Chapter

Nanoparticles as Drug Delivery Systems

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Abstract

This chapter presents a review on the design of nanoparticles which have been proposed as drug delivery systems in biomedicine. It will begin with a brief historical review of nanotechnology including the most common types of nanoparticles (metal nanoparticles, liposomes, nanocrystals and polymeric nanoparticles) and their advantages as drug delivery systems. These advantages include the mechanism of increased penetration and retention, the transport of insoluble drugs and the controlled release. Next, the nanoparticle design principles and the routes of administration of nanoparticles (parental, oral, pulmonary and transdermal) are discussed. Different routes of elimination of nanoparticles (renal and hepatic) are also analyzed.

Keywords: nanoparticle, drug delivery, insoluble drug, controlled release, route of administration

1. Introduction

Nanomedicine is a relatively new discipline that arises from the intersection between nanotechnology and medicine. It is based on the control of matter at the nanometer scale for applications in the field of human health. The use of materials in this range has been a great advance for the pharmacology by modifying fundamental properties of the drugs such as solubility, diffusivity, half-life in the bloodstream and drug release and distribution profiles [1–4]. Although the production and use of nano-sized matter dates from hundreds of years [5, 6], nanomedicine as a modern interdisciplinary science was first established at the end of the last century. Many authors consider the beginning of nanotechnology in the famous lecture of the physicist and Nobel laureate Richard P. Feynman in 1959 for the American Physical Society entitled: "There's Plenty of Room at the Bottom: An Invitation to Enter a New Field of Physics" [7]. In it, Feynman presented a futuristic vision of technology that leads towards the atomic scale and towards the final limits established by physical laws. Revolutionary ideas were put forward, such as reducing the integrated circuits of a computer to diameters between 10 and 100 atoms. To understand the scope of his predictions, suffice it to remember that, at the time of presenting these ideas, a computer occupied an entire room if not several. However, the word (or the prefix) nano was not mentioned even once in his presentation, Feynman stuck to describing the miniaturization of machines and its possible applications. The honor of having coined the term "nano" is awarded to Norio Taniguchi, for his presentation "On the basic concept of nanotechnology" in 1974 [8].

It is important to emphasize that the term nanotechnology applied to the study of nanoparticles simply consists in renaming the study of colloidal dispersions, in which field the contributions of renowned scientists such as Michael Faraday stand out, who in 1857 disseminated the first synthesis of gold nanoparticles and other metals [9]. In the paper, Faraday reveals his amazement at the changes in the optical properties of metallic colloidal dispersions. These properties were later explained in

Drug	Company	Application	Date of approval
Lipid-based			
Doxil	Janssen	Kaposi's sarcoma, ovarian cancer, multiple myeloma	1995
DaunoXome	Galen	Kaposi's sarcoma	1996
AmBisome	Gilead Sciences	Fungal/protozoal infections	1997
Visudyne	Bausch and Lomb	Wet age- related macular degeneration, myopia, ocular histoplasmosis	2000
Marqibo	Acrotech Biopharma	Acute lymphoblastic leukemia	2012
Onivyde	Ipsen	Metastatic pancreatic cancer	2015
Vyxeos	Jazz Pharmaceuticals	Acute myeloid leukemia	2017
Onpattro	Alnylam Pharmaceuticals	Transthyretin- mediated amyloidosis	2018
Polymer-based			
Oncaspar	Servier Pharmaceuticals	Acute lymphoblastic leukemia	1994
Copaxone	Teva	Multiple sclerosis	1996
PegIntron	Merck	Hepatitis C infection	2001
Eligard	Tolmar	Prostate cancer	2002
Neulasta	Amgen	Neutropenia, chemotherapy induced	2002
Abraxane	Celgene	Lung cancer, metastatic breast cancer, metastatic pancreatic cancer	2005
Cimiza	UCB	Crohn's disease, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis	2008
Plegridy	Biogen	Multiple sclerosis	2014
ADYNOVATE	Takeda	Hemophilia	2015
Inorganic			
INFeD	Allergan	Iron-deficient anemia	1992
DexFerrum	American Regent	Iron-deficient anemia	1996
Ferrlecit	Sanofi	Iron deficiency in chronic kidney disease	1999
Venofer	American Regent	Iron deficiency in chronic kidney disease	2000
Feraheme	AMAG	Iron deficiency in chronic kidney disease	2009
Injectafer	American Regent	Iron-deficient anemia	2013

Table 1.
FDA-approved nanomedicines for drug delivery. Adapted from reference [16] with permission of Springer Nature.

1908 by Gustav Mie who would give a solution to Maxwell's equations for particles with a finite volume [10]. In 1925, Richard Zsigmondy would be awarded the Nobel Prize in Chemistry for his demonstration of the heterogeneous nature of colloidal dispersions [11]. His contributions in methodological terms have become fundamental for the study of modern colloidal chemistry and nanotechnology.

