guidelines for regulatory bodies are quite poor.

4) Manufacturing. In large-scale manufacturing, controlling stability and physicochemical properties is challenging. Regular pharmaceutic industry does not have experience with these products. This implies a limitation to the manufacturing and scaling-up of nanomedicine.

Reproducibility is crucial to ensure nanomaterials are accurate for their use, which is why understanding its behaviour helps to know how to approach their preparation. There are two types of approach to prepare NPs: [7]

- Top-down: manufacture nano structure from larger ones.

- Bottom-up: mix or arrange small components to achieve a functional assembler.

Automatization of the process should be the next step in the fabrication chain. This could solve both production problems between batches and the heterogeneity between NPs. Nevertheless, this requires huge investment from industries, and generally this only happens when they know it would be profitable for them. Therefore, there will still be some years of waiting.

Advantages

1) Easy elimination. As nanomaterials in medicine are thought to treat diseases, an important characteristic they should present is biodegradability and biocompatibility. Besides, due to their small size, they do not present any problem to be eliminated following natural processes such as through kidneys via urine. [10]

2) Drug delivery. It consists in the transport and delivery of drugs using a NP as a vehicle. This leads to the possibility of targeting specific locations in an active or passive way. The difference between these two strategies is that in the former a vector is added in order to direct the particle to the wished tissue. **(Fig.3)** Hence, it produces less side effects in the patient, whereas in the latter it depends on the circulation time. [11] This transport includes some critical steps to consider since it has to be stable enough during the route, then stick to the tissue and finally penetrate it. Anyway, recently smart particles that can go from stealthy to sticky have been designed. They use polymers such as polyethylene glycol (PEG) or zwitterionic ones. [6]

3) Precision in diagnosis and treatment. Having a good understanding in biomarkers in every disease state improves therapy. Thus, using NPs in order to detect and label noxious cells is essential for good diagnosis. This contributes to a better treatment that can be carried out in an early stage. Besides, NPs can be used in imaging as contrast, like in magnetic resonance, and this approach can enhance the detection sensitivity in imaging. [12] [6]

4) Targeting and selectivity. A controlled release can be beneficious to decrease the impact of toxicity and improve the selectivity of drugs. There are various methods to achieve this, for instance through pH-sensitive groups or by temperature, as well as magnetic fields. [6]

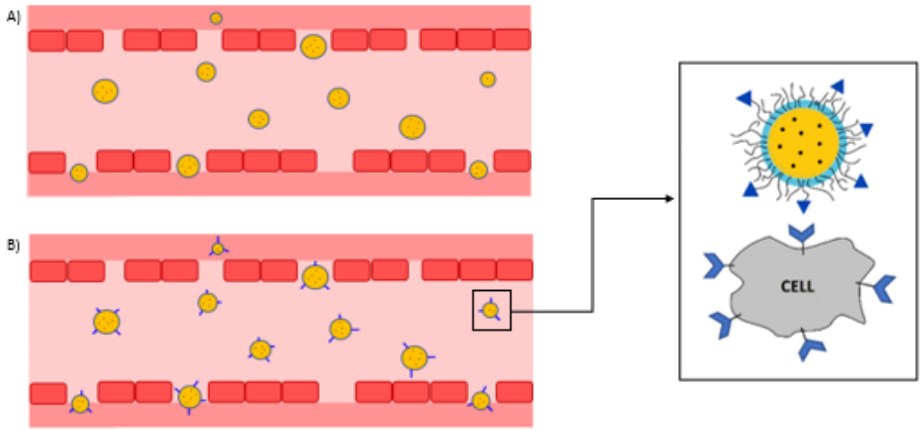


Figure 3. A) Passive targeting where NPs are introduced by diffusion via a leaky vascularization. B) Active targeting where NPs bind to specific cells using vectors

5) Innovation and improvement. Despite the fact that research has been mainly focused on drug delivery and cancer tumour, nanomaterials can be used in an extensive number of scenarios such as improving synthesis of drugs, enhancing drug solubility or monitoring devices. It can be used for organ implants too. [12]

All in all, nanomaterials present drawbacks as well as advantages. They should be contemplated to see if it weighs more in the benefits side. In that case, it should be taken advantage of nanomaterials when treating medical problems.

4.3. CURRENT SITUATION OF NANOMEDICINE

The study of nanomedicine has significantly increased according to the difference in the amount of publications between 1980s and 2004. [13] It has been enhanced in more than one hundred articles per year, with United States being the leader in patenting while Europe holds the first place in research. The most researched field was drug delivery with 59% of papers about it.

In a more recent study, it was observed that most part of research is still focused on the same topic, being the most of it addressed specifically to applications for cancer treatment, having almost two-thirds parts of the investigation, although it is reasonable considering that it is the worldwide main cause of death. [12]

The number of nanoparticles that have been developed should be highlighted. In this field, two kinds can be differentiated: hard or soft nanoparticles. Hard nanoparticles are those formed by iron oxide, silver, gold or ceramic. [12] On the contrary, soft NPs include liposomal, micelles, dendrimers or emulsions. Obviously, these materials have been showed as biologically compatible, the difference between using one material or another being the prevalence in the human body. Their size also plays a role in their prevalence in the human body, for example liposomes smaller than 70 nm stay less time than those who are 200 nm. [10] Generally, NPs are accumulated in liver and spleen.

As far as administration is concerned, there is special focus on emulsions and liposomes and heavy interest in intravenous administration. [12] There are other studied methods such as intracellular, subcutaneous or oral administration. Nevertheless, travelling mechanism and targeting are very sophisticated. There are two ways to approach this issue, one is through an active targeting which consist in biodistribution to a tissue without mattering its size. The other is passive targeting that depends on the geometry and size of the NPs. [12]

The main targeting mechanism is functionalizing nanoparticles in order to go unnoticed until they reach their goal, so that tricks the human body. This can be done by using ligands that receptors of the interested matrix would associate, coating NPs with sugars or any other substances that may be of interest to the tissue or cells that we want to attack. This mechanism using receptors is a clear example of active targeting which is currently used in some tumour treatments.

Nowadays, targeting is quite a successful field. Some smart NPs have been designed which can switch from a stealthy to sticky nature, just like some viruses do. [6] It has been able to mimic this mechanism using polymers with strong antifouling properties, the most common example of this being pegylated NPs. They are based on polyethylene glycol, a hydrophilic polymer that is added on the NPs surface in order to suppress protein adsorption due to steric hindering. [8] This polymer is biocompatible and functions as a barrier to the components present in the blood, creating a water barrier to avoid aggregations. Pegylated NPs present a high circulation time which boosts systemic delivery, but this behaviour can be affected by molecular weight or surface density. [14] These particles can be functionalized and enhance accumulation in tumour cells by a passive targeting.

