ACTIVE TARGETING OF DRUGS

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Abstract. *Nanotechnology and nanomedicine are on the verge of realizing the concept of the "magic bullet" first proposed a century ago by the Nobel Prize laureate and founder of chemotherapy, Paul Ehrlich. The last 2-3 decades have been notable for the scope of research in this field, aimed at achieving more efficient, even personalized therapy for patients with various diseases. The great challenge was to achieve treatment options that are much more effective than traditional therapy, initially for cancer. Subsequently, the concerns of specialists in the field - chemists, biologists, doctors, pharmacists - have also expanded to treat practically any disease of the human body. The achievement of such therapy involves the association of the drug with a nanometric carrier, capable of being administered into the body, especially by injection, loaded with the drug, and decorated on the outside with functions (ligands) capable of being recognized by the typical receptors overexpressed in the membrane of cells specific to each organ. The paper aims to review the literature of the last decade, in particular, presenting the types of known nanocarriers, how to obtain them, how to functionalize them with ligands recognized by specific cellular receptors, as well as the medical applications that, at least potentially, can benefit from the advantages of targeted therapy.*

Keywords: drug targeting, nanocarriers, functionalization with ligands, applications of targeting polymeric systems

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1. Introduction

It has been a century since the founder of chemotherapy, Paul Ehrlich, received the Nobel Prize in Physiology or Medicine, being the one who introduced the concept of the "magic bullet," which underpins the development of targeted therapies, initially conceived for more effective cancer treatment. Progress achieved over the past few decades has led to a better understanding of disease pathology and improved treatment strategies. Combined with significant technological advances, these developments have enabled the development of therapeutic systems capable of delivering specific drugs "to the target," thereby preventing and/or treating not

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only malignant conditions but also numerous benign disorders and other diseases [1–3].

What were the factors that imposed the development of new therapeutic forms involving targeted drug delivery?

Conventional drug administration, although still the most widely used therapeutic method, presents major limitations such as low bioavailability, nonspecific distribution in the body, and inability to maintain a constant therapeutic concentration of the active principle for a prolonged time at the site of disease, which often requires frequent administration of high doses, with the risk of systemic side effects. These factors gradually led to the emergence of a new interdisciplinary field - targeted drug delivery systems - at the interface of pharmacology, bioengineering, chemistry, and nanotechnology [4].

The purpose of these systems is to transport the active substance in a controlled and selective manner to the exact site where therapeutic action is needed, while maintaining optimal concentration for a defined period. The ligand functionalizing the nanocarrier guides it to the overexpressed receptor on the target cell, enabling selective internalization of the drug and increasing local concentration at the site of action [5,6]. This approach enhances treatment efficacy and reduces toxicity to healthy tissues.

This paper aims to provide a brief overview of this relatively new research field, which seeks to improve human health and thus quality of life—a domain that has mobilized numerous research teams worldwide over the past 2–3 decades, with an almost exponential increase in scientific publications reporting increasingly valuable results.

2. General principles of targeted delivery

The considerations that led to the design of targeted delivery systems can be summarized as follows [7]:
- Reduced stability of conventionally formulated drugs (tablets, capsules, injectable systems, ointments, etc.)
- High capacity of the active molecule to bind to cell membranes, influencing absorption
- Often limited absorption, in small quantities
- Low solubility of many drug types
- Biological instability of the drug
- Low specificity
- Large volume of drug biodistribution

Targeted delivery systems are based on the idea of controlled transport of the drug to a specific anatomical or cellular region where it exerts its maximum therapeutic

effect. Unlike conventional administration, where the active substance is nonspecifically distributed throughout the body, these systems use mechanisms that increase selectivity and reduce toxicity. Thus, the main goal of targeted drug delivery systems is to deliver the therapeutic agent's pharmacological action only to affected organs without harming healthy ones.

Targeted delivery is an advanced method of administering drugs to patients in a directed sequence that increases drug concentration in targeted organs, tissues, or cells, thereby improving treatment efficacy by reducing side effects. It is a specialized form of drug administration in which the active pharmacological agent is selectively directed to its site of action or absorption [7].

Traditional drug administration through various routes does not allow directing them solely to the site of disease. To achieve this goal, drugs must be associated with a carrier whose physicochemical characteristics (shape, size, chemical structure, functional groups, etc.) facilitate targeting and concentration in the desired area. In recent years, the successful development of nanotechnology—especially the emergence of new nanomaterials—has provided new ideas and potential methods not only for treating many major diseases but also for early diagnosis and monitoring during treatment [8,9].

Drug nanocarriers for targeted release belong to the category of drug delivery systems (DDS) introduced a few decades ago. Compared to classical DDS, nano-DDS can enhance therapeutic efficacy by improving the pharmacokinetic and pharmacodynamic properties of encapsulated drugs, including drug stability, and achieving targeted release at specific body locations due to their special size, shape, and material characteristics [10,11]. To achieve high targeting efficiency, nano-DDS must remain in the physiological system long enough to reach specific cells and tissues for drug release, avoiding destruction by the immune system [12]. Nanoparticles can improve the stability and solubility of the encapsulated payload, facilitate transmembrane transport, and prolong circulation times, thereby improving safety and efficacy [13]. They can enter the bloodstream through blood vessels and act at specific sites within them to treat intravascular diseases. They can also cross the vascular endothelium or reach target tissues, thereby enhancing treatment efficiency for various extravascular diseases. Despite the obvious advantages of nano-DDS and research progress, it must be acknowledged that the precise delivery of therapeutic drugs to the target site remains a significant challenge that requires further investigation [14].

The concept of targeted delivery is based on two main mechanisms: passive targeting and active targeting.

Passive targeting (often called non-targeting) relies on the physicochemical properties of the active molecule and tissues (e.g., increased permeability of tumor

vessels) as well as physiological phenomena such as the Enhanced Permeability and Retention (EPR) effect. In principle, the effect of systemically administered drugs (via injection, oral, nasal, vaginal, rectal routes, etc.) is based on passive targeting, with the drug being biodistributed throughout the body but partially reaching the site of disease [15].

Active targeting is based on the specific recognition between a ligand and receptors expressed on the surface of target cells. It involves attaching recognition molecules (ligands) to the surface of the nanocarrier, such as antibodies, peptides, aptamers, protein fragments, polymer fragments, or creating organic functional groups that selectively bind to specific receptors expressed on target cells. Through these mechanisms, targeted delivery ensures biodistribution, concentration of the active principle at the site of disease, and a significant reduction in adverse reactions [16]. A schematic diagram illustrating how gold nanoparticles loaded with a drug and functionalized with a ligand interact with tumor cell receptors after being injected into the human body, demonstrating active targeting, is shown in Fig. 1 (B) [17]. For comparison, the way the same non-functionalized nanocarrier reaches diseased cells without stably anchoring to their surface is illustrated in Fig. 1 (A). While passive targeting relies on the penetration effect of nanoparticles mediated by tumor vascularization, many of which do not reach the target, active targeting refers to the specific binding of ligand-decorated nanoparticles to tumor cell receptors, through an interaction that can be vividly compared to that between a magnet and a magnetic/magnetizable material.

The literature mentions several types of active targeting, including inverse, dual, double, and combination targeting, as well as physical, chemical, and biological targeting.

Physical targeting refers to situations in which the active molecule is localized at the target based on the nanocarrier's size or content, rather than on ligand–receptor interaction. Moreover, the release of the active molecule occurs in response to a physical stimulus (ultrasound, UV radiation, an external magnetic field, or light).

Chemical targeting involves delivering specialized prodrugs to specific sites, where the active molecule is released through chemical or enzymatic processes that depend on the local environment. Targeting that uses biomolecules (antibodies, peptides, proteins) associated with the active molecule is called *biological targeting*. Gene expression can be targeted to specific regions using vector systems that employ cells, tissues, or specific promoters [19,20].