In 1981, Eric Drexler [12] proposed what is now known as a bottom-up approach, where atoms are self-assembled to create higher-order structures. It contrasts with the approach proposed by Feynman, who conceives the beginning of nanotechnology from a top-down approach, building smaller and smaller machines that, ultimately, are used to manipulate matter with atomic precision. In particular, the bottom-up approach is of great interest in nanoparticle synthesis, where self-assembly properties, the product of natural chemical and physical interactions between molecules, can be exploited to produce defined characteristics. It is this concept that opens a wide range of possibilities towards the synthesis of nanoparticles with a wide variety of functionalities. From this point of view, nanoparticle engineering is based on "programming" with predetermined instructions the self-assembly of atoms or molecules in such a way that the desired nanoparticles are the final product.

Different authors see the paths of nanotechnology and medicine intertwined in 1986 when Matsumura and Maeda [13] observed that an anticancer protein bound to polymeric nanoparticles exhibited greater accumulation in tumor tissues than in healthy tissues. This discovery led to the theory of enhanced permeability and retention (EPR) as a consequence of tumor physiology and the size of nanoparticles (<200 nm), which are capable of penetrating tumor cells due to their reduced size and, at the same time, being retained [13]. The discovery lays the foundation for the development of different theories on targeted delivery via passive transport to tumor tissues and a large cascade of advances in the design of drug nanocarriers. In 1995, the first liposome-based nanostructure for the delivery of doxorubicin, an important anticancer drug, was approved by the FDA (Food and Drug Administration, USA) under the trade name Doxil® [14]. Since then and until April 2016, more than 50 nanomedicines of different kinds have been approved by the FDA and this is expected to be only the beginning of the near future [15]. **Table 1**, which has been adapted from reference [16], summarizes the FDA-approved nanomedicines used for drug delivery up to date. At the time of writing this chapter, one of the most conservative market capitalization estimates that the value of all nanomedicines is comprised of $ 47.5 billion and is expected to rise to $ 164 billion by 2027 driven by the crisis SARS-CoV-2 [17].

2. Types of nanoparticles

Modern and advanced synthesis techniques have led to the preparation of a great variety of nanoparticles with different shapes and sizes, together with the use of a great variety of materials. The classification of nanoparticles can be based on different physical and/or chemical parameters. This is a brief summary of the most important characteristics and functions of different types of nanoparticles used in biomedicine, classified based on the materials used in their synthesis.

2.1 Metal nanoparticles

Metallic nanoparticles have attracted great interest for use in medicine as anticancer agents [18], imaging contrast agents [19], and drug carriers [20]. One of the most exploited properties of these nanoparticles is the increase in molar absorptivity that colloidal dispersions present due to the intensity of their surface plasmon

resonance [21], classic examples of this being nanoparticles of metals such as gold, silver or copper. Plasmon resonance can radiate light (Mie scattering), a process that finds great utility in the optical and imaging fields, or it can be rapidly converted to heat (absorption). The latter mechanism can be used to convert metallic nanoparticles into light-activated heat sources for use in medicine in selective laser photothermolysis of cancer cells [22–24]. The properties of the resonance plasmon can be tuned by modifying the size, morphology and nature of the metals used for the synthesis of nanoparticles, thus being able to serve different purposes [18]. Their optical properties and high capacity to catalyze reactions and electron transfer also give them applications as biosensors [21] which, through ingenious modifications, are capable of significantly amplifying signals [25].

Metallic nanoparticles are also of interest as vehicles for the administration of drugs and other active principles due to their high surface-volume ratio, stability, functionality through chemical modifications of their surface and relative harmlessness. For example, Libutti et al. [26] functionalized the surface of 27 nm gold nanoparticles with tumor necrosis factor-α and polyethylene glycol. The nanoparticles managed to passively accumulate in the cancerous tissues avoiding healthy tissues. This allowed the researchers to administer doses of tumor necrosis factor-α that were previously considered toxic. Iron oxide nanoparticles have been approved by the United States Food and Drug Administration (FDA) for the treatment of anemia [27]. Recently, molecular docking studies propose the reuse of these nanoparticles to combat the current global pandemic of SARS-CoV-2 [28]. The studies revealed that both Fe_2O_3 and Fe_3O_4 nanoparticles interact effectively with the different proteins and glycoproteins of the virus. These interactions associated with conformational changes in proteins are expected to result in the inactivation of the virus.

However, despite the great boom in metallic nanoparticles due to their long history and simplicity in terms of their synthesis, they present toxicity problems in prolonged use as they cannot be biodegraded [29–31]. In addition, different authors have already expressed their concerns regarding the neurotoxicity of these particles as they are capable of crossing the blood–brain barrier [32, 33].

2.2 Liposomes

Liposomes are spherical vesicles composed of one or more concentric membranes of lipid bilayers with an internal compartment that normally contains water. Liposomes have the ability to encapsulate both lipophilic molecules in their membrane and hydrophilic in their internal cavity. The size of these vesicles can vary from a few nanometers to several microns. However, liposomes applied for medical use range between 50 and 450 nm [34]. Liposomes were discovered in the 1960s [35] and claim to be the first nanoparticles to be used for the delivery of nanomedicines after Doxil® was approved by the FDA in 1995 [14]. At present, there have been technological advances that have managed to use various natural or synthetic lipids, as well as surfactants to modify the physicochemical properties of liposomes, giving rise to the second and third generation of them [36]. Changes in the physicochemical properties of liposomes influence their interaction with cells, their half-life in circulation, their ability to penetrate tissues, and their final fate in vivo [36]. For example, through the exchange of a phospholipid bilayer in liquid phase for a bilayer in solid phase in liposomes, by incorporating cholesterol (bilayer tightening effect) or sphingomyelin, the retention of the drug loaded in the liposomes increases, delaying the release.