The greatest progress in targeted antitumor therapy was thanks to the enhanced permeability and retention (EPR) effect that was first discovered in 1986 by Hiroshi Maeda, a Japanese chemist and pharmacologist. The study concluded that solid tumours present some blood vessels with defective structures producing an extensive amount of vascular permeability factors. This permeability allows for the tumour growing since nutrients, oxygen and other molecules reach it. [15] **(Fig.4)** Thus, EPR effect depends on the particles' molecular weight since larger molecules remain longer in circulation and big molecules have a selective accumulation in tumour tissues rather than in normal ones. Pegylated liposomes take advantage of this and are used for drug delivery. [16] In some studies, it was concluded that glucose in carbon nanotubes present a low cytotoxicity in vitro and in vivo and fructose has a high affinity and selectivity for breast cancer cells and healthy ones. [17] [18]

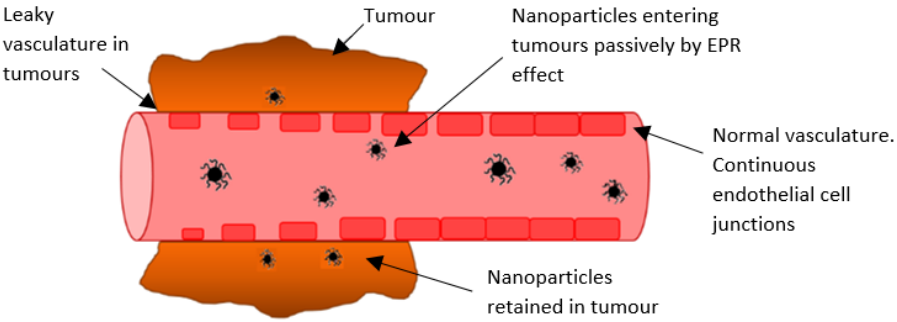


Figure 4. Schematic representation of the enhanced permeability and retention effect in solid tumours

Moreover, there are some other applications for NPs in the imaging field which are very practical in diagnosis. The most common material in imaging are quantum dots which are stable under light irradiation and presents long fluorescence time. [19] [6] Using luminescent particles allows for having more contrast. Magnetite or iron oxide NPs can be used as contrast agent in magnetic resonance since they change spin – spin relaxation time and are already accepted by the FDA. Gold nanoparticles are useful for contrast in X-Ray imaging and have been demonstrated to be capable to treat tumour under laser excitation. [6] [12] This could be a promising clinical use in the future in case of their approval. Moreover, a new NP has been recently designed to detect copper in the human body, which is a crucial pathogenic in Parkinson disease. [19]

All in all, there are still some existent problems with the lack of a clear definition and classification for nanomedicine products through FDA, nanomaterials synthesis is a complex process and manufacturing is expensive. However, it cannot be denied that there is a remarkable potential in this field and that for such a short time, important developments and applications which are only a scratch in the surface have already been achieved. Nanomedicine is a really futuristic and promising approach that is worth exploring further.

5. NANO-APPROACHES TO FIGHT ALZHEIMER'S DISEASE

5.1. NEURODEGENERATIVE DISEASES AND ALZHEIMER'S DISEASE

Medicine has greatly evolved through the years overcoming serious handicaps along the way. However, nowadays there are still some question marks involving really common diseases such as neurodegenerative ones. Neurodegenerative disease is a general term which englobes different afflictions mainly related to the central nervous system (CNS). They produce a progressive degradation of neurons or even their death, provoking several problems in our body such as cognitive loss or, in the worst-case scenario, dementia. There is a wide range of these diseases such as Alzheimer's disease (AD), Huntington disease or amyotrophic lateral sclerosis and unfortunately there is not a cure for them yet.

Apart from being classified as neurodegenerative diseases they can also be englobed as a conformational. These are characterized for being non-infectious, caused by an alteration in a protein most of the times due to a mutation. This can derive to form protein aggregations or destabilization in their native structure. The most common conformational disease is Alzheimer followed by Parkinson. Other examples are type II diabetes or Creutzfeldt-Jacob disease.

Alzheimer's disease was first studied in the 1900s by Alois Alzheimer, a German psychiatrist and neurologist. It is one of the most common pathologies around the world, affecting thousands of people. According to the World Alzheimer's report, in 2018 there were 50 million people affected by it and it is expected to increase to 82 million by 2030. There is the estimated rate that every three seconds a person is diagnosed with the disease. [20] This disease presents problems with

language, disorientation and mood swings as some of its symptoms. It is considered by the World Health Organization a public health priority due to the vast number of people suffering it. [21]

AD attacks progressively different zones of the brain. Firstly, it affects the frontal and temporal lobes, in concrete, atrophying hippocampus causing problems with language and memory, making it very difficult to remember some things or learning new ones. [22] [23] The hippocampus is located in the brain's temporal lobule, it is quite small and presents an elongated form. **(Fig.5)** Afterwards, it damages cortex surface which can evolve to the loss of emotional control, and lastly it affects the brainstem, which is in charge of automatic functions such as respiration or cardiac rhythm.

There are environmental and genetic factors involved in the appearance of AD. Its origin can be from familial or sporadic causes, and it has been seen that the prevalence due to familial causes is around 4-8% [24]. AD is an age-dependant disease, being twice more common in women than in men, contrary to what happens with Parkinson. [25] According to some studies the prevalence of AD in people above 60 years old is higher in the Middle East and Africa whereas Europe presents the lowest rate. This could be related to different lifestyles. [26] AD normally appears in people around 65 years old although sometimes it can come in the late 40s or early 50s, which is an early on set that only happens between 1-5% of the times. [27] According to some epidemiologic studies, AD derives in dementia in approximately only 1% of the cases between 60-65 years old. However, this rate increases exponentially with age. Moreover, there are other risk factors linked to dementia. For instance, obesity or smoking as well as vascular diseases. [28] [27]

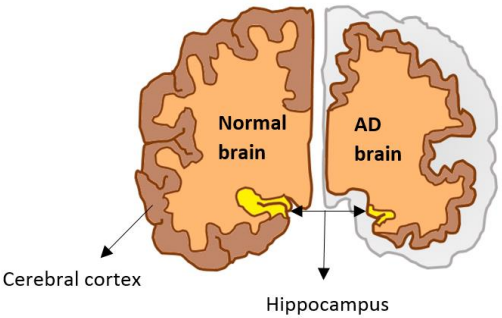


Figure 5. Representation of the structural changes in Alzheimer diseased brain

The impact of AD in the economy is huge. Just in the United States, it was estimated to cost US $ 818 billion in 2015. The cost of a patient is divided in two sections: formal services which

are in exchange of money, and informal ones, which are normally given from family or friends. [21] This disease not only affect the patient himself, but it also has a strong implication for his relatives in a financial, emotional and psychological manner.

Parkinson disease is the second most prevalent neurodegenerative and conformational disease, affecting around one percent of the population in 2014. This affliction is related to an amyloid protein, α-synuclein, which associates with others forming what are called Lewis bodies, contributing to the appearance and development of it.

5.2. ETIOLOGY OF ALZHEIMER'S DISEASE

There are some unknown aspects of Alzheimer's disease that are still under study. The one that will be discussed is the cause by which is originated. There are several hypotheses about it although one of them is more accepted than the others and it is thought as the most probable and accurate one. This will be the one explained in depth. [29]

Tau protein hypothesis

Tau protein is a soluble cytoplasmatic protein that binds to tubulin when it polymerizes in microtubules in neurons, therefore it is related to axonal transport. [30] This theory consists in the association with one another following paired helical fragments producing neurofibrillary tangles. The production of these tangles can be enhanced due to mutations in the mitochondrial DNA replication and insufficient ATP production. This protein is quite related to Alzheimer's disease since it also appears in a considerable amount in those people affected.

Mitochondrial cascade hypothesis

Mitochondrial dysfunction is caused by stress or proteasome dysfunction which leads to a low production of ATP due to mutations. This helps neuronal degeneration or synapsis abnormalities. Nevertheless, it has been observed that mitochondria play an important role in clearing aggregated proteins from itself. Therefore, it has a disposal trash function. Unfortunately, Aβ protein attached in synaptic mitochondria damages synapses. [31] This is due to a fragmentation and therefore, a change in structure in mitochondria.

Amyloid cascade hypothesis

This theory explains how it is due to a mutation in a gene, mostly the amyloid precursor protein (APP), although it can happen to presenilin-1 or presenilin-2 gene. There is an increase in the production of the Aβ42 peptide which presents an amyloidogenic nature. [23] [30] This means

that it has a tendency to form oligomers which would cause serious damages in synapsis processes. In addition, there is an imbalance between Aβ production and clearance. Firstly, it generates benign deposits to be then transformed into fibrils plaques. This provokes a neurotic dystrophy that widespread leading to cell death and dementia.