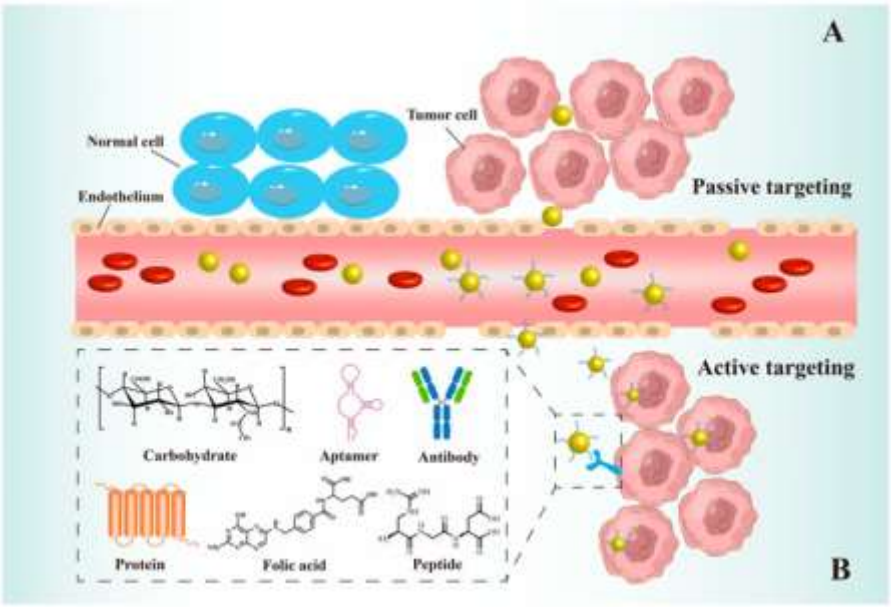

Fig. 1. Suggestive comparison between passive targeting with non-functionalized gold nanoparticles (A) and active targeting with nanoparticles functionalized with various ligands that enable their specific binding to tumor cell receptors (B) [18].

Other techniques for achieving active targeting have also been developed over time. An example of recent advancements in medicine is the development of hybrid nanocarriers. These nanocarriers are polymer-based nanoparticles that contain metal or metal oxide nanoparticles with superparamagnetic properties, such as iron oxides (e.g., maghemite or magnetite) and gadolinium oxide. These materials encapsulate a therapeutic agent. When administered intravenously into physiological fluid (blood), these nanocarriers can be directed to the site of disease using an external magnetic field. This targeted method focuses the nanocarriers on the specific location, ensuring drug release occurs precisely at the intended site [21].

Another example is the development of protected carriers, usually with a membrane or polymer matrix resistant to an aggressive environment (typically acidic), capable of transporting drugs sensitive to high acidity, thus overcoming the gastric barrier without significant loss or degradation. This makes it possible to administer orally certain drugs intended for colon disorders (especially cancer), which practically pass intact through the gastric barrier and are predominantly released in the colon [22]. The specialized literature also reports combined active targeting techniques, for example, using nanocarriers that are both functionalized and loaded with magnetic nanoparticles [23]. This paper aims to provide a concise overview of the current state of research in the field of active drug targeting, primarily based on literature published over the past few decades.

3. Types of carriers used in active targeting

The various systems for targeted drug delivery are the subject of an actual design process that considers several aspects: the nature of the drug, the site of action, the route of administration, the mode of interaction between the nanocarrier and the drug, its ability to protect the drug until reaching the target and prevent premature release, the biocompatibility of the material from which the nanocarrier is built, the possibility of functionalizing it with ligands recognizable by cellular receptors, and so on.

A systematization of the main types of nanoparticles usable for targeted drug transport, the basic criterion being their chemical nature, as well as methods of preparing nanocarriers to ensure targeted drug delivery (with bibliographic references), is presented in Table 1.

Table 1. Types of nanocarriers for targeted drug delivery

The chemical nature of the nanocarrier	*Exemples*	*Active targeting preparation mode*	*Ref.*
Metals	- gold - silver - platinum - palladium	Coating with poly(ethylene glycol) (PEG) or polysaccharides and functionalization with ligands (antibodies, peptides, folate)	[18, 24-28]
Metal oxides	- Fe oxides (maghemite, magnetite) - titanium dioxide - zinc oxide	- Fe oxides are frequently coated with a dextran/PEG/polymer layer, or silica coating, followed by (silanization) or diacyl-phosphonate treatment for anchoring, allows for ligand conjugation and external magnetic targeting. -Ti and Zn oxides are silanized or coated with polymers. Functionalization is achieved by phosphonate linkages and by adsorption/covalent bonding of molecules with hydroxyl groups.	[29-34]
Inorganic compounds	Ceramic - silica, - alumina	- functionalization by silanization by introducing $–NH_2$ and/or –SH groups to facilitate ligand attachment. - alumina is treated with organosilanes, organophosphates, or by polymer absorption to introduce reactive groups; subsequently, conjugation with the ligand is performed.	[9, 35]
	quantum dots	stabilization by coating with a functional polymer (COOH, NH_2, maleimide), followed by PEGylation and conjugation with antibodies, aptamers, or peptides for targeting	[36-38]

	carbon - fullerenes - graphene - nanotubes - carbon dots - diamond	- modification by creating covalent bonds with the advantage of bond stability but the disadvantage of structural modification: controlled oxidation (introduction of –COOH), followed by activation with carbodiimide and N-hydroxysuccinimide for amidation. For fullerenes, the Prato reaction is used, which involves diazotization of the amino group and subsequent modification with folic acid. - coating with chitosan derivatives and subsequent functionalization with a ligand. - modification by creating non-covalent bonds: π–π interactions (aromatic derivatives, polypeptides), surfactant/polymer adsorption (pluronic, PEG-derivatives), with the advantage of preserving intrinsic properties but of reduced stability under physiological conditions.	[39-44]
Organic compounds	lipid	- PEG coating to increase circulation time ("stealth"). - functionalization with ligands by covalent conjugation (antibodies, aptamers, sugars, folate) or insertion of modified amphiphilic lipids	[45, 46]
	lipozomes	coating by PEGylation followed by conjugation by amidation reactions, maleimide-thiol, or click chemistry with folate, transferrin, or antibodies.	[47-51]
Polymers	dendrimers	-introduction of functional groups (–NH_2, –OH, –COOH) followed by conjugation with folate, peptides, antibodies, saccharides.	[52]
	micelles	- constructed from amphiphilic copolymers, often PEG, to the end of which the ligand (peptides, folate, aptamers, antibodies) binds	[53-59]
	spherical (full) nanoparticles	functionalization by activated reactions of carbodiimide and N-hydroxy succinimide, click chemistry, maleimide-thiol, followed by ligand binding (antibodies, peptides, aptamers, lectins)	[40, 60-69]
	nanocapsules	creation of a polymer membrane (PLGA, chitosan, Eudragit) and functionalization with ligand (folate, hyaluronic acid, transferrin, monoclonal antibodies)	[70-73]
	polymersomes	constructed from PEG-based block copolymers, to which a ligand (antibody, peptide) is attached	[74]

Hibride	magnetic polymer nanoparticles	functionalization with folic acid and loading with magnetic nanoparticles (ensures dual targeting)	[21]
	magnetic polymer nanocapsules	functionalization with peptides and loading with magnetic nanoparticles (ensures dual targeting)	[23]
	magnetic liposomes	coating by PEGylation followed by conjugation by amidation reactions, maleimide-thiol, or click chemistry with folate, transferrin, or monoclonal antibodies	[51]

Therefore, active targeting nanocarriers can be made from a wide range of materials: inorganic (metals, metal oxides, ceramics, carbon-based nanoparticles), organic, and polymers. Each of these, regardless of their chemical nature, can be functionalized with appropriate ligands to ensure preferential anchoring to receptors on the surface of diseased cells (Table 1). Obtaining inorganic nanoparticles is relatively simple and well established (except for Carbon Dots, which require more advanced technology), but developing other types of nanoparticles requires more complex techniques (except for low-density lipoprotein nanoparticles obtained via nanoprecipitation from solution in an ultrasonic field). Some examples are illustrated below.