Despite all the hopes for conventional liposomes, they have presented various problems and pharmacological implications over the years. A major drawback of

conventional liposomes is their rapid capture by the reticuloendothelial system [37]. Liposomes accumulate mainly in the liver and spleen, due to their abundant blood supply and the abundance of phagocytic cells resident in these tissues [38]. The marked increase in the retention and accumulation of liposomal drugs in these organs may delay the clearance of lipophilic anticancer drugs from the circulation [39]. Furthermore, during chemotherapy, it can lead to partial depletion of macrophages and interfere with important host defense functions in these cell types [40].

2.3 Nanocrystals

Nanocrystals are perhaps the simplest forms of nanomedicine, i.e., nanoparticles made up of 100% of the drug. The large surface/area ratio offered by the nanometric scale increases the dissolution rate, allowing improved pharmacokinetic profiles. The small size of the nanoparticles increases the penetration of the nanocrystals to biological barriers such as the digestive tract, thus increasing the bioavailability of insoluble drugs. The production of crystalline nanoparticles has been applied to both organic drugs and inorganic materials [41–43]. Although the inorganic crystalline nanoparticles approved by the FDA (year 2016) are limited to hydroxyapatite and calcium phosphate for use as substitutes for bone grafts and iron oxide, iron oxide nanoparticles have been used for the treatment of glioblastoma and anemia, due to iron deficiency in kidney diseases [15]. Solubility problems associated with several pharmacological compounds have been improved by conversion to nanocrystals and are marketed for a variety of indications [43]. The pearl mill developed by Elan Nanosystems was used to produce the first three FDA-approved nanocrystals: Rapamune®, Tricor® and Emend®, and is expected to be almost universally applicable to a variety of drugs with low solubility, estimated to be 70–90% of potential drug compounds [41].

2.4 Polymeric nanoparticles

Polymeric nanoparticles are colloidal particles of solid nature that, depending on the preparation method, can form two types of structures: nanospheres or nanocapsules [44]. Nanospheres consist of a matrix system in which the drug can be adsorbed on the surface or co-precipitated with the polymer [45], while in nanocapsules the drug is contained in an internal cavity surrounded by a polymeric membrane [46]. Natural polymers like carbohydrates and proteins vary in their properties between hydrophilic, hydrophobic and even amphiphilic. On the other hand, synthetic polymers are mostly hydrophilic in nature and can be present in a prepolymerized form or be polymerized during the nanoparticle synthesis process. Synthetic polymers, in turn, can be subdivided into two classes, biodegradable and non-biodegradable. Polylactic-co-glycolic acid (PLGA) is a biodegradable polymer widely used for drug delivery [47, 48]. On the other hand, polyacrylates are non-biodegradable polymers that have also been studied for drug delivery [49, 50], although to a lesser extent compared to biodegradable polymers for clear biocompatibility reasons [51].

Polymeric nanoparticles have immense potential as drug carriers, since they can deliver them in different organs, they protect drugs against degradation in vitro and in vivo, they release the drug in a controlled manner and also offer the possibility of passively targeting drugs to tumors or other tissues actively [44]. The use of polymeric nanoparticles for drug delivery is a universal approach to increase the therapeutic performance of those that are poorly soluble in any route of administration.

3. Advantages of nanoparticles for drug delivery

Nanoparticles bring a new level of engineering and control to the field of medicine by being able to modify parameters such as solubility, diffusivity, half-life, toxicity, pharmacokinetics and biodistribution of drugs and diagnostic agents. The applications of nanoparticles are very diverse and are expected to increase with the advancement of technology. In recent years, numerous studies have demonstrated their ability to act as sensors [52], drug carriers [53–55], and diagnostic agents [1, 56]. Recent efforts have managed to integrate treatments and diagnoses in a single application, giving rise to the procedures known as "theranostic".

The justification for the use of nanoparticles as drug delivery systems lies in at least three mechanisms: (i) Enhanced Penetration and Retention (EPR) of nanoparticles in solid tumors; (ii) The possibility of transporting insoluble drugs in the blood through stable colloidal systems and (iii) the controlled release thereof. In this section, the possible advantages of each of these points will be developed.

3.1 Enhanced permeability and retention (EPR)

The term EPR was coined by Matsumura and Maeda in 1986 [13]. In their work, the researchers observed that the anticancer protein neocarzinostatin, conjugated to a polymeric matrix, exhibited greater accumulation in tumor tissues than free neocarzinostatin. By applying labeled macromolecules to tumor-bearing mice, they observed that their concentration was up to 5 times higher in tumor areas than in blood over a period of 19 to 72 hours [13]. The authors affirm that the passive accumulation of these macromolecules in tumors is due to the abnormal physiology associated with tumor masses: fenestrated hypervascularization with increased permeability to macromolecules (or nanoparticles) and poor recovery through blood vessels or lymphatic vessels [57]. Subsequently, it was shown that other plasma proteins greater than 40 kDa are capable of passively and selectively accumulating in tumor areas [58]. The EPR effect can be demonstrated in mice with the intravenous injection of the Evans Blue marker, which binds to plasma albumin forming a complex that demonstrates differential accumulation in tumor areas [59], as shown in **Figure 1**.