There is also another issue, worth to mention, which is oxidative stress. The central nervous system presents a high energy consumption rate, and this leads to free radicals such as reactive oxidative species or reactive nitrogen species which have been demonstrated to play an important role in AD. [32] [33] The CNS homeostasis includes oxidative stress regulation which is in charge of astrocytes. Oxidative stress occurs when there is a decompensation between production of free radicals and antioxidant processes in CNS.

All in all, the impact of these theories separately was promising when they were discovered, nevertheless neither of them explains completely why and how AD is caused. It is thought to be a combination of all of them, since there is evidence of how each one might be related to AD. For instance, it has been showed how in AD brains there are Aβ fibrils as well as tau proteins in a quite high proportion. [19] [34] Amyloid cascade hypothesis is the one that will be explored in this work since it is considered the most relevant one.

Nonetheless, the fact that there is one theory more popular than the others, does not suggest that the others should be ignored. Actually, this indicates that there are several factors implied in this pathology but focusing only on one issue and understanding it is more likely to end up in success rather than taking all of them at the same time.

5.3. HYPOTHESIS OF THE AMYLOID CASCADE

Around 1982 the term *prion* was first coined by Stanley Prusiner, an American neurologist, who defined it as a transmissible agent of spongiform encephalopathy with unconventional properties the mainly component of which is a protein. In the latest years, the term *prion-like* has appeared to define a misfolded protein with a diseasing mechanism. [34] Prions present various common properties, although they may be different substances since they can exist in a soluble conformation or in an aggregate form. They present a globular domain, a part of which has an unstructured sequence and asparagine and glutamine residues in a high rate. [35]

The amyloid cascade hypothesis emerged in 1984 from an isolation of a N-terminal amino acid sequencing a 43 amino acid peptide with a single carboxyl terminal threonine residue. [36]

There are more than 200 atomic structures of amyloid proteins or peptides in a fibrillar or crystalline conformation. However, all of them present a common β - structure. [37] At the beginning, it was difficult to determine their structure but thanks to solid state NMR and cryoelectronic microscopy techniques it was possible to know it in an atomic resolution. [38]

Alzheimer's disease β – protein, referred to as Aβ, accumulates in spherical microscopic deposits known as senile plaques, forming an aggregate. [30] These oligomers, which are macromolecule complexes with non-covalent bonding, might be one of the causes of AD, since they interrupt synaptic integrity. In the 1980s, a biochemist was able to isolate Aβ proteins from a post-mortem diseased brain and a high concentration of these assemblies was observed. However, it has also been proved that people without any symptom present cortical deposits of Aβ, but they are overall diffuse forms of amyloid plaques. [23] [30] **(Fig.6)** Furthermore, there is the inexplicable and disturbing fact of the lack of correlation between the amount of amyloid deposits and the advancement degree of the disease.

A correct folding allows for proteins being biologically stable; nevertheless, amyloid aggregation has been showed to present an important resistance to denaturalization. The conditions of temperature, pH or hydrostatic pressure that must be applied to achieve so are much rougher that the ones needed to denaturalize a globular protein. [38] In spite of this, Li et al. demonstrated how a sample in presence of a denaturant agent of amyloid fibril became sensitive to it and when it was removed the sample was degraded. Besides, in another sample, an amyloid seed with a denatured protein lead to a reassembly of amyloid fibrils reverting the degradation effect. [38]

Even though amyloid fibrils are toxic to neuronal cells, it is still not conclusive that they are the cause of pathogenesis since it is possible that their precursor such as protofibrils or low molecular weight oligomers is the responsible species. [35] Surrounding Aβ aggregates there are smaller diffusible assemblies that cannot be discarded as source of neuronal dysfunction. Some investigations agree that oligomers present a higher toxicity than protofibrils as well as Aβ peptides rather than mature fibrils. [39] Furthermore, according to a study, dimers and trimers of soluble Aβ oligomers disrupt learning behaviour in rats and might be responsible for pathogenesis in Alzheimer's disease. [40] [41]

Thus, it is yet to be proven that amyloid fibrils are the only cause of AD, since there are various unsolved aspects and unanswered key questions about oligomer toxicity and propagation. Anyway, there seems to be an important link between these two facts hypothesizing a crucial role for Aβ aggregation.

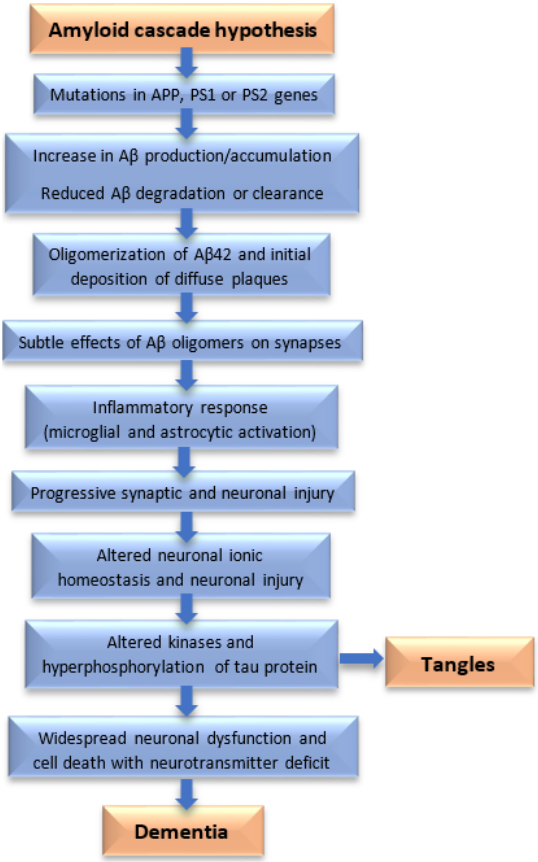


Figure 6. Sequence of pathogenic events according to amyloid cascade hypothesis

5.4. AMYLOID AGGREGATION

Proteins present a coded sequence of amino acids that allow them to fold in the correct position in order to be well conformed, have a long-term stability and fulfil their biological functions. As it was said before, there are some cases where this does not happen, and proteins can present a misfolding structure, which is what happens with amyloid fibrils.

The following process which is considered is a nucleation dependent mechanism where amyloid precursor protein (APP) cleavage is performed by a sequence of various secretase enzymes. APP is quite extensive, with some parts being located intercellularly whereas others are intracellularly, and they are cleaved in different subunits, such as Aβ40 or Aβ42. **(Fig.7)** There is a tendency in the aggregate formation in which the peptide goes from a α-helix structure to β-sheet, hence losing its native structure. [42] The resultant amyloid fibril assembly outcomes in a heterogenous-ordered sheet structure, in β-plaques, and presents a remarkable insolubility. [37] [30] [29] Its conformation and stability are due to a linkage of non-covalent bonds, concretely hydrogen bonds, that provides thermostability and kinetic stability. **(Fig.8)**

There are several factors such as ionic strength or temperature that affect the fibrillation process.[43] Other factors are net charge due to the fact that it may hinder self-association and the hydrophobicity of the side chain. It has been proven that protein sequences have evolved to avoid forming cluster of hydrophobic residues since the amino acid substitutions play a crucial role in the aggregation. According to Chiti et al. a decreasing in positive net charge of a protein provokes an acceleration in β-sheet formation. [35] Besides an enhanced hydrophobicity membrane can increase Aβ binding, and depending on the interaction that takes place, a fibril polymorphism can happen, overall in vivo since there are several cell membranes and macromolecules which can prompt it. [44]