- ***Liposomes*** are spherical vesicles of colloidal size (20 nm - 10 μm) formed by a double layer of molecules with surface-active properties, namely lipids (e.g., phospholipids). The molecules are arranged so that the hydrophilic, polar part is directed outward, while the hydrophobic part is directed inward (see Fig. 2), thus creating a lipid membrane that separates the vesicle from the external liquid environment. When the lipid membrane consists of a single double layer, we speak of unilamellar liposomes, with diameters ranging from 25 to 200 nm. When the membrane is composed of several concentric double layers, we talk of multilamellar liposomes, with diameters ranging from 200 to 3500 nm.

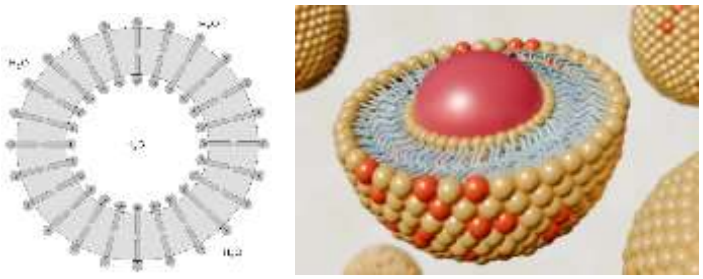

Fig. 2. Representations of a liposome
(https://ro.wikipedia.org/wiki/Utilizator:Madalinus/Lipozom_subpagin%C4%83)

Liposomes can simultaneously transport both hydrophilic drugs in the vesicle core and lipophilic drugs in the lipid membrane layer(s). They are obtained by several methods [75] that allow control of size and dimensional polydispersity. In addition to these properties, liposomes interact specifically with biological membranes and cells. If the constituent lipid is previously functionalized with a ligand, the result is a directly functionalized liposome capable of targeting cells that possess the corresponding receptors [47,48].

- ***Dendrimers*** are hyperbranched three-dimensional polymer structures that are radially developed to form spherical assemblies, practically exhibiting monodispersity in size. They were discovered by F. Vögtle (1978) and by Tomalia and collaborators (1980) [76]. Figure 3 shows the successive stages of dendrimer size growth starting from a trifunctional monomer [77,78].

Nanometric dimensions (adjustable), monodisperse size distribution, the presence of an immense number of functional groups on its surface, compact structure, ability to form conjugates with drugs having complementary functional groups, ability to include drug molecules between its branches and transport them to the target, easy functionalization with ligands, and other features make dendrimers ideal nanocarriers, including for targeted drug delivery [77, 78].

Polymeric micelles are spherical nanostructures with diameters ranging from 10 to 100 nm. They are obtained from amphiphilic block copolymers when their concentration in aqueous solution reaches a certain value (critical micelle concentration – CMC). Morphologically, the micelle has a core–shell structure: the hydrophobic part of the copolymer orients inward, forming a hydrophobic core, while the hydrophilic block orients toward the aqueous medium, forming a protective corona. A suggestive image is shown in Fig. 4 [79] [Fig. 4 (A)]. The self-assembly process of such copolymers is governed by the hydrophobic effect, which reduces the Gibbs energy of the system by removing hydrophobic blocks from the aqueous medium.

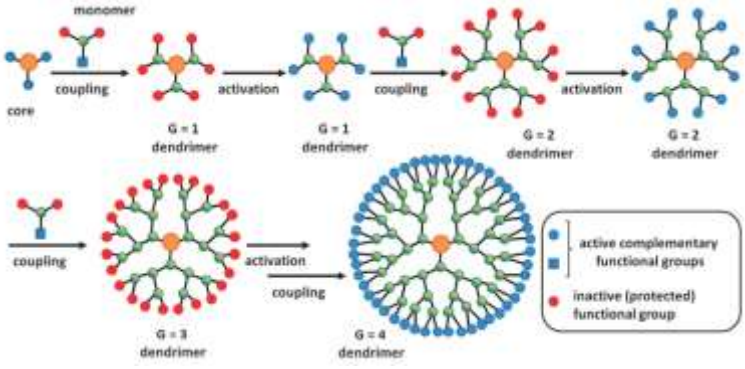

Fig. 3. Schematic representation of dendrimer formation starting from a trifunctional monomer [77].Reproduced with permission from Sowińska, M.; Urbańczyk-Lipkowska, Z., *New Journal of Chemistry*, 2014, 38, 2168. © Royal Society of Chemistry.

The hydrophobic arms of the micelle are later functionalized with ligands to ensure active targeting [Fig. 4 (B)] [80]. Although obtaining polymeric micelles functionalized with ligands involves fairly complex chemistry, their small size and the variety of polymers that can provide the hydrophilic arm allow the functionalization with ligands of different chemical structures and, consequently, targeted drug delivery to any organ in the body.

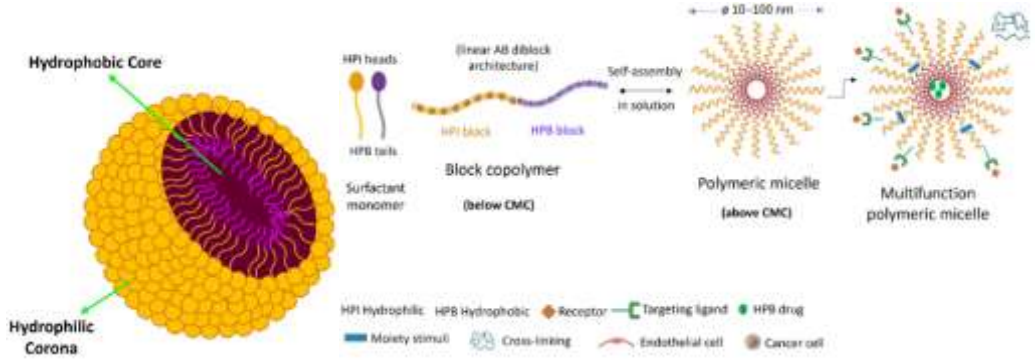

Fig. 4. Suggestive schematic representation of micelle morphology (A) and the steps involved in synthesizing multifunctional micelles modified with ligands for active targeting (B) [80].

- ***Polymeric nanoparticles*** have a high potential to increase the therapeutic efficacy of drugs, on the one hand by facilitating their release at the specific site of action (targeting) and, on the other hand, by protecting them until they reach the site of action. Current methods for obtaining them, the relatively wide variety of polymers from which they can be constructed, the possibility of producing them in various, controllable sizes, with different surface chemical groups that allow functionalization with ligands, and their ability to respond to stimuli associated with the biological environment make polymeric nanoparticles the most usable carriers in targeted therapy [81].

Some advantages of using them as carriers are: (***i***) they increase the solubility of poorly water-soluble drugs; (***ii***) they protect the therapeutic agent from degradation before reaching the target; (***iii***) they prolong circulation time in the body; (***iv***) they ensure controlled drug release; (***v***) they facilitate precise targeting of diseased tissues/cells with the drug [40].

However, numerous challenges need to be addressed, such as stability, biocompatibility, and biodegradability, which depend mainly on the nature of the polymer used to construct the particles and the manner in which they are obtained, often involving complex chemistry that requires the use of toxic reagents, which are difficult to remove from the final product. On the other hand, surface characteristics such as electric charge, hydrophobicity, and the presence of chemical functional groups determine how the particles behave in the biological

environment. These properties may be intrinsic to the polymer(s) used to obtain them or may be modified through appropriate chemical reactions. Table 2 presents, in a concise form, the main methods for obtaining polymeric nanoparticles, with brief references to the procedures used, advantages, limitations, and a selective bibliography.

From the perspective of the method/technology used to obtain nanoparticles, we can speak of physical and chemical processes.

Physical processes: nanoprecipitation, emulsion–solvent evaporation, assembly/coacervation, spray drying (including nanospray drying and supercritical CO_2-assisted spray drying), freeze drying, and fluidized-bed drying.