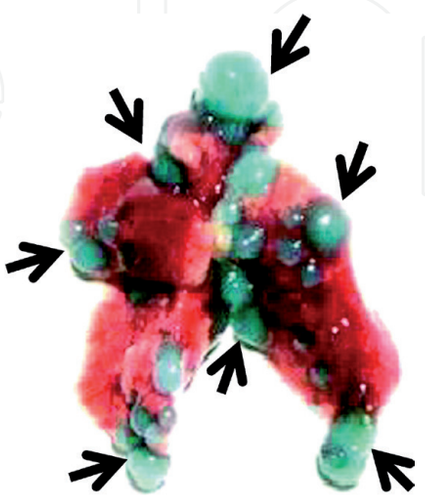

Figure 1.
Image of a metastatic lung cancer originating from 26 colon tumors implanted in the dorsal skin of a mouse. The mouse was sacrificed 3 months after implantation and 10 hours before sacrificing, a solution of Evans blue (5%) was injected intravenously to allow the EPR effect to become visible. Albumin-Evans blue complex (70 kDa) preferentially accumulated in metastatic tumor nodules, as in primary tumors. Arrows point to metastatic tumor nodules. From reference [60] with permission of Elsevier.

For passive accumulation through the EPR effect to be important, different requirements are needed. On the one hand, the nanoparticles must remain in circulation for a time greater than 6 hours [60, 61]. This can generally be achieved by functionalizing the nanoparticles with polyethylene glycol (PEG) [62]. On the other hand, the mechanism also depends on the particles being small enough to penetrate biological membranes but large enough to be retained. Yuan et al. [63] measured the microvascular permeability of several macromolecules in human colon adenocarcinoma LSI74T transplanted in mice with immunodeficiency and the results indicated that the cut-off size of the pore is around 400-600 nm, depending on physicochemical properties such as charge and hydrophobicity of the nanoparticles. Regarding the minimum size, Maeda et al. [58] estimated that the nanoparticle size must be greater than 40 kDa to show significant retention in the tumor area.

Vascular extravasation is also highly dependent on the morphology and the specific type of tumor. Scanning electron micrographs of normal vascular epithelium and two epithelia associated with different tumors are shown in **Figure 2**. As can be seen, tumor-associated epithelia have significant pores (fenestrations) and their size depends on the type of tumor. Smith et al. [64] studied the extravasation capacity of quantum-dots (20-25 nm) and single-walled carbon nanotubes (2-3 x 200 nm) in tumors implanted in mouse ears. The surface of both types of nanostructures was modified by PEG to avoid differences in charge or surface chemistry and that the results were only due to the morphology of the particles. The authors found that spherical quantum-dots are capable of extravasation of the endothelium of LS174T tumors, whereas cylindrical nanotubes are capable of extravasation in U87MG tumors. Surprisingly, the authors were not able to see the extravasation of the nanomaterials in normal endothelium. This suggests that the morphology of the nanoparticles may be a determining factor for penetrating certain tumors, while healthy endothelium could prevent nanoparticle transfer.

Although the EPR model has been tested in rodents with large induced tumor masses [59, 65], these models differ widely in morphology and physiology of possible human tumors and, for these reasons, there is still much controversy regarding it [55, 60, 66]. Firstly, tumors of up to 10% of body weight have been reported in mice. If we make an analogy with a 70 kg human, the tumor would be the size of a basketball [67], when they actually have a size between millimeters and centimeters at the time of diagnosis and treatment [68]. Such tumor masses filter out a significant proportion of the injected drug dose and act as a reservoir, enhancing efficacy while mitigating toxicity. In addition to this, the

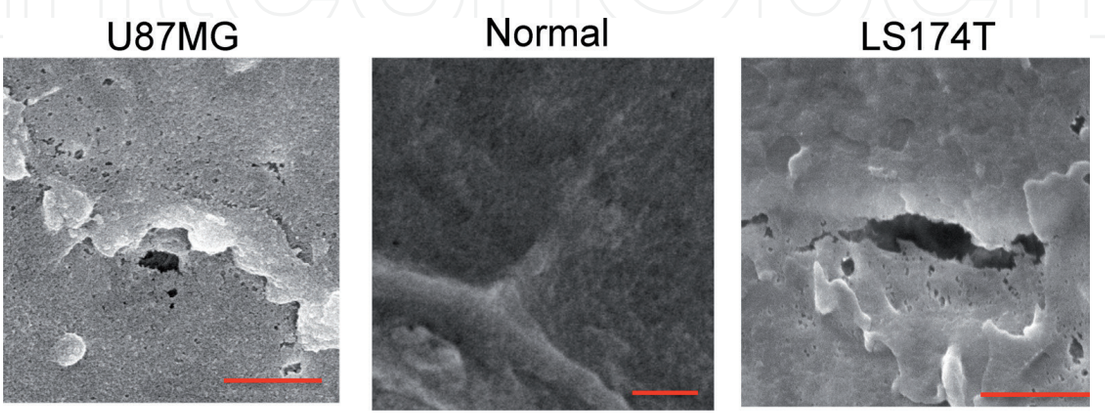

Figure 2.
Scanning emission microscopy (SEM) of tumors and normal blood vessels. SEM images show pores in the U87MG and LS174T tumor vasculature at the apparent border between endothelial cells. No pores are seen at the border of the vasculature of a tumor-free mouse ear. Scale bars: U87MG (500 nm), Normal (1 μm) and LS174T (1 μm). Reprinted with permission from reference [64]. Copyright 2012 American Chemical Society.