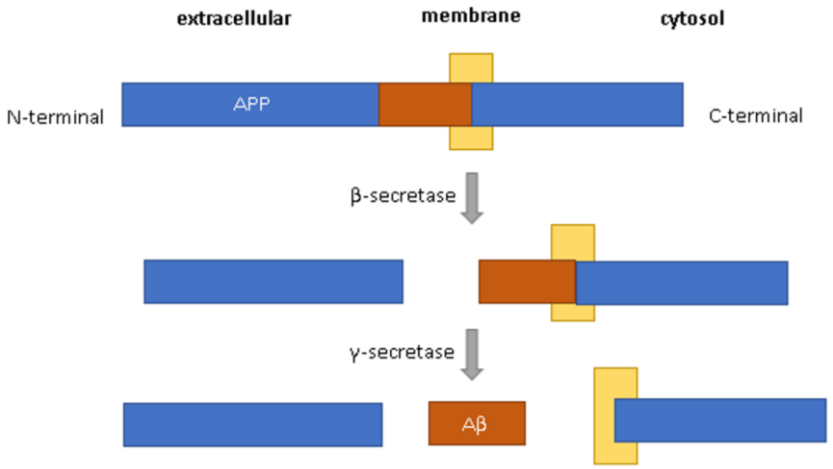


Figure 7. Cleavage of APP transmembrane protein by secretase enzymes to obtain Aβ peptide

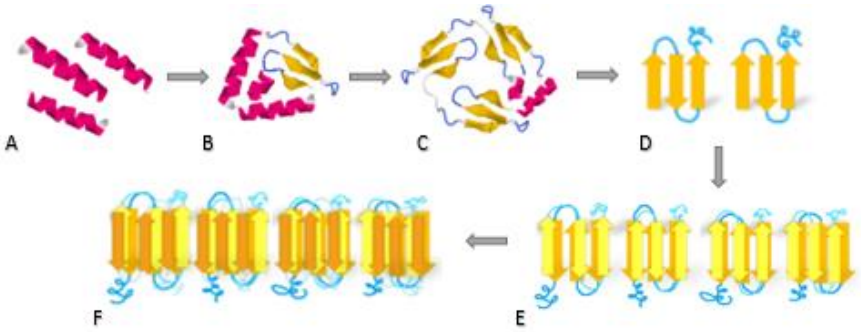


Figure 8. Process of amyloid aggregation. A) Native peptide α-helix chain B) Intermediate partially misfolded. Conversion of α-helical structure to β-sheets C) Final misfolded peptide D) Soluble protein oligomerization E) β-structure aggregate (protofibrils) F) Amyloid fibrils

5.5. NANOMEDICINE TO ADDRESS ALZHEIMER'S DISEASE TREATMENT

Nowadays, there are some drugs tested and approved by the FDA in the market in order to treat AD. None of them are curative though, but they alleviate symptoms. These medicaments are basically in charge of controlling the motor regulation to combat cognitive loss. [29]

Amyloid cascade hypothesis considers oligomers as the most toxic species hence their inhibition is of great importance. [22] [30] Alzheimer's patients revealed neuronal loss and cerebral atrophy due to the presence of extracellular plaques and intracellular neurofibrillary tangles, therefore tau protein is involved as well. In fact, Aβ42 and tau protein are the two most abundant peptide species related to AD. [22]

Furthermore, considering that accumulation of amyloid plaques is not directly the reason of dementia but of neuritic dystrophy and synaptic loss, it is crucial to reduce this formation through a drug than would be able to reduce neuroinflammation, disaggregate amyloid plaque and repair dystrophy [45]. Thanks to the developments in the nanomedicine field and considering the advancements that can take place, academics have been investigating several approaches to treat the issues mentioned before.

This path consists in the application of nanoparticles as a possible therapeutic approach but in order to do so it is important to understand one of the principal properties and interactions NPs might suffer, such as protein corona or blood brain barrier. These considerations are needed in order to have the best possible result.

Protein corona

Protein corona is an ensemble of biomolecules condensed on the nanomaterial's surface. [46] Proteins are active surfaces which present some affinity to adhere to other surfaces. In fact, those substances more similar between each other will displace those who has lower affinity. In addition, this adsorption can affect protein stability. Note that the soft and flexible particles interact stronger with proteins because of the possibility to establish several non-covalent interactions due to their secondary structure changes, whereas, hard particles, depends on either electrostatic or hydrophobic forces. [43]

Bearing this in mind and considering that NPs are currently one of the most important approaches to attack amyloid fibrils aggregations, there have been many different strategies and designs of them, but only some will be exposed below.

Nanoparticles, a possible therapeutic approach

Nanoparticles are the order of the day when it comes to nanomedicine and therapeutic purpose since diagnosis requires really complex pharm kinetic properties. There are various kinds of NPs and some are more promising than others. [22]

- NPs to detect AD. There are two nanoparticles to be highlighted. The first one is a dye thioflavin-T NP based on fluorescent emission in order to detect amyloid fibrils. The other one is a protein-based NP coated with a specific antibody and sialic acid to detect early stages of AD through magnetic resonance imaging. [22] This one was used in mice brains.
- Curcumin loaded NPs. Curcumin is an anti-amyloid protein even in micromolecular concentration. Nevertheless, it has poor stability due to its easy hydrolyzation. Thus, a derivative was developed and showed that in its planar structure presents a high affinity with Aβ42 fibrils. [47] Besides, curcumin encapsulated in a polymer NPs is capable of destroying Aβ aggregates without any observable toxicity.
- NPs-mediated chelation. It is reported that amyloid plaques present a higher concentration of Cu^{2+} and Zn^{2+} than in a healthy brain. [23] The presence of metals provokes a dysregulation in brain tissue due to oxidative stress. Therefore, toxic hydroxyl radical is released. This metal ion free radical formation makes chelation an attractive approach, although with limitations since there is no elimination metal chelator process.

- Metallic NPs. They can be from selenium, cerium or gold. The last ones, under in vitro conditions have the ability to couple with Aβ fibrils and can dissolve plaques if they are incubated for a week under a microwave irradiation. Moreover, research using zebrafish larvae coupled with a kind of casein did not show any harmful effects. [48] On the other hand, AuNPs can reduce aggregation in insulin fibrils, which for the type II diabetes mellitus, another neuropathology related to β-sheets, opens the possibility to consider it as an in vivo inhibitor. [49]

All these nanoparticles open a huge range of possibilities and looks like treatment is not in a so-distant future. In addition, there is another promising application for NPs that mimics molecular chaperones. These molecules can be found inside cells, for instance in endoplasmic reticulum, aiding proteins to fold correctly. They protect prone regions from aggregation and some of them are capable of solubilising some aggregates. [50] This strategy would be exposed later.

Nevertheless, getting to use NPs for drug administration in vivo is really complicate to accomplish due to the blood brain barrier (BBB). This issue is probably one of the most important ones since it has caused so much trouble.

Blood brain barrier

This barrier is a semipermeable border constituted by three microvasculature elements which are endothelial cells, astrocyte end-feet and pericytes. [51] **(Fig.9)** It accomplishes the function of not letting solutes from blood trespass the extracellular fluid of the central nervous system. This system is highly selective. Some crucial molecules necessary to neural function are allowed to pass such as amino acids, glucose or nutrients following a selective transport whereas other substances go by passive diffusion.

To overcome this obstacle nanomedicine is a key strategy. The problem to be faced though is that 98% of small molecules do not cross BBB and either almost the totality of large ones. [45] Considering the BBB handicap for drug delivery, it has been tried to avoid it by using other strategies. However, none of them are efficient enough to what was expected. For instance, nasal delivery must overcome nasal barriers like liposoluble small molecules transported via diffusion and water-soluble ones do not cross unless there is an injury in the membrane.