Chemical processes: polymerization in mini- or nanoemulsion, crosslinking in inverse emulsion (with variants such as simple crosslinking and double crosslinking).

From the perspective of the starting raw material, we can speak of:

-Processes of polymerization or polycondensation starting from *monomers.*

-Processes starting from preformed *polymers,* especially polysaccharides.

Regardless of the method of preparation, nanoparticles can subsequently be functionalized with ligands to ensure targeting and concentration in the desired area.

Table 2. Methods for obtaining polymeric nanoparticles for targeted administration

Obtaining procedure	*Short description*	*Advantages*	*Disadvantages (limitations)*	*Ref.*
Mini- or nanoemulsion polymerization	formation of micro/nano-particles by polymerization of monomers in an organic phase dispersed in an aqueous continuous phase	- controlled dimensions, - narrow dimensional polydispersity/homogeneity - in the case of templating, control over morphology, allowing the production of porous particles	- requires the complete removal of monomers and initiators that can induce toxicity	[82-84]
Nano-precipitation	precipitation of a polymer from aqueous solution with a miscible organic solvent (alcohol) in the presence of surfactants, in an ultrasonic field, followed by drying by lyophilization	- a simple method that does not require high temperatures and is suitable for heat-sensitive compounds.	- requires fine control of mixing speed and solvent /water ratio.	[85,86]

Emulsion-solvent evaporation	-the polymer and drug are dissolved in a slightly volatile organic solvent immiscible with water, which is then emulsified with water containing surfactant, after which the solvent is evaporated, forming solid particles	- applicable for water-insoluble polymers (PLGA, PLA).	- difficult size control/high parameter dependence -possible loss of active/ hydrophilic molecules - aggregation upon drying - traces of solvent, sometimes toxic	[87]
Assembly/ coacervation	phase separation by coacervation of the polymer in solution, forming increasingly concentrated particles in the polymer	- does not require organic solvents - suitable for sensitive bioactive molecules	pH and temperature sensitivity	[88]
Inverse emulsion crosslinking -single crosslinking -double crosslinking	emulsification of the aqueous solution of polymer and drug in an organic solvent, followed by crosslinking with bifunctional compounds (simple crosslinking) or with covalent and ionic crosslinkers (double crosslinking)	- easy adjustment of size and dimensional polydispersity by playing with reaction parameters - direct inclusion of the drug from the synthesis stage, or later by diffusion - high biocompatibility by reducing the amount of covalent crosslinker	- advanced purification of nanoparticles to remove organic solvent and traces of crosslinker - double crosslinking can only be used in the case of polyions	[60, 89, 90]
Spray drying - nano spray drying - supercritical CO2-assisted spray drying	-the polymer nano-suspension is atomized in a hot air stream, causing instantaneous evaporation of the solvent and deposition/collection of the powder. - one variant is supercritical fluid technology, which consists of replacing the solvent with supercritical CO2,	- the size and morphology of the nanoparticles depend on the inlet/outlet temperature, air flow rate, and the nature of the stabilizing agent.	-in the first variant, thermal degradation of temperature-sensitive active principles may occur	[91, 92]

	which evaporates instantly			
Freeze drying	rapid freezing of the nanoparticle suspension and removal of the solvent by sublimation under vacuum (lyophilization)	- redispersible powders are obtained, preserving the pharmacokinetic and biofunctional properties of the nanoparticles.	wide dimensional polydispersity	[93, 94]
Fluidized-bed drying	-a stream of hot air maintains the particles in a nanosuspension (fluidized state), ensuring intense and uniform contact between the gas and the solid.	-it can be used both for drying and for coating or granulating nanoparticles, transforming nanosuspensions into redispersible powders.	-possible thermal degradation of heat-sensitive drugs	[95, 96]

- ***Polymeric nanocapsules*** – these are vesicular systems in which the drug is loaded into a cavity consisting of a liquid core surrounded by a polymer membrane [97]. More generally, they can be defined as nano-vesicular systems in which the drug constitutes the core, being surrounded by a polymer membrane or a polymer coating [98]. Two suggestive images illustrating the morphology of a polymeric capsule are shown in Fig. 5.

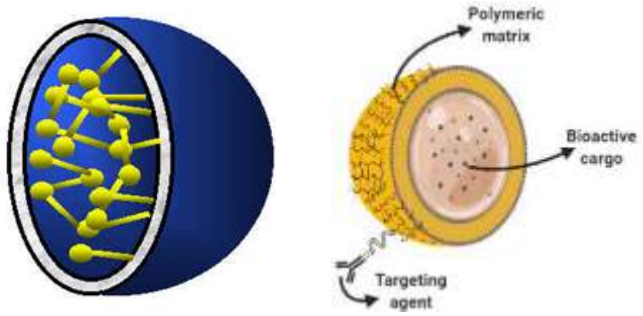

Fig. 5. Suggestive images showing the construction of a non-functionalized polymeric nanocapsule (A) and one functionalized with a targeting ligand [99].

Several methods are known for obtaining nanocapsules, either starting from monomers or from preformed polymers.

The emulsion–solvent evaporation method is one of the most widely used methods, starting from a double emulsion (water/oil/water) containing the polymer dissolved in a volatile solvent, which constitutes the oily phase, and the drug (lipophilic or hydrophilic). Upon solvent evaporation, a nanocapsule is obtained with either a

liquid or a solid core (if the solvent is less volatile or the drug is liquid) and a polymer shell [100].

The nanoprecipitation method (solvent interdiffusion) consists of dissolving the polymer and the drug in a solvent miscible with water, then gradually adding the solution, drop by drop, without stirring, to an aqueous phase. The polymer precipitates in a controlled manner at the droplet–water interface, around the internal phase containing the drug. It is a rapid, heat-free process that is highly suitable for encapsulating thermolabile drugs [97].

The coacervation method (simple or complex) consists of separating the polymer-rich phase (coacervate) from the solution, which deposits on the surface of the internal (oily) phase containing the drug. Coacervation can be induced by changing pH, temperature, or by electrostatic interactions between polymers with opposite charges. This method offers good control over shell thickness and allows the production of biocompatible nanocapsules [101].

The double emulsion method involves creating a primary emulsion by emulsifying an aqueous polymer solution in an immiscible organic solvent containing the drug, followed by re-emulsification in another organic solvent to obtain double emulsion droplets consisting of the aqueous phase containing the polymer intercalated between two organic phases. By diffusion of an appropriate crosslinker (a bifunctional compound such as glutaraldehyde) from the organic phase to the aqueous phase, polymer crosslinking occurs, forming a membrane around the liquid core containing the drug. To strengthen the nanocapsule walls and avoid using large amounts of covalent crosslinker, often toxic, double crosslinking, both covalent and ionic can be applied. This method allows simultaneous encapsulation of hydrophobic drugs (in the nanocapsule core) and hydrophilic drugs (in the polymer membrane) [102,103].

Interfacial polycondensation consists of the diffusion of condensation-capable monomers from two immiscible phases toward the interface, where they polycondense to form a polymer membrane. The drug can be dissolved in one of the two phases, remaining encapsulated within it. This technique enables the production of highly stable nanocapsules with controlled permeability [104].

Interfacial condensation is based on the reaction between functional groups on the chains of two polymers soluble in immiscible solvents, with one solution introduced, under vigorous stirring, in fine droplets into the other polymer solution. As the polymers diffuse toward the interface, their complementary functional groups react upon contact, resulting in the formation of a polymer membrane that surrounds the core containing the drug dissolved in one of the phases. This method requires polymers with highly reactive functional groups (even at room temperature), such as anhydride groups, which readily react with amine or hydroxyl groups [105]. Nanocapsules have the advantage of being able to carry larger

amounts of drug than solid nanoparticles of the same diameter, and their shell protects sensitive molecules (RNA, peptides) more effectively.