tumor microenvironment in humans presents important physiological differences compared to murine tumors: (i) lack of fenestrations in the tumor endothelium for the entry of nanoparticles, (ii) heterogeneity of blood flow through tissues, which causes the regions to become acidic or hypoxic [69], (iii) lower pericyte coverage, (iv) heterogeneous basement membrane and (v) higher and heterogeneous density of the extracellular matrix. This leads to high interstitial pressure and therefore the main mechanism of matter transport is by diffusion and not by convective transport, which is more efficient [69, 70].

For these reasons, it is not possible to directly transfer the results obtained in rodents to humans, mainly because cell penetration depends on the nanoparticles go from the point of application to the tumor mass and be able to interact with cells to be internalized. Currently, different methods are being investigated to increase the EPR effect. For example, Fang et al. [71] developed agents which can selectively generate vasodilator molecules (carbon monoxide) in tumor areas, achieving an increase in the concentration of the nanocarrier between 2 and 3 times higher in these, while an increase in tissues healthy was not detected. Similar results have been achieved with nanocarriers that can release nitric oxide [72, 73]. The increase in blood pressure results in an increase in the osmotic pressure, which promotes the filtration of the particles towards the tumor areas so that when angiotensin II is co-administered with the nanocarriers, an increase in the transfer and accumulation in the tumor areas can be observed [74].

In contrast to the passive accumulation of drug nanocarriers in tumor areas by the EPR mechanism, active targeting is presented, which is based on the functionalization of the nanoparticle surface with recognition molecules such as antibodies [75, 76] or ligands [77, 78] which can specifically bind to molecules overexpressed at the target site [79]. In the active targeting strategy, two cellular targets can be distinguished: (i) targeting cancer cells, which present overexpression of molecules such as transferrin, folate, epidermal growth factor receptor or glycoprotein receptors, and (ii) targeting tumor endothelium, which have overexpression of vascular endothelial growth factors (VEGF), $\alpha_v\beta_3$ integrins, vascular cell adhesion molecule-1 (VCAM-1), or matrix metalloproteinases [66, 80]. In some cases, both receptors are overexpressed in cancer cells and endothelium and can be exploited simultaneously [80]. In addition, the design of nanocarriers as active targeting systems may involve the coupling of recognition molecules as surface receptors which are able to initiate endocytosis, and hence to increase cell internalization in contrast to simple accumulation [81]. Not only would this increase the antitumor efficacy of many drugs, but it could also be used for the delivery of genetic material [82].

3.2 Insoluble drug transport

Most orally administered drugs that are soluble in water and capable of penetrating biological membranes during the passage of the gastrointestinal tract will eventually become bioavailable in the body. In contrast, water-insoluble drugs will generally not be bioavailable after oral ingestion as they cannot dissolve and pass through the gastrointestinal barrier. Along the same lines, due to their low solubility, they cannot be administered intravenously and parenteral administration does not always increase bioavailability [83]. It is estimated that 90% of drugs in development are insoluble in water, while only 40% of drugs on the market share this characteristic [84]. These statistics could indicate that many drugs in development do not reach their administration to patients due to their low solubility in water. This not only means less capital invested in research and development but also lost treatment opportunities. The development of a drug in 2011 was estimated at between 92 million and 1.8 billion dollars [85], lasting for a period of between 11.4

and 13.5 years on average [86]. Considering these, we can see that low water solubility represents a formidable challenge and opportunity for nanotechnology.

Three factors govern the speed and degree of absorption of orally administered drugs: (i) dissolution rate, (ii) solubility and (iii) intestinal permeability, which are grouped according to the biopharmaceutical classification system (BCS, Biopharmaceutical Classification System) in the categories [87]:

Class I: High Solubility - High Permeability.
Class II: Low Solubility - High Permeability.
Class III: High Solubility - Low Permeability.
Class IV: Low Solubility - Low Permeability.

The criterion established by the BCS classifies a drug as soluble when it is capable of dissolving an entire therapeutic dose in 250 mL of water, being this volume equivalent to the average amount of water found in the stomach [87].

As can be deduced, the possibilities of entering the market for a class I drug are substantially greater than that of the rest of the categories, however, a possible solution to these problems lies in the development of drug carriers which can transport them in a stable colloidal dispersion and with particles capable of crossing biological membranes [88]. As an example, Atovaquone (Wellvone®) is an antibiotic used for the treatment of *Pneumocystis carinii*, leishmaniasis and *P. falciparum* malaria, however, its low solubility limits its absorption. By formulating a dispersion of nanoparticles of this drug, it was possible to increase absorption from 15 to 40% with a 3-fold lower drug dose [89]. Xie et al. [90] prepared curcumin-loaded silk fibroin nanoparticles (SFN) to increase the dissolution rate of the drug and the mass of the drug in dispersion. Recent results from our research group revealed that SFN are an excellent vehicle for the transport of the natural drug naringenin, with anti-cancer properties [91], which has low solubility in water. The results indicated that this drug loaded in the nanoparticles is 1.7 times more effective in reducing the viability of HeLa cells than by itself. These results can be attributed to the low solubility and slow dissolution of free naringenin which, when loaded in the SFN, remains stable in dispersion, increasing its cellular penetration and improving the dissolution profile.