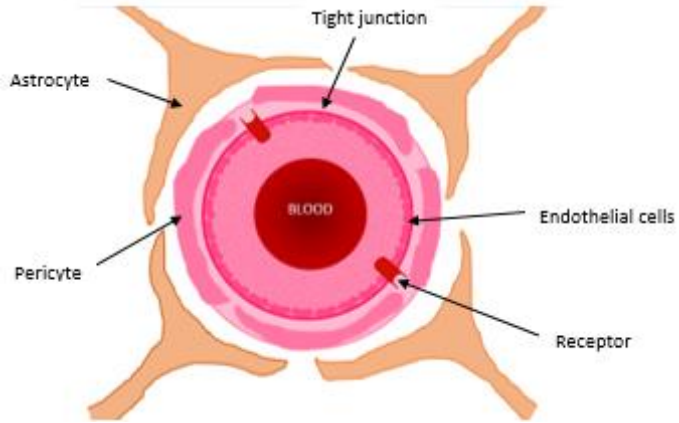


Figure 9. Schematic representation of the blood brain barrier

Stem cells could be a good path to therapy since they are derived from nervous system and they renew themselves. In spite of this, it is still not very clear if they are capable of crossing the BBB, because although it is quite cited, several experiments suggest that this takes place by a minimal transport. In addition, drug injection through cerebral spinal fluid (CSF), which bathes the brain's surface, is different from the brain itself. The former presents a choroid plexus barrier which is leakier than BBB. In the beginning, it was thought to be a proper strategy since it was believed that nutrients passed from blood to CSF and afterwards to the brain. Unfortunately, it was discovered that this was false, since drugs enter brain directly from blood. This opened a new perspective, administrating drugs to the cerebral spinal fluid and expecting it to arrive to the brain. It was concluded though, that through CSF a natural transport implies a very slow diffusion and a decline in drug concentration while a bulk flow presents a more uniform distribution that only arrives to the surface. [45]

In the end, there is a wide range of different strategies to avoid BBB, but neither is truly successful. Besides, apart from the BBB problem, there is also the problem of insertion, since there are technological limitations as well as ethical issues. [20] The wished solution is to find a suitable method to deliver drugs straightforward to the brain through blood brain barrier.

There are some NPs which have showed to cross BBB. There is a highly biocompatible polymer NP with silicon quantum dots or magnetic NPs that under an electromagnetic field crosses BBB or polymer NP with chitosan to protect hydrophobic drugs. It can be coated with

apolipoprotein, a transport molecule that can leave the brain by efflux pump, facilitates uptake and can act as drug delivery. [52]

Moreover, it was showed that a crocetin and cyclodextrin complex attacked Aβ aggregates and was able to pass the blood brain barrier. [53] Crocetin is a natural active substance that can cross BBB, yet it is insoluble in water. Cyclodextrins are oligosaccharides that can trap or encapsulate lipophilic molecules in order to protect water soluble complex. [54] From the different types of cyclodextrin, the γ-cyclodextrin has the lowest toxicity and a large cavity to encapsulate crocetin. In conclusion, this complex was proved to be capable of inhibiting Aβ fibrils and promote their degradation without being toxic to neuronal cells or AD cells.

In a nutshell, BBB and protein corona are important obstacles to consider in order to find an effective treatment, but these issues are currently being under study with successful results. Thus, it is just a matter of time before we can perfect nanoparticles and control their behaviour.

6. NANOMODULATION OF AMYLOID AGGREGATION

6.1. PROTEIN – NANOPARTICLE INTERACTION AND PROTEIN CORONA

Nanoparticles seem like a great opportunity to fight conformational diseases. Nevertheless, designing correctly these particles is crucial. As NPs are commonly administrated to the organism through bloodstream, a key point to consider is their interaction with proteins and the factors that may influence their behaviour. These considerations are explained below.

First of all, NPs present a high surface area to volume ratio, which when they are found in biological fluids it is more likely to provoke an interaction between serum proteins and other particles which can alter them. [55] There can happen two processes, which are in competition, a protein – protein interaction or a protein – NP one. [56] **(Fig.10)** As in the biological fluids are loads of proteins, which can adhere to NP surface and form protein corona, it is important to know the effects it might produce. This formation depends on factors such as exposure time, concentration and surface since kinetics and affinity are key factors to the binding. [57] The binding that takes place between protein and NPs can form two differentiate parts called the hard

corona or soft corona. The former happens when the attachment of proteins is irreversible whereas the latter is a quick and reversible process with weak bonds. [50] [57]

It has been showed that sometimes proteins can suffer conformational changes in secondary and tertiary structure when they interact with one another. [58] [59] Sometimes proteins go through the loss of part of their α - helical structure, although they maintain most of their native conformation. [60] This can make them unstable and more susceptible to denaturant agents, which would be a problem for the accomplishment of their biological function. [50]

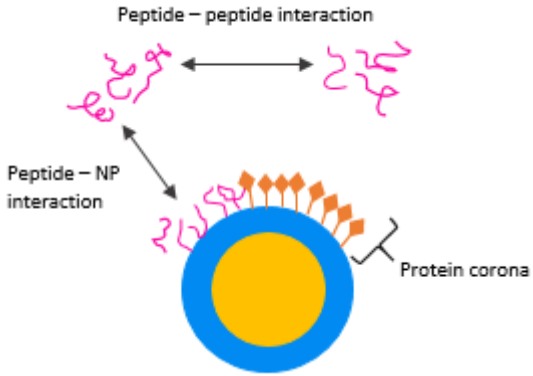


Figure 10. Schematic representation of nanoparticle - corona system. Peptide - peptide and peptide - NP interaction competition.

Another aspect to consider is surface curvature. The smaller a particle is, the higher its curvature. Note that high surface curvature can favour native protein structure. Following the importance of surface properties, it is noteworthy that when there is an elevated protein surface density, meaning that NP surface presents a significant amount of proteins attached, there is a higher tendency for a protein – protein interaction and this can protect proteins from unfolding. [60] [61] In addition, some other factors that can influence adsorption are pH, temperature, surface hydrophobicity and peptide/nanoparticle concentration ratio.

First of all, pH can affect adsorption if its value is far from the peptide isoelectric point, since that would derive in charged zones. Furthermore, high temperature is the main cause of denaturalization, whereas hydrophobic surface functionalization decreases the energy barrier needed to displace surface water in order to let protein pass. [61] Moreover, concentration ratio is another factor because it would affect peptide local concentration. This implies that if there are

too many NPs and the same amount of peptide, the local peptide concentration would be low, and it is more bound to interact at high concentration levels than in low ones.

In conclusion, interactions between proteins and nanoparticles are of huge importance to understand protein corona and how NPs can change or how to design them for a useful biological application. According to this and following the goal of this work, the interaction and the effect than can take place between amyloid proteins and NPs are the next point to consider.

6.2. EFFECTS OF NANOPARTICLES ON AMYLOID AGGREGATION

As it has been exposed, NPs interact with proteins, and under specific conditions their behaviour might change. This can be applied to amyloid proteins, which is something to consider in order to treat Alzheimer or other conformational diseases.

The NP – protein system can lead to acceleration, inhibition or have no effect at all in the amyloid peptide aggregation. [62] Related to protein corona, it has been reported that it creates a shell that protects NP surface, which reduces access to Aβ fibrils. However this does not prevent that another oligomerization process or Aβ nucleation could take place, depending on the affinity between protein corona and Aβ monomers. [46] Therefore, following the amyloid cascade hypothesis, it is a vital step to fight AD by studying the circumstances which can lead to the different outcomes in amyloid fibrillation using NPs.