These systems offer numerous advantages, including high loading capacity for lipophilic molecules, controlled release profiles, and a stable protective shell that protects sensitive cargos, such as RNA or peptides. Nevertheless, they also present certain limitations. For example, an unstable membrane may lead to premature cargo release, and some preparation techniques may involve the use of surfactants or organic solvents that must be thoroughly removed. Furthermore, scaling production to an industrial level can pose significant technical challenges. Notwithstanding these constraints, these systems are extensively employed in the delivery of hydrophobic pharmaceuticals, particularly in oncology, as well as in various gene and RNA delivery methodologies.

- ***Polymersomes*** are self-assembled nanoarchitectures formed from amphiphilic copolymers, spherical or cylindrical, depending on the ratio of hydrophilic to hydrophobic arms. Spherical ones have a structure analogous to that of liposomes, but with thicker, more resistant membranes. The copolymers used can be di- or tri-block (with end arms either hydrophilic or hydrophobic) or graft copolymers with a hydrophilic backbone and hydrophobic grafts. In aqueous media, these copolymers naturally assemble into a polymer bilayer that encloses an inner compartment, allowing the encapsulation of water-soluble substances in the lumen and hydrophobic substances in the membrane layer. The difference between copolymers and lipids (liposome constituents) lies in the polydisperse nature of copolymers, even though recent polymerization methods allow products with nearly monodisperse characteristics. Amphiphilicity is the driving force that determines the self-assembly of both lipids and amphiphilic copolymers into vesicles [106,107]. Properties such as biocompatibility, colloidal stability, and responsiveness to external stimuli (pH, temperature, redox reactions, enzyme action) impart versatility to these nanocarriers while providing precise control over permeability and release time. Surface functionalization with specific ligands (antibodies, peptides, aptamers) enables active targeting of pathological cells, especially in oncological treatments and personalized nanotherapy.
- ***Hybrid nanoparticles.*** This category includes organic or polymeric nanostructures associated with nanoparticles of another chemical nature, usually magnetic. The purpose of this association is to achieve dual targeting: on the one hand, determined by ligand functionalization, and on the other hand, achieved by directing the nanocarrier introduced into the body using an external magnetic field. The methods of preparation and functionalization are the same as for simple nanoparticles.

Magnetic hybrid nanoparticles are reported in the literature [21], incorporating magnetic nanoparticles—usually maghemite or magnetite (Fe_3O_4)—into a polymer matrix. They are obtained either by coating previously synthesized maghemite nanoparticles (via

nanoprecipitation or emulsion–solvent evaporation) with a polymer or by incorporating them into matrices formed by in situ polymerization [23,108,109].

Magnetic polymeric nanocapsules are core–shell polymer nanostructures that contain magnetic nanoparticles, either dispersed in the solid core along with the drug or integrated into the polymer membrane that protects it. They are obtained by interfacial polycondensation or condensation, by the double emulsion method with solvent evaporation, or by interfacial polymerization in inverse emulsion [110].

Magnetoliposomes contain magnetic nanoparticles either in the lumen (core) or in the lipid bilayer. They are obtained by: (***i***) incorporating magnetic nanoparticles into a lipid film followed by hydration and extrusion [111,112]; (***ii***) encapsulation during liposome formation by sonication/evaporation; (***iii***) post-formation functionalization (anchoring with modified lipids) [51].

4. Ligands for Active Targeting

Active targeting is undoubtedly one of the most advanced areas in modern pharmaceutical nanotechnology, focusing on the selective delivery of drugs to specific cells or tissues while also minimizing systemic side effects. Active targeting differs from passive targeting by utilizing specific molecular interactions. While passive targeting relies on physiological accumulation phenomena, such as the Enhanced Permeability and Retention (EPR) effect, active targeting uses ligands attached to the surface of nanocarriers that bind to receptors overexpressed on pathological cells. But what is the importance of ligands attached to nanocarriers for targeting? By functionalizing the nanoparticle surface with *ligands* (biological or synthetic), the system gains *molecular specificity and active recognition capability*. Thus, nanocarriers can accurately deliver loaded drugs to targeted cells, improving therapeutic effectiveness while reducing toxicity to healthy tissues [113]. The choice of an appropriate ligand is not random; it is dictated by a series of factors that critically influence colloidal stability, release kinetics, biodistribution, and cellular internalization mechanisms of the delivery system. Moreover, the simultaneous use of several ligand types enables *multimodal targeting*, offering advantages in combined therapies and personalized nanomedicine.

Ligands are the central element that transform nanocarriers from simple passive structures into intelligent delivery systems capable of directed interactions, adaptive responses to biological stimuli, and precise molecular recognition of pathological tissues. Below is a brief overview of the types of ligands used to functionalize drug nanocarriers for active targeting:

- ***Monoclonal antibodies***

Monoclonal antibodies offer highly specific targeting by recognizing cellular antigens, such as tumor receptors and surface markers. They can attach to the nanoparticle surface either covalently through conjugation or non-covalently,

directing accumulation at the desired target [17]. Nanoparticles thus functionalized combine the advantages of nanocarriers (delivery of larger amounts of drug to the affected area) with improved selectivity and release efficiency [114,115]. In the bloodstream, functionalized nanoparticles can interact with biological molecules, forming a protein corona that triggers an immune response and affects the targeting ability of the nanoformulation [116].

Antibody fragments / nanobodies are structures that retain antibody specificity but are much smaller, which improves tissue penetration. They are more stable and easier to express and engineer. They are often used for nanoparticle conjugation in therapeutic and imaging applications**.** [117,118].

- ***Peptides***

Short peptides that recognize surface receptors (integrins, neuropilin, etc.) are widely used to target tumors and tumor vasculature, offering advantages such as small size, low cost, and ease of conjugation to functional groups on polymer chains [119]. RDG (targets integrins αvβ3/αvβ5) is one of the most widely used targeted peptides and is found in adhesive cellular matrices. It is known for its high affinity for the αvβ3 heterodimer receptor, which is overexpressed in neoplastic endothelium [120,121]. Other ligands in this group include TAT and iRDG. TAT (Trans-Activator of Transcription) derives from the transactivator protein of HIV-1 and is a cell-penetrating peptide (CPP) composed of 11 amino acids (typical sequence: YGRKKRRQRRR). It can cross cell membranes via endocytosis or direct translocation without affecting membrane integrity. When conjugated to the surface of liposomes, dendrimers, nanoparticles, or polymeric nanocapsules, TAT increases cellular penetration efficiency (internalization), including in tumor cells and neurons of the central nervous system [122,123]. iRGD recognizes integrins αvβ3 and αvβ5, overexpressed in tumor vessels. It is considered one of the most efficient ligands because, after binding to integrins, it undergoes proteolytic cleavage, generating a fragment that binds to the neuropilin-1 (NRP-1) receptor, triggering transcytosis and deep penetration into tumor tissue. It is used for functionalizing liposomes, polymeric nanoparticles, metallic nanoparticles, or silica nanoparticles [124].

- ***Aptamers***

Aptamers are oligonucleotides (or oligoribonucleotides) that can adopt a 3D conformation to recognize a target protein with high affinity and specificity. Their main advantages include being non-immunogenic, easily chemically modified, and even synthesized. An important application is their use in functionalizing theranostic nanoparticles as an alternative to antibodies [125,126].

- ***Small Molecules / Vitamins***

Small molecules (folate, biotin, certain vitamins or inhibitors) can recognize (or be recognized by) receptors overexpressed in certain cell types. Among these, folate

is the most widely used, with many in vitro studies proving its efficacy compared to other types of ligands (especially endosomal trapping).

In vivo biodistribution studies revealed issues that may arise from folate receptor targeting, including rapid liver uptake, subsequently reducing the nanoparticles' tumor uptake [127–129].