3.3 Controlled release

Nanoparticles can be used as drug reservoirs for their controlled release over time, which offers numerous advantages compared to conventional administration of multiple doses. Among them, it can be highlighted the improvement in efficacy and reduction of toxicity and patient cooperation [92]. The former can be considered as the increase in therapeutic activity compared to the intensity of the side effects, while the latter offers the advantage of reducing the number of applications required during treatment.

Controlled release is especially beneficial for those drugs whose half-life in the blood is relatively low due to a high rate of metabolism and elimination by the body. This effect can be observed in **Figure 3**, where the concentration of a drug in blood applied by a conventional method (red line) is represented against a controlled release system (blue line). As can be seen, the drug administered in a conventional manner is only a fraction of the time in the zone considered therapeutic, while fluctuating between subtherapeutic concentrations and above the maximum tolerable level. On the other hand, the controlled release system takes longer to reach the therapeutic concentration window but remains stable within it. The goal of the system is to match the rate of clearance to that of release in the

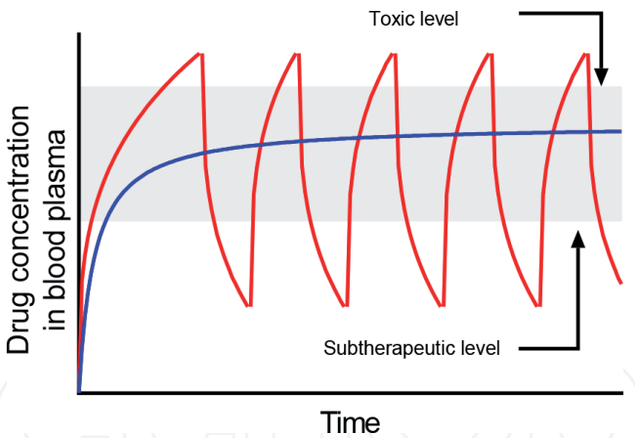

Figure 3.
Diagram of the blood concentration of a drug after multiple administrations as a conventional injection (red line) and as a controlled release system (blue line).

therapeutic concentration zone. In the clinic, this translates into numerous benefits, for example, in the case of administration of analgesics, the concentration could be prevented from falling to subtherapeutic levels and therefore the patient feeling pain. This is transferable to a large number of drugs including anti-inflammatories, antibiotics, anesthesia, hormones, chemotherapeutics, etc.

There are different mechanisms by which polymer nanoparticles can allow controlled drug release. On the one hand, the release can be delayed by using a water-soluble polymer as a matrix, whose dissolution rate is slow and consequently releases the drug at the rate of dissolution of the polymer. In the case of insoluble polymers, they can act as a diffusion barrier, slowing down the release of the drug from inside the nanoparticle to the medium. The release can also be controlled by an osmotic flow generated by a semipermeable membrane, which is itself the nanosystem, as is the case with liposomes. Finally, a delivery system that responds to internal or external stimuli could be achieved, which would be very useful, for example, in diabetic patients in which the nanosystem would release insulin on demand of the blood glucose concentration [93]. Volpatti et al. [94] have succeeded in synthesizing nanoparticles whose insulin release is sensitive to glucose levels by adding glucose oxidase and catalase to them. These researchers demonstrated that a single subcutaneous injection provides 16 h of glycemic control in diabetic mice. Cheng et al. [95] developed SFN capable of loading the antitumor drug paclitaxel (3%) and delivering it sustainably for 14 days.

4. Nanoparticle design

From the point of application to the site of action, nanoparticles face a host of challenges. In the first place, they are diluted in approximately 5 L of blood that circulates at 5 L/min through the circulatory system about 106 km long, where the velocity in each blood vessel can be between 1.5-33 cm/s [96] hindering the interaction between nanoparticles and the target tissue. Interstitial fluids have a much lower speed, just a few μm/s, where interactions would be favored. However, reaching them means crossing biological barriers, which is not an easy task. Finally, to all of the above, it is added that when nanoparticles enter the body they are treated hostilely by the immune system. For these reasons, different design principles are applied to nanoparticles to try to get around different obstacles depending on their final application.

As mentioned above, as soon as the nanoparticles enter the body, they are exposed to the mononuclear phagocyte system which consists of a system of phagocytic cells, predominantly macrophages resident in the spleen, lymph nodes and liver, which sequester the nanoparticles immediately after administration [97]. This process begins with the opsonization of the nanoparticles based on the adsorption of plasma proteins, including albumin, complementary system proteins, pattern recognition receptors and immunoglobulins. This process is relatively fast and can occur in a period as short as 30 seconds [98]. This "natural functionalization" is known as the formation of the protein crown and clearly can alter the function or fate of nanoparticles by disturbing different parameters such as size, charge and surface chemistry, as well as hydrophobicity. This protein crown can even mask the receptors or ligands attached to the nanoparticles [99].