First of all, it should be noted that polypeptides chains present a tendency to form ordered structures, although the more stable a protein is, a slower fibril formation will have. Nonetheless, NPs surface acts, in a way, as a catalyst for peptide aggregation following a mechanism of two steps. Firstly an association process occurs onto NPs surface, leading to an increase in local peptide concentration and oligomerization, and afterwards there is a reordering into β-sheets structures by hydrogen bonds. [63]

The amyloid binding to NPs depends on how strong the interaction might be. A strong attraction leads to formation of unstable fibrils that influences negatively the peptide structural rearrangement for the conformation of β-sheets. This means that there is inhibition of the necessary conformational changes for the amyloid aggregation to happen. [56] If, instead, the interaction between peptide - surface is weak, the NP presence hardly influences fibrillation process, since peptide - peptide attraction dominates. [64] This can also happen when speaking of electrostatic attraction. [65] Amyloid beta peptide presents negative and positive residues that

can be bound through local electrostatic attraction to the surface. If they are tightly bound to the surface, then the fibril growth is inhibited. **(Fig.11)**

Following the interactions matter, peptides can bind through hydrogen bonds which are a key factor in inhibiting fibrillation, since if there is a low capability to bonding, there is decrease in adsorbing proteins. Moreover, it results in a tight binding, therefore an incapability for monomer or early aggregates to grow. [66] In addition, hydrophobic interactions are also important, due to the fact that amyloidogenic peptides usually contain large amounts of hydrophobic residues and they present high affinity to hydrophobic surfaces. [67] [62]

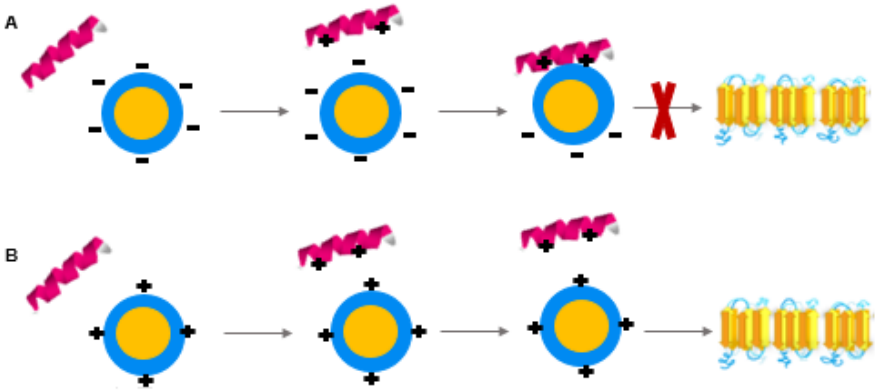


Figure 11. Representation of A) negative NP inhibiting amyloid fibrillation.
B) Positive charged NP not affecting fibrillation

On the other hand, nucleation process of fibrillation is highly dependent on peptide concentration. However, sometimes a high local peptide concentration does not always implies an acceleration of fibril growth as it might be thought. [56] This can be due to the presence of an interface. [68] [69] Note that adsorption of proteins only happens if the protein - surface interaction is favourable.

Furthermore, NP - protein ratio is another thing to consider. If there is a high concentration of NP, peptides would bound to its surface. A low amount of peptide monomer will remain in solution and the elongation process of amyloid aggregation would not happen or if it does, it would be in a minor proportion. [70]

Another factor to keep in mind is surface roughness, which plays a key role in self-assembly of Aβ42 molecules through dynamic diffusion. [71] [56] This means that kinetics is affected since

a rough surface blocks fibril mobility and they require peptides molecules for diffuse to grow. Besides, surface charge is also important to inhibit fibrillation owing that amyloid fibrils present charged residues. [65] It has been showed that negatively charged gold NPs or silica ones are efficient to do so, although the latter presents quite low cell viability due to its toxicity. This inhibition is caused by the positive residues of amyloid fibrils that can adsorb onto NPs surface. As both materials were successful, it was concluded that the inhibition dependent factor is charge instead of material, at least in NPs of 15 nm. [72]

Nevertheless, the material used to synthetize NPs is crucial, since its toxicity and functionality are factors to always keep in mind. For example, in the metal sector there are lots of possibilities although iron and titanium present less toxicity than zinc, whereas gold is a highly biocompatible material. In fact, it has been reported that large AuNPs accelerate Aβ fibrillation, whereas small ones can postpone the process in a significant way or even be capable of its inhibition. [73] This is explained by surface curvature, which can limit spatial arrangements that can be incompatible with fibrillation dynamics. [74] [63] In addition, when Sen et al. tried to show if from coated AuNPs with glutamic acid and human serum albumin, there were significant differences between the use of enantiomeric products for fibrillation. It was concluded that D - glutamic acid was more effective. Hence, surface chirality may be a property too. [75]

All in all, proteins and NPs are connected with one another in a way that cannot be ignored. Achieving proper hydrophobicity and electrostatic interactions are crucial to ensure an accurate interaction and supress protein aggregation. Hence, studying these interactions will lead to a better understanding which will be in our favour in order to design new treatments. As NPs cooperate so much with proteins, maybe instead of trying to inhibit amyloid fibrillation, they can help to convert these fibrils to their native structure.

6.3. PROTEOSTASIS NETWORK AND NEURODEGENERATIVE DISEASE

Most of proteins, as it is well-known, present a defined three-dimension structure in charge of their biological function. Nonetheless some do not present a unique conformation and under some circumstances like stress condition, such as inflammation or heat, they can become susceptible to form a non - native structure.

Proteins present polar or charged surface and hydrophobic domains hidden in their core, but these regions become exposed when they unfold. So, in order to assure that these molecules do

not cause any harm to the organism and everything functions correctly, it exists a mechanism responsible for taking care of them. It is called protein quality control and it is composed by chaperones and the ubiquitin proteasome system (UPS). They work in synergy with one another since those proteins that cannot be refolded are destined to degradation by the UPS or autophagy. [42] [76] In case of environmental stress conditions, there could be a higher synthesis level of chaperones and proteases which can favour the removal of aggregates.

Molecular chaperones are defined as an assembly of proteins, present in prokaryotes as well as eukaryotes cells, which are found overall in the endoplasmic reticulum. [50] They play vital functions such as helping proteins to fold, by protecting prone regions to aggregate, as well as helping in assembly proteins or in cellular transport, since they can be used for translocation of polypeptides across membranes. [77] It is proposed, as a general mechanism, that chaperones present a two-step process. It consists in the capture of a denatured protein and assisting it to refold, to then, be released in its corresponding bioactive form. [78] **(Fig.12)**

Molecular chaperones can be divided in chaperonins, although they are commonly called chaperones, and nuclear proteins like histones. Most of them are called heat shock proteins, which there are a wide range of different ones, owing that they are activated by thermal stress.

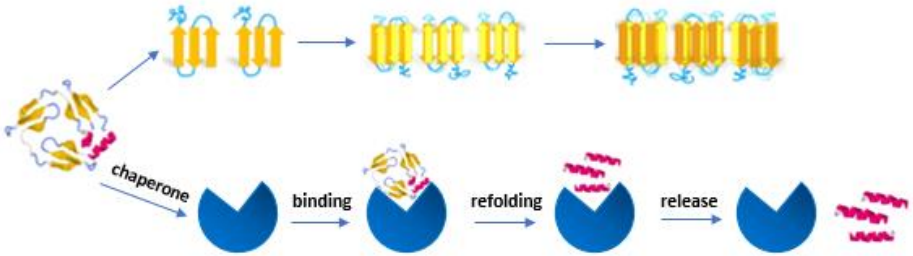


Figure 12. General mechanism followed by a chaperone to refold unnatured proteins in order to prevent aggregation. A misfold protein is bound by the chaperone through hydrophobic or electrostatic interactions. Once it is refolded, the protein is released.

Chaperones can be classified by their size or according to the mechanism they followed in, three categories: holding, folding or disaggregating. [79] [80] The first action mode consists in holding the unfolded client protein until it spontaneously folds itself, the folding one is by using ATP to unfold stable misfolding proteins and convert them to their active species, whereas the

last one is based on ATP hydrolysis in order to solubilize aggregates to achieve their native conformation.

One reason for which neurodegenerative disease occurs is the accumulation of protein aggregates and the imbalance between production and clearance of these assemblies. This means that ubiquitin protein system activity is reduced and simultaneously, chaperone activity is not high enough.

As it can be seen, these proteins present a quite interesting nature that can be explored in order to get a possible medical application. It has already been explained how-thanks to nanotechnology a huge advancement in medicine has taken place. However, there are still some handicaps as far as conformational diseases are concerned. Thereby, it could be possible to combine both nanotechnology and chaperone's knowledge to develop new approaches to treat these pathologies.