Biotin, also known as vitamin B7 or vitamin H, is an essential water-soluble vitamin from the B complex, with a high capacity to actively target tumor cells [130]. Polymeric nanoparticles functionalized with biotin exhibit enhanced internalization in brain endothelial cells, demonstrating the ability to cross the blood–brain barrier (BBB). Tripathi et al. provide a critical review of the mechanisms by which biotin conjugates can be taken up—essential for understanding whether "biotin-targeting" is mediated by SMVT or other pathways [131]. Veszelka et al. show that biotin can increase nanoparticle internalization in brain endothelial cells, providing a useful BBB model for brain delivery applications [132]. An interesting comparison between the efficacy of nanoparticles decorated with folic acid and those doubly decorated with folic acid and biotin is reported by Jurczyk et al. [133]. Biotin has also been used to functionalize PAMAM-based dendrimers to modulate release and enhance anti-proliferative and apoptotic effects of drugs intended for tumor cells [130].

Vitamin B12 (cobalamin) is an interesting example of a ligand, leading to the development of polymeric nanocarriers designed to target the CD320 receptor (overexpressed in certain cancers) and deliver a genetic payload (miRNA) with therapeutic effect [134].

- ***Carbohydrates / Glycoconjugates***

Several polysaccharides or their derivatives can bind lectin receptors, suggesting their potential as ligands for targeted therapy. A relatively recent review summarizes these aspects [135]. For example, hyaluronic acid and other oligosaccharides bind to lectin receptors and are used for cellular targeting and selective release; hyaluronic acid is frequently used for targeting CD44-positive cells in cancer [136].

Mannose is recognized by mannose receptors (CD206), which are mainly expressed on macrophages, dendritic cells, and some tumor lines. Nanoparticles decorated with mannose can thus be directed to these cells, facilitating targeted delivery of vaccines, immunotherapeutic agents, or anti-inflammatory drugs. This approach is particularly useful for nanovaccines and treatments for infectious diseases and cancers associated with the immune system [137,138].

The major advantage of using oligo- and polysaccharides as targeting ligands lies in their abundance, both quantitative and structural, and their intrinsic biocompatibility.

- ***Endogenous proteins***

Endogenous proteins such as transferrin and albumin can be successfully used to exploit physiological transport pathways. Transferrin binds to the TfR1 receptor, which is overexpressed on many tumor cells and the blood–brain barrier (BBB), thereby facilitating barrier overcoming and targeting the central nervous system [139,140]. On the other hand, albumin serves as a natural ligand for the transport of hydrophobic molecules. It can be used to stabilize and prolong the circulation of nanocarriers in the bloodstream, as well as to facilitate selective tumor accumulation through receptor-mediated recognition [141].

5. Applications of targeted drug delivery systems

The development of targeted therapies represents one of the significant directions of modern medicine, aiming, as stated several times in this manuscript, to deliver active substances selectively to diseased tissues or cells while minimizing unwanted systemic effects. This concept underpins personalized nanomedicine, which combines advances in nanostructured materials with molecular biology and advanced pharmacology.

Targeted therapies can treat diseases that fall into several main categories, depending on their pathological mechanisms and the type of therapeutic targeting involved.. A review of these and how they can be treated using targeted nanomedicine will be presented below.

- ***Oncological diseases***

Cancer treatment is the greatest challenge of current medicine. Cancer has therefore become the most studied field of targeted therapies, as tumor cells exhibit overexpression of receptors (e.g., HER2, folate, transferrin, EGFR) or specific tumor microenvironments (acidic pH, enzymes, hypoxia). Nanocarriers can thus be programmed for active recognition (via specific ligands) or local activation (via pH/redox-responsive mechanisms) [142–144].

Nanoparticles functionalized with antibodies, peptides (such as RGD, TAT), folic acid, biotin, or aptamers are extensively studied for targeted delivery of chemotherapeutic agents, gene inhibitors, or photodynamic drugs. These systems allow selective accumulation in the tumor microenvironment, reduce systemic toxicity, and can cross biological barriers such as the dense tumor barrier [133,134].

Triple-negative breast cancer (TNBC), highly metastatic, often exhibits multidrug resistance, which complicates drug uptake and promotes its expulsion from cells. Conventional antitumor treatments face challenges, including limited drug targeting specificity, short retention times in tumor tissue, and harmful effects on both tumor cells and surrounding healthy tissue. Studies have concluded that designing drug

nanocarriers of small size, comparable to the scale of biological molecules, can improve therapeutic effects [145]. Samrat *et al*. report the development of a system consisting of submicron particles based on poly(vinyl alcohol) obtained by coacervation, encapsulating bis-(isothiocyanatomethyl) benzene, known for its antitumor activity. The average nanoparticle diameter is 300 nm, and their encapsulation efficiency reached 68%. In vitro tests on tumor cell lines (MDA-MB-231) showed reduced viability and higher apoptosis rates compared to hormone-responsive MCF-7 cells. Molecular docking studies revealed strong binding affinity of PC for the estrogen receptor (ER), demonstrating its antagonist activity and suggesting its potential for targeting aggressive breast cancer, which exhibits both cytotoxic and anti-metastatic properties [146].

Malignant tumors can occur in several endocrine glands (thyroid, adrenal, pancreatic, parathyroid, and pituitary glands). Although not common, parathyroid carcinoma is notable for releasing parathyroid hormone, which leads to hyperparathyroidism. Adrenocortical carcinoma (from the adrenal tumor category) is another example of a highly aggressive tumor, known for frequent recurrence and limited chemotherapy effectiveness. An interesting and comprehensive recent review presents possibilities for targeting these types of cancer using nanocarriers decorated with specific receptors [147].

Pancreatic cancer is one of the most lethal forms of cancer due to its uniquely aggressive and hypoxic tumor microenvironment. Tumor hypoxia is a characteristic of adenoid pancreatic carcinoma, consisting of an insufficient oxygen supply, significantly lower than in normal pancreatic tissue. This effect is caused both by abnormal tumor vascularization and by the dense stromal content, being the determining factor of therapy resistance in this type of cancer [148].

Over the past 15 years, various nanoparticulate systems have been developed to specifically target this type of cancer, using nearly all the nanocarriers discussed in this paper [149]. The major challenge is to develop a theranostic platform, that is, a multifunctional nanocarrier capable of targeting, transporting the drug to the target, releasing it in a controlled manner, and subsequently monitoring treatment progress.

It is anticipated that in the near future, the number of nanoformulations approved by the FDA will increase, contributing to enhanced chemoprevention and treatment options to cure and/or prolong survival for those affected by this relentless form of cancer.

Lung cancer is one of the malignant tumors with an increasingly high mortality and morbidity rate in recent years. It is therefore no coincidence that research into new possibilities for detection, diagnosis, and especially treatment has intensified in recent years, focusing mainly on targeted therapy using nanocarriers (liposomes, polymeric nanoparticles, micelles, solid lipid particles, or biomimetic nanosystems) functionalized by conjugation with ligands for overexpressed receptors (EGFR, αvβ3, folate receptor,

transferrin receptor, etc.) [150,151]. *In vitro* and *ex vivo* studies demonstrate improved pharmacokinetics and the ability of functionalized nanocarriers to accumulate at the tumor site (including via the EPR mechanism), thereby reducing systemic toxicity. In this context, it should be noted that other pulmonary conditions can also be targeted with functionalized nanocarriers: pneumonia, chronic obstructive pulmonary disease, cystic fibrosis, and tuberculosis are generally caused by viruses, bacteria, and fungi. Systems such as vesicular carriers (nano- and microcapsules [73]) or micro-/nanospheres based on carboxymethyl chitosan and poly(vinyl alcohol) [61], functionalized on the surface with both the peptide (CGSPGWVRC) and indolicidin, have been reported as effective ligands for active targeting of both alveolar capillary endothelial cells and bacterial cells. Both types of carriers were evaluated for their effects on human lung cells (WI-38) and mouse macrophages (RAW 264.7), demonstrating biocompatibility (lack of toxicity) and the ability to internalize at the intracellular level.