Different design strategies have been developed to avoid opsonization and subsequent clearance by the immune system. This evasion of the immune system tries to increase the circulation time of the nanoparticles in the body and, consequently, the chances that they find the target tissue while they circulate through the bloodstream. One of the easiest and most direct strategies is PEGylation, based on the functionalization with polyethylene glycol (PEG) molecules on the surface of the nanoparticles where the polymer units form very strong associations with the water molecules, generating a hydration layer and a steric barrier to opsonization [100, 101]. An alternative strategy may be to functionalize the nanoparticles with endogenous signals normally present in healthy cells. Rodríguez et al. [102] functionalized viral particles with the CD47 membrane protein, which acts as a "non-phagocytizing" signal [103], thus prolonging the circulation time. Another similar strategy is to cover the particles with biomimetic molecules such as cell membranes, to hide the particles from the immune system [104, 105]. Another way to increase circulation time is the one proposed by Nikitin et al. [106], which is based on a slight and transient suppression of the mononuclear phagocyte system through the administration of anti-erythrocyte antibodies. They were able to increase the circulating half-life of different nanosystems up to 32 times through the suppression of ca. 5% of hematocrits.

Silk fibroin exhibits unique low immune response properties, allowing it to evade the immune system. This can be exemplified by the study by Catto et al. [107], who implanted tubular matrices based on silk fibroin in mice, detecting few macrophages labeled with anti-ED1 antibodies, which was indicative of a low inflammatory response. The absence of T lymphocytes (anti-CD4 antibodies) demonstrated that there was no cell-mediated immune response. Recently, under a state-of-the-art design, Tan and colleagues [108] have designed a doxorubicin delivery nanosystem using silk fibroin as a Trojan horse. The researchers synthesized drug-loaded amorphous calcium carbonate nanoparticles and coated them with silk fibroin. It prevents the premature release of doxorubicin and helps evade the immune system. Thanks to the EPR mechanism, nanoparticles are accumulated in cancerous tissues and, finally, internalized by lysosomes. The acidic pH of the latter promotes the generation of CO_2 from calcium carbonate, resulting in the bursting of the lysosome due to the expansion of the gas and the release of doxorubicin inside the target cell. Results in mice revealed that silk fibroin-coated nanoparticles are more effective in reducing tumor mass and preventing side effects in mice compared to free doxorubicin or uncoated calcium carbonate nanoparticles. In addition, the immunotoxicity tests indicated that the nanoparticles did not initiate an immune response by not increasing the amount of T cells ($CD4^+$ and $CD8^+$) or IgM, IgG and IgA compared to the control group. More information on intracellular drug release can be found in the review by Fenghua et al. [109].

5. Routes of administration of nanoparticles

SFN have proven to be extremely versatile for the transport of therapeutic compounds such as small drugs, proteins and DNA molecules [110]. The functionality of these compounds is closely related to the route of administration. For example, nanoparticles can be injected into the bloodstream and make use of the EPR effect for passive accumulation in metastatic tumors or they can be injected directly into the tumor mass [111]. On the other hand, they can be applied topically for the treatment of skin cancer [112] and in a similar way for lung treatments [113, 114]. SFN have been used in a wide variety of routes of administration [54]. For the sake of simplicity, only the main routes of administration will be mentioned: parental, oral, transdermal and pulmonary, and some of the studies that address the use of SFN for these different routes of administration will be cited as examples.

5.1 Parenteral

Parenteral administration forms are intended for administration by injection, which can be subdivided into intravenously (into a vein), intramuscular (into the muscle), subcutaneous (under the skin), or intradermal (into the skin). Parenteral administration acts faster than topical or enteral administration, and the onset of action often occurs in a range of seconds to minutes. Essentially, the bioavailability of the injected drug is 100% and its distribution is systemic, which means that it is potentially capable of reaching the entire body. This last concept paired with the EPR effect as mentioned above is of special interest for the treatment of tumor masses. For example, ZhuGe et al. [115] prepared SFN with their surface functionalized by proanthocyanidins and loaded with indocyanine green. Indocyanine can absorbe near-infrared light (650-900 nm) and producing a thermal effect both in vitro and in vivo. This photothermic compound is approved by the FDA and can be used to kill cells by photothermolysis. To test their functionality, the researchers injected the loaded nanoparticles intravenously into mice bearing C6 glioma. The pharmacokinetic study showed that the nanoparticles managed to reach the gliomas after intravenous administration in vivo, while the pharmacological study demonstrated inhibition of tumor growth after irradiation with near-infrared light. On the other hand, nanoparticles also offer temporary release control. Recently, in another study, Zhan et al. [116] administered Celastrol-loaded SFN to rats intravenously. The results showed that an increase in the total exposure time to the drug is reduced by increasing its residence time and reducing its metabolism.