Overall, nowadays there are only some drugs in the market that slow down their progression or have palliative effects and 95% of developed drugs for AD treatment end up in failure. [68] For this reason, being able to study different approaches and points of view is so important. Therefore, these kinds of molecules responsible for proteostasis open up several opportunities to aim for a new approach in therapy, especially against conformational diseases.

6.4. HEAT SHOCK PROTEINS

Heat shock proteins (HSP) are a kind of molecular chaperones the function of which is generally activated by thermal stimulus, although it is not always required. Their main goal is helping proteins to refold from abnormal structures or denature conformations. **(Fig.12)** HSP malfunction or altered expression in the central nervous system has been detected in many pathological conditions such as epilepsy, trauma or neurodegenerative diseases. [81] Therefore, targeting HSPs might be a good focus to prevent these afflictions. There are many different heat shock proteins but only some of them will be exposed below since they are the most important ones related to conformational diseases.

First of all, HSP60 is a protein localized in mitochondrial matrix although it can also be found in cytosol, plasma membrane and peripheral blood. It is constituted by two heptameric rings which present three domains: apical, intermediate and equatorial. HSP60 interacts with ATP and HSP10, as a cochaperone, and acts as a folding structure. [82] There is some evidence that

HSP60 presents an efficient capacity to inhibit Aβ42 amyloid fibril. Although in theory HPS60 and Aβ are not localized in the same place, under pathological conditions, they can colocalize and interact with each other through hydrogen bounds, according to a computational study. [83]

In addition, another chaperone is HSP70, which is modulated by HSP40 and has a substantial role in guiding misfolding proteins to UPS. [84] Alzheimer's patients presented high levels of HSP70 accumulated in extracellular senile plaques, but this fact is not totally understood since HSP70 does not contain a peptide signal for its secretion. [84] One hypothesis explains that it might be released through extracellular vesicles whereas other hypothesis states that the export of HSP70 to extracellular media is due to the expression of an enzyme responsible for degrading Aβ, called TFG – β1. [85] [81] HSP70 is important because it has been showed to present direct binding to pathogenic proteins as well as being able to inhibit aggregation of aggregation-prone proteins such as Aβ or α-synuclein. [80] [86] It can reduce Aβ early stages of oligomerization in Alzheimer's disease, although it is not effective in dealing with fibrils, at least in in vitro conditions. [87] This is explained following the Aβ-oligomerization hypothesis, where there is an initial Aβ complex which is the precursor of Aβ oligomerization, and it is thought that HSP70 may interfere in that. However, it is not clear the way in which HSP70 does this, if it is through a stabilization of the complex making it unable to oligomerize or if HSP70 interaction with the initial complex is reversible. [88]

Moreover, HSP90 is the most abundant chaperone in cells and it is composed by a N-terminal domain, then a C-terminal and a middle domain which is the domain that functions as a cochaperone for the binding. [81] HSP90 can be found in cytosol and endothelial reticulum and it is present in many cellular processes including clearance of tau species, although it facilitates microtubules association too. [89] [90] This fact might convert HPS90 in an interesting target. However, since it is involved in so many other processes, not having any side effect would be really unlikely. [91] This chaperone is usually coupled to inflammation response which is a hallmark in AD. Besides, HPS90 seems to be more selective for client proteins than other chaperones, since it recognizes structural elements of a client instead of a particular primary sequence. [91] HSF-1 can be found in the HSP90 complex protein. It is a factor that under stress conditions dissociates from the complex, translocates into nucleus and binds to the promote region of a molecular chaperone gene to induce its expression. [92] Therefore, this factor induces HSP70 and HSP40 expression as well as the mechanism that cells use for protection under stress conditions. [90]

Observing how important HSP are it could be thought to overexpress them for a therapeutic strategy. There is some evidence that in experiments with mice was effective. [42] Nevertheless, this overexpression has to be controlled because it can have side effects. Therefore, this method may not be the best one to prevent aggregation. Thus, a question emerges from this issue which is if perhaps NPs can solve it. Exploring this possibility is really appealing and that is what will be explained next.

6.5. NANOPARTICLES ACTING AS CHAPERONES TO PREVENT AMYLOID AGGREGATION

There has already been demonstrated that some nanoparticles have the ability to inhibit Aβ peptide oligomerisation and aggregation, having a promising use. Note that there are already various well-known anti-amyloidogenic substances identified. For instance, curcumin, sialic acid or peptides. Unfortunately, only a small fraction of them can be used in NPs in vivo due to poor chemical stability or low solubility. [93] For this reason, searching for a new approach to avoid these obstacles and fight conformational diseases is necessary.

As it has been exposed, apart from protein aggregation problems there is also the issue of denatured proteins. Thus, in the latest years, the idea of fighting Aβ peptides before aggregates form has arisen. This means preventing the aggregation in an earlier stage. It would not be necessary to inhibit or slow down the formation of amyloid fibrils if exists the possibility to refold those proteins. Therefore, those proteins which lose their native conformation and cause harmful damages to the organism would not become such a problem. It would not matter whether Aβ peptides appear or if they are in high concentration, because they could be folded again to a functional structure. In fact, there are already some methods to refold proteins, although they are not based on NP, and all of them present low yields, around 15 - 25% of the total protein. [78] Thus, it was needed to find another approach.

Hence, an alternative strategy appeared consisting in creating synthetic chaperones in order to mimic the natural ones since some are known for their ability to refold denatured proteins. To do so, the best option is synthetizing something with similar properties and size. That is the reason why nanoparticles come into play. Proteins present dimensions of around ten nanometres and nanoparticles go from 1-100 nm. In addition, the surface of NPs can be modulated to different composition and characteristics to control their interactions. Some examples are showed below.

First of all, when a nanoparticle-based synthetic chaperone (so-called nanochaperone) is going to be designed is crucial to have in mind some features, such as surface hydrophobicity and charge, since as mentioned previously, these factors are quite important to avoid unwished interactions and to obtain better results. However, the protein - NP interaction can be manipulated and exploited through polymer NPs with high selectivity. [94] [95] Nanochaperones mainly have been inspired taking HSP70 as a role model since it is responsible of refolding denatured proteins. The general mechanism of refolding consists in binding the chaperone to hydrophobic residues of non-native proteins, sequestering them and preventing aggregation from forming a stable complex. [96] Afterwards, chaperones release the client substrate once it is correctly refolded.

For instance, there is a case that tested if high charged NPs could serve as refolding agents, as well as preventing aggregation, by interacting with charged residues on denatured proteins. [97] Rotello et al. demonstrated it by using functionalized gold core NPs with 2-(10-mercaptodecyl) malonic acid to assess the refolding protein. Those proteins used in the experiment where α-chymotrypsin, papain and lysozyme, which present positive residues whereas the functionalize AuNP is highly anionic. [98] Proteins then can be released from the electrostatic interaction increasing their ionic strength by increasing salt concentration. [99] This follows a catch-and-release mechanism.