Glioblastoma is a highly aggressive form of brain cancer, with the significant challenge for its treatment being the need to cross the blood–brain barrier (BBB), characterized by high selectivity and thus difficult to overcome. The most productive strategies involve nanoparticles [152] functionalized with ligands that use receptor-mediated transcytosis pathways (e.g., transferrin/transferrin receptor, anti-PDGFRβ aptamers, peptides that mediate transcytosis). Transferrin-conjugated liposomes [153] have demonstrated significant increases in intratumoral accumulation and improved therapeutic response in preclinical models, showing high potential for glioblastoma treatment. Li et al. [154] prepared liposomes functionalized with transferrin, loaded with elemene and cabazitaxel, and subsequently modified with cell membrane proteins of RG2 glioma cells, thus obtaining active-targeting biomimetic liposomes (Tf-ELE/CTX@BLIP). In vitro tests demonstrated a high capacity to target specific brain tumor cells compared to non-functionalized liposomes. In vivo tests showed that modified liposomes could cross the BBB and accumulate in the brain, leading to increased survival time and reduced tumor volume in treated animals.

Aptamer-functionalized nanosystems have also shown, through *in vivo* testing, the ability to cross the BBB and deliver specific antitumor drugs for glioblastoma treatment [155]. Albumin-based nanoparticles functionalized with low-molecular-weight protamine, crosslinked with oxidized gellan, and loaded with polyphenols, exhibiting high antioxidant activity and potential for glioblastoma treatment, were recently reported by the group led by Iurciuc et al. [156]. In vitro tests using an artificial membrane simulating the BBB demonstrated that this system can cross it.

Skin Cancer

Two types of skin cancer are known: melanoma and basal cell carcinoma (BCC).
Melanoma is a malignant tumor derived from melanocytes, the pigment-producing cells of the skin. It has high metastatic potential and aggressive progression. It is associated with UV radiation exposure and mutations in genes such as BRAF,

NRAS, or KIT. It requires complex treatments, such as traditional surgery and immunotherapy. Targeted therapy with nanoparticles (liposomes, polymeric nanoparticles, microenvironment-sensitive systems, nanovaccines, and platforms for combined therapy with immunotherapy) aims at both tumor cells (via ligands for specific receptors or tumor antigens) and the tumor microenvironment (e.g., to overcome hypoxia or interstitial pressure that hinders drug penetration). Modern approaches combine targeted delivery with immunotherapy (checkpoint inhibitors) or theranostic systems (imaging and therapy) and have shown promising results in preclinical models [157,158]. All published studies emphasize that ligand design (specificity, affinity, density on the nanoparticle surface), size, and surface properties determine the success of active targeting and biodistribution.

Basal cell carcinoma (BCC) originates from basal cells of the epidermis. It is the most common type of skin cancer, but with low aggressiveness and rare metastases. Conventional treatment (excision, cryotherapy, imiquimod, Hedgehog inhibitors) is effective, but nanotherapies can improve local drug penetration (e.g., vismodegib, 5-fluorouracil) in extensive lesions. Research results from the group coordinated by Popa have demonstrated the potential of liposome-type nanocarriers functionalized with aptamers [48,49], as well as nanocapsules based on chitosan and poly(vinyl acetate-alt-maleic anhydride) functionalized with the same ligand and loaded with 5-fluorouracil, to target BCC through topical administration of a hydrogel in which the nanocapsules were dispersed. The aptamer used is recognized by cellular receptors such as nucleolin, which is highly expressed in the membrane of tumor cells (BCC) [71].

Ex vivo tests demonstrated the nanosystem's ability to penetrate chicken skin (a model of human skin) and thereby increase the bioavailability of the antitumor drug. In vitro cytotoxicity assays on the TE 354.T (ATCC® CRL-7762™) human basal carcinoma cell line showed that the formulations loaded with 5-fluorouracil exhibit significant cytotoxicity.

- ***Cardiovascular diseases***

Targeted delivery systems are being investigated for the local administration of thrombolytic, anti-inflammatory, or regenerative agents to atherosclerotic plaques or ischemic tissue. Nanoparticles can be directed using specific ligands for endothelial adhesion molecules (VCAM-1, ICAM-1) or by external magnetic fields.
Atherosclerosis and *atherothrombosis* are the main factors contributing to cardiovascular diseases, constituting the leading cause of death worldwide. Current pharmacological therapies are associated with side effects or are insufficient to halt atherosclerotic progression effectively. Nanomedicine offers a way to treat these conditions more efficiently. Functionalized nanoparticles are used to deliver anti-inflammatory, antithrombotic, or reparative agents directly to the affected

endothelium, atherosclerotic plaques, or ischemic tissues. Active targeting is achieved through peptides and antibodies decorating the nanocarrier, directing it to adhesion molecules (receptors such as VCAM-1, ICAM-1, selectins), activated platelets, or macrophages; another approach involves using an external magnetic field to guide hybrid nanocarriers (loaded with magnetic nanoparticles) to the target [159]. Distasio *et al.* report the development of PEGylated peptide-targeted coatings employed to bind gene delivery NPs (NP-VHPK) to VCAM-1, an essential target in atherosclerosis [160]. In vivo tests revealed preferential binding of nanoparticles to inflamed endothelial cells (VCAM-1^high) in atherosclerotic plaque regions from the aorta and aortic sinus of mice. Administered via intravenous injection, they localized in the thoracic region near the heart and blood vessels in a mouse model of atherosclerosis, demonstrating targeting efficiency and the potential use of this nanocarrier as an alternative to conventional treatment for certain cardiovascular conditions. Pickett *et al.* highlight in their work that vascular cell adhesion molecule-1 (VCAM-1) is a critical contributor to atherosclerosis and consequently an attractive therapeutic target for anti-atherosclerotic drug candidates, reviewing recent literature on the possibility of targeting these cells with drug nanocarriers [161]. Noting the discovery two decades ago of VCAM-1 as an endothelial adhesion receptor exploitable for drug nanocarrier targeting, Castro et al. present the use of peptides, antibodies, or aptamers as targeting ligands [162].

- ***Neurological disorders***

Targeting the central nervous system to treat conditions such as Alzheimer's, Parkinson's, epilepsy, dyslexia, chronic headaches, stroke, etc., involves overcoming the blood–brain barrier (BBB), which is highly selective and thus poorly permeable [163]. Most drugs used to treat these conditions do not cross, or cross only briefly, the BBB, so strategies have been developed using receptor-mediated transcytosis pathways (e.g., transferrin/TfR, ApoE/LDLR, insulin/INSR) or cell-penetrating peptides (CPPs). Thomsen et al. provide a synthesis of data reported in the literature up to 2022 on TfR-mediated nanoparticle transport [164].

Physical approaches combined with chemical strategies (e.g., FUS + microbubbles) are also reported to facilitate nanoparticle passage through the BBB to deliver small molecules, mRNA/siRNA, proteins, or cytotoxic agents to glioma. Wu et al. review methods for BBB modulation and the use of nanocarriers, including ligand-functionalization to enhance targeting, as well as the use of physical and chemical stimuli for this purpose [165].

Nanoparticles decorated with transferrin, lactoferrin, apolipoprotein E, TAT peptides, or insulin facilitate receptor-mediated transcytosis, as previously mentioned. These allow controlled delivery of drugs for Alzheimer's, Parkinson's, multiple sclerosis, or glioblastoma. A study investigated the pharmacokinetics and pharmacodynamics of

chitosan-based nanoparticles functionalized with transferrin and loaded with ziprasidone, administered intravenously compared to intranasal administration in rats [166]. It was found that intranasal administration of the nanosystem led to greater nanoparticle and thus drug accumulation in the brain, recommending their development as targeted therapeutics for neuropsychiatric disorders.

- ***Metabolic and genetic disorders***

Functionalized nanocarriers are used for targeted delivery of mRNA, plasmid DNA, antisense oligonucleotides, and therapeutic enzymes. RNA therapeutics have shown potential in various medical applications, including virus vaccines, cancer immunotherapy, and gene editing, but their major problem is instability. Several strategies have been proposed to increase stability, but the most viable seems to be inclusion in lipid vehicles [167]. These are composed of lipid mixtures, including ionizable lipids that complex with RNA and facilitate endosomal escape. Based on this technique, two LNP-based mRNA vaccines (BNT162b2, Pfizer-BioNTech, and mRNA-1273, Moderna) were successfully developed and obtained FDA authorization in 2020.