5.2 Oral

Oral administration is the most common route and probably the one preferred by patients when receiving medications. However, conventional formulations, such as tablets and capsules, can release drugs in a rapid and poorly controlled manner, which can result in degradation and alteration of the drug due to the environment of the gastrointestinal tract (variations in pH and the presence of digestive enzymes and microbiota). Furthermore, the common mechanism of drug absorption through the gastrointestinal tract is passive diffusion. Consequently, most of the initial dose is not absorbed but is metabolized and excreted. SFN possess favorable characteristics to overcome the aforementioned problems and become candidates of interest for the oral administration of therapeutic compounds. Firstly, due to their mucoadhesive capacity, SFN can adhere firmly to the gastrointestinal mucosa or intestinal epithelial cells (Peyer's lymphatic M cells), followed by cell

internalization via endocytosis [117]. Thus, encapsulated drugs can enter the bloodstream effectively and intact. Zhan et al. [116] increased more than doubled the absolute bioavailability of Celastrol from 3.14% to 7.56% by loading the drug in SFN and administering it orally to rodents.

5.3 Pulmonary

The lung is a potential target for drug delivery for both local and systemic treatments. Locally, lung and respiratory diseases, e.g., lung cancer or tuberculosis, can be treated with a reduced dose and fewer side effects compared to conventional dosage forms. At the systemic level, due to the large surface area of the lung, the drug can be absorbed quickly and efficiently without being degraded by the first-pass metabolism as in oral administration [118]. In 2015, Kim et al. [113] prepared cisplatin-loaded SFN for the treatment of lung cancer. The particles showed compatibility with the human lung epithelial cell line A549. The results indicated that the cisplatin loaded in the particles increases the cytotoxicity concerning the drug applied alone. The researchers concluded that the particles showed a high aerosolization performance through in vitro lung deposition measurement, which is at the level of commercially available dry powder inhalers.

5.4 Transdermal

The transdermal administration of drugs improves their bioavailability and is useful for systemic and local treatment as in pulmonary application. Takeuchi et al. [119] evaluated the in vivo permeability of 40 nm SFN through the skin using mice and demonstrated that the particles are capable of reaching the dermis in 6 hours in addition to the stratum corneum, hair follicles and epidermis that surrounds them.

6. Nanoparticle elimination

The use of nanoparticles in humans raises great doubts about their safety and their elimination capacity. If the removal is very fast, the nanoparticles will not reside long enough to fulfill their function. On the contrary, if the retention is very high, the concentration of nanoparticles can increase to the point of becoming harmful. Consequently, a relevant question in the use of nanoparticles in humans is how these biological systems can eliminate nanoparticles once their functions have been fulfilled. The properties of nanoparticles that affect their removal are mainly based on size, shape, composition, charge, and surface chemistry. These aspects will be briefly discussed within the two main elimination routes, (i) renal and (ii) hepatic elimination, to obtain a global vision of the process.

6.1 Renal elimination

The kidneys have the potential for rapid removal of particles from the vascular system without the need for decomposition. Renal elimination involves the mechanisms of glomerular filtration and tubular secretion to end in urinary excretion [120]. The nanoparticles reach the nephrons through the afferent arteriole, where they meet three endothelial barriers: the fenestrated endothelium; the highly negatively charged glomerular basement membrane; and the podocyte extensions of glomerular epithelial cells. The fenestrated epithelium has pores with a functional physiological diameter of between 9 and 10 nm, and a few (ca. 1%) pores of 15 to 23 nm in diameter [121], which means that nanoparticles with diameters less

than 10 nm can spread freely regardless of the charge of the particle. The second barrier presented by the glomerular basement membrane filters particles between 6 and 8 nm depending on the electrostatic interactions between the nanoparticle and the membrane [122]. In this way, low-charged or positively charged nanoparticles can diffuse more freely. After glomerular filtration, the nanoparticles enter the lumen of Bowman's capsule, where they can be reabsorbed. Because the proximal tube epithelium is negatively charged, positively charged nanoparticles can be more easily reabsorbed.

Choi et al. [123] administered quantum-dots (inorganic nanoparticles) intravenously to rodents to study their renal elimination. The results indicated that particles with a hydrodynamic diameter less than 5.5 nm present rapid elimination and the increase in this diameter is inversely proportional to the retention time of the quantum-dots in the body.

6.2 Hepatic clearance and the reticulum endothelial system

Those nanoparticles that are too large to be excreted by the renal system must be eliminated by the hepatobiliary system. In 1924, Karl Albert Ludwig Aschoff coined the term reticuloendothelial system (RES) to describe a functional cellular system widely distributed in the body, composed of sessile and circulating macrophages of mesenchymal origin. These cells have a marked phagocytic capacity towards particulate matter. Macrophages stored in the RES can be found in the central nervous system (microglia), in the spleen, lymph nodes, tonsils, in the bone marrow (reticular cells) and, particularly, in the liver (90% of all macrophages) [124]. The exogenous structures are subjected to very intensive phagocytosis by the RES as well as the foreign proteins of higher molecular weight. Total blood flow must pass through the liver, making it a central organ to monitor the blood for endogenous, foreign substances and particles that must be removed for physiological reasons. In order to perform their functions, RES cells have special abilities such as: phagocytosis, pinocytosis, the release of signaling substances (cytokines, eicosanoids) and elimination of endotoxins, among others [124]. In addition, these are equipped with numerous pores of various diameters, depending on their different functions, which gives them the ability to filter larger molecules and particles, keeping them away from the liver parenchyma. The Kupffer cells and the endothelial sinus are in a privileged position to engulf any colloid foreign to the body. For this purpose, Kupffer cells are equipped with a branched and ciliated surface that act as capture mechanics