Furthermore, Ma et al. conducted a research where they successfully synthetized a nanochaperone with charged and hydrophobic domains, formed by PEG chains to stabilize it in the aqueous media. They were mixed shell polymeric micelles (MSPMs) and it was shown that hydrophobic regions were essential to the refolding of denatured proteins, owing that without their presence the capture was ineffective. They concluded that electrical repulsion weakens binding affinity which is beneficious since this gives the protein flexibility to refold. Afterwards, it can be detached from the nanochaperone through this same exact electrostatic repulsion once the protein achieves its native structure. [78] In the case that the interaction is too strong the refolding would be blocked and if it is too weak there would not be any binding, so it is crucial to have a proper interaction. [100]

In addition, Huang et al. studied how charge affected the chaperone activity of MSPMs to refold lysozyme. These mixed shell micelles were synthetized with a hydrophobic core and block copolymers segments with thermal responsive. This means that at high temperature is hydrophobic, but in cold ones it changes to hydrophilic. Neutral MSPMs were the most efficient in

the refolding rate, because of the positive and negative presented excessive electrostatic interactions. [101] Moreover, there are some polymers which can transform spontaneously its hydrophobic nature, and combined with PEG in the mixed shell, this MSPM is able to block aggregation by trapping Aβ oligomers and monomers forming a MSPM-Aβ complex, which is highly susceptible to be degraded. [96] Furthermore, these nanochaperones can be reused, since they can go up to several heat and cold cycles. [94]

The promising properties of MSPMs were demonstrated as well by Yang et al. using polycaprolactone core and a mixed shell of two biocompatible and biodegradable polymers. This nanochaperone was effective in in vivo experiments with mice alleviating and decreasing amyloid deposition owing that they are able to mitigate cell membrane adhesion, thus protecting neurons from Aβ toxicity. [102] Furthermore, it was showed that they presented certain selectivity for Aβ peptide instead of other proteins such as ubiquitin or bovine serum albumin.

In addition, these nanochaperones can also be used to prevent aggregation according to Qu et al. that created MSPMs with modified KLVFF peptide, which is a hydrophobic pentapeptide region of Aβ, onto the end of PEG chains. It was concluded that KLVFF onto its surface interacts with Aβ fibrils disassembling them. Additionally, hydrophobic regions of the nanochaperone could capture Aβ fragments reducing neurotoxicity. [97] [103] Moreover, Zhao et al. demonstrated as well that if KLVFF was added to a nanocomposite's surface, it allowed for eliminating toxic Aβ oligomers and protected hippocampal neurons against apoptosis. [104] Furthermore, another study concluded that α-casein presents a chaperon-like activity against Aβ(1-40) aggregation, and Javed et al. demonstrated how β-casein coated AuNPs with chaperone activity, similar to HSP due to its ability to bind with partially folded proteins, inhibited Aβ42 fibrillation with no harmful effects. [48] [105]

Alternatively, in the last years nanogels have attracted quite interest because of their drug delivery properties and their application in medical fields. Moreover, in aqueous media they act as soft nanoparticles. [106] Due to this, they have studied their chaperon-like activity. Several studies revealed that amphiphilic nanogels such as cholesteryl group bearing pullulan (CHP) nanogel act as artificial chaperones. [107] [108] [109] It was compared to the GroEL, a chaperone belonging to HSP60 family, behaviour and efficacy. [110] This nanogel had a strong bound to the denatured protein and could prevent its aggregation folding it again in a similar time that GroEl. Besides, this nanogel had cyclodextrin as a modulator, to control its binding ability as well as the

rate release of the protein, therefore the mechanism followed is similar to the catch and release present in some molecular chaperones. [100] Fei et al. showed how CHP nanogel with β-cyclodextrin, could refold carbonic anhydrase B under heating conditions. [60]

There is another kind of substances that can act as chaperones called chemical chaperones. They are mainly osmolytes, which are low molecular compounds such as amino acids or organic solutes that accumulate inside the cell to counter cellular stresses and provide protection against cell shrinkage or swelling. Osmolytes are called chaperones owing their ability to stabilize in vitro native or native-like proteins. [84] Pradhan et al. concluded that glutamine and proline nanoparticles presented chemical chaperone activity, since they were effective in inhibiting protein aggregation. [93] Moreover, there are as well some other ways to target misfolding proteins such as pharmaceutical chaperones. [111] These kind of molecules are more specific in targeting and require lower concentrations than osmolytes for being effective in assisting refolding or stabilizing non-native structures. [112] They can be combined with chemical chaperones in order to upregulate chaperone synthesis.

In conclusion, although hydrophobicity and charge are properties to keep in mind when nanochaperones are being designed, other properties cannot be forgotten such as surface, biodegradability, biocompatibility, and being able to cross BBB. Therefore, it is not a simple task to achieve. However, results show that nanochaperones present a hopeful future and being able to refold amyloid proteins not only would be significant to Alzheimer's disease but also to many other pathologies with the same problem. It seems like we are following the right pathway, although it is difficult to pinpoint when this will happen.

7. CONCLUDING REMARKS

Throughout this work, different aspects of nanotechnology have been exposed, with a special focus on their therapeutic application in neurodegenerative diseases. As these pathologies affect so many people around the world and do not have cure yet, to fight them is a vital issue. Nanoparticles have an open new range of possibilities from diagnosis to some treatments. In fact, there are some treatments based on nanoparticles which are currently in use, although unfortunately none of them is being used for neurodegenerative pathologies.

However, NPs have been studied in depth and they can be manipulated to interact less with serum proteins or to adapt their electrostatic interactions, hydrophobic domains, adding peptides and even cross blood brain barrier. All of this is with the purpose of using NPs to eliminate toxic Aβ peptides, slow down the fibrillation process or even try to inhibit it. Thus, there are several ways to tackle amyloid protein aggregation. Nevertheless, not only is already really difficult to prevent it, but it also has to be considered that Alzheimer's disease presents various factors involved in its pathology such as oxidative stress, tau aggregation, etcetera. Hence, it is a multifactorial disease. So, instead of focusing on just only a pathway, it is recommendable to consider a multitargeting treatment, for instance, cocktails with diverse mixed drugs to interact with aggregates which present several attacking mechanisms.

Therefore, with so many aspects to consider, the real question is whether it is possible to reach an effective treatment for conformational diseases. As far as this question is concerned, nanochaperones look like a promising solution owing to the fact that there are already some advancements with their design and creation with good results. Thus, the most crucial part is if these particles are suitable to be used in vivo following the corresponding regulations of toxicity, biocompatibility, functionality and many more, from agencies like FDA or the European Commission.

Furthermore, time is something to consider since the earliest a treatment comes, the better, but passing the preclinical and clinical stages until its approval are long processes. Overall, that

there are still some unknown aspects since nanochaperones are still a quite recent approach in nanomedicine. Therefore, a sensible assumption to this issue is that there is still a long way to go.

Nevertheless, nanochaperones present a promising future specifically the mixed shell polymer micelles (MSPMs). They are synthetized with hydrophobic core and block copolymers onto the surface which have demonstrated their effectivity. In addition, gold nanoparticles present right qualities to be used in vivo, they have been useful for therapy and they can be coated with polymers. Hopefully, MSPMs as well as AuNPs could be studied more in depth to adapt and design them to accomplish perfectly with a chaperone function. They could even be combined to attack amyloid aggregates from different approaches. After all, there is no reason to think otherwise.

It has been suggested that nanochaperones are the answer to all the problems, that perhaps, once and for all, a cure to conformational diseases would be achieved. All in all, putting so much effort to be successful in this method must be useful for something. Nevertheless, it is a huge statement to affirm so. It is true that if they can refold proteins, specifically Aβ peptides, there would be no possibility to form aggregates, but one cannot forget that this just follows amyloid cascade hypothesis and there are still other factors that influence these diseases. Thus, maybe it is really the solution for these pathologies, or perhaps it just a huge step in the right direction and a very efficient way to treat the main reason for which this affliction appears. Only when it will become a reality, we will be able to affirm so.

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9. ACRONYMS

FDA: Food and Drug Administration

EMA: European Medicines Agency

NPs: nanoparticles

AuNP: gold nanoparticle

BBB: blood brain barrier

PEG: polyethylene glycol

EPR effect: enhanced permeability and retention effect

AD: Alzheimer's disease

APP: amyloid precursor protein

CNS: central nervous system

NMR: nuclear magnetic resonance

CSF: cerebral spinal fluid

CRT: crocetin

CD: cyclodextrin

UPS: ubiquitin proteasome system

HSP: heat shock protein

MSPMs: mixed shell polymeric micelles

CHP: cholesteryl group bearing pullulan