For liver disorders, targeting is achieved using ligands such as galactose (for ASGPR) and glycyrrhetinic acid (GA) (for GA-R) to specifically bind receptors overexpressed on liver cells, facilitating the targeted delivery of therapeutic genes via receptor-mediated endocytosis. These optimize gene therapies for metabolic diseases such as familial hypercholesterolemia or Gaucher disease. Novel strategies include dual-targeting with multiple ligands to increase specificity and cellular uptake for diseases like hepatocellular carcinoma [168].

For *genetic therapies*, nanocarriers that protect the cargo (LNPs, cationic polymers) are used, enabling targeted transfection (hepatocytes). A typical ligand easily recognized by liver tumor cell receptors is the folate radical [21]. Hybrid nanoparticles, which contain encapsulated maghemite nanoparticles and are based on chitosan functionalized with folic acid, have demonstrated a high in vitro ability to target a liver cancer-specific cell line.

- ***Ophthalmological disorders***

Active targeting is being studied for ophthalmological conditions with increasing incidence not only in older individuals but also in younger generations: macular degeneration, glaucoma, and diabetic retinopathy. Nanoparticles enable controlled drug delivery to the anterior and posterior ocular chambers. Liposomes, polymersomes, and nanostructured lipids functionalized with anchoring peptides and mucoadhesive ligands (hyaluronan or lectins) increase ocular retention [169]. Such systems, when applied topically, subconjunctivally, or intravitreally, improve penetration and prolong release time, with progress reported in systems delivering

anti-VEGF agents, corticosteroids, or therapeutic mRNA [170]. A review summarizing recent achievements in targeted therapy for ocular diseases was published by Barar et al. [171].

- ***Inner ear disorders***

The inner ear is an extremely sensitive organ, and various chemical, physical, or biological factors can cause conditions such as hearing loss, Ménière's disease, and ototoxicity. Access to the inner ear (cochlea) is difficult; strategies include transtympanic administration or through the round window membrane (RWM) using nanoparticles/gels/hydrogels that ensure penetration and prolonged release. Particles can be functionalized to target sensitive cells (hair cells) or local macrophages and can transport otoprotective agents, antibiotics, or regenerative factors. Controlled drug release can be achieved by decorating nanocarriers with stimuli-responsive functionalities [23]. Recently, the development of nanoparticulate systems with the potential for dual targeting of the inner ear has been reported, consisting of magnetic nanocapsules functionalized with peptides capable of being recognized by receptors of inner ear-specific cells (TAT, Tet1), as well as functionalized magnetic cationic liposomes. The nanocarriers were loaded with dexamethasone with the goal of gradually releasing the drug at the level of inner ear cells to prevent and treat inflammation. [23,51].

The approach to this type of treatment is schematically shown in Fig. 6.

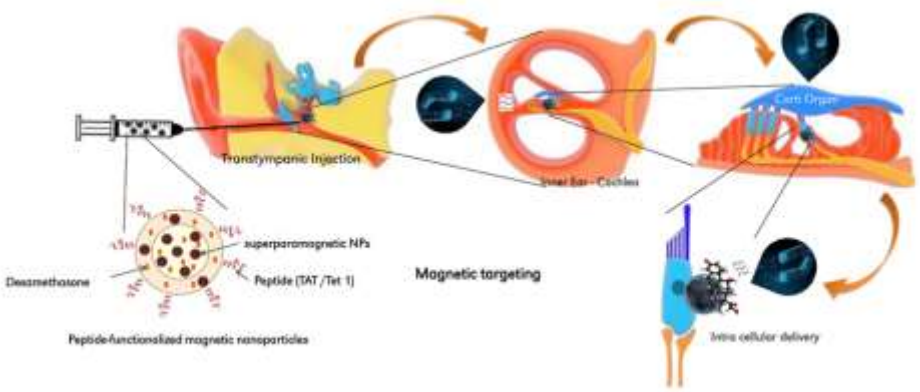

Fig. 6. Schematic representation of the possibility of treating inner ear disorders using nanocarriers (nanocapsules and/or liposomes) with dual targeting (magnetic and via ligands specific to receptors expressed in the membranes of inner ear-specific cells).

- ***Dermatological disorders***

Although drug transport and release through the skin remain challenging, recent research has shown that this method of treating dermatological conditions is feasible with the use of polymeric or lipid nanocarriers functionalized with suitable ligands.. The literature reports the possibility of using functionalized nanocarriers

for targeting skin conditions such as psoriasis, atopic dermatitis, acne, BCC, and melanoma. Liposomal, micellar, and polymeric nanoparticles are used for local delivery of anti-inflammatory agents, antibiotics, or antineoplastic agents, enhancing transdermal penetration and increasing local retention; functionalization with muco-/bio-adhesive ligands, hyaluronic acid, collagen, adhesive peptides, or bioadhesive polysaccharides targeting hair follicles/keratinocytes can boost selectivity and effectiveness [172,173].

Functionalized nanosystems with appropriate ligands allow, for example, selective delivery of anti-inflammatory, immunomodulatory, or antitumor drugs into the dermal layer or at the follicular level, where receptors or local conditions enable active recognition. This is particularly relevant in diseases such as atopic dermatitis, psoriasis, skin cancer, and chronic lesions, where the skin barrier is compromised, and nanoparticles can provide controlled release and active targeting within the affected tissue. Additionally, functionalizing nanoparticle surfaces with muco-adhesive groups, anchoring peptides, or molecules that recognize specific skin cell markers enhances local retention and internalization, leading to improved therapeutic effectiveness and lower toxicity.

Hyaluronic acid-based nanoparticles have been developed for the topical treatment of psoriasis by accumulating in inflamed skin, suppressing immune responses, and restoring barrier function, according to studies using in vivo skin penetration and psoriasis animal models [174]. Ammar et al. report the development of a system based on lipid nanoparticles functionalized with folic acid for skin targeting and propose an in vivo model for skin hydration/antioxidation [175].

Studies have found that the CD44 protein is highly expressed in certain skin diseases such as psoriasis. HA is a natural ligand of the CD44 protein and can potentially be used to develop targeted nanodrug transdermal delivery systems [176]. Zhao et al. propose different types of functionalized nanocarriers for targeted administration to treat various dermatological conditions. Hyaluronic acid nanoparticles and functionalization ligands are highlighted, with systems tested in vivo for psoriasis treatment [173]. A recent review also discusses types of nanocarriers, ligands, functionalization methods, and applications of systems tested in vivo for various skin conditions [177].

Conclusions

Targeted therapy using nanocarriers offers an adaptable and selective strategy for a wide range of diseases, not just cancer. Functionalization with molecular ligands is the key to specificity and efficiency, but long-term safety and industrial-scale translation remain major challenges.

The main advantages of these systems include: increased therapeutic efficiency, reduced doses and toxicity, controlled and prolonged release, and the possibility of combining multiple drugs. However, significant challenges remain, including ensuring biocompatibility and controlled carrier degradation, managing high synthesis and formulation costs, overcoming hurdles to obtaining clinical approvals, and addressing stability within the body and potential immunological reactions. It is evident that targeted drug delivery systems represent one of the most promising directions in modern medicine. They offer the potential to optimize treatments by combining therapeutic precision with patient safety. Current and future research focuses on creating intelligent systems capable of responding to multiple stimuli or delivering drugs in controlled sequences. Functionalized nanoparticles with multiple ligands for combined targeting, bio-inspired systems based on exosomes or cell membranes, medical nanorobots, and personalized therapy platforms adapted to the patient's genetic profile are being developed.

Although there are technological and clinical challenges, the rapid progress of nanotechnology and bioengineering indicates a future in which therapies will become increasingly personalized, effective, and free of significant adverse effects.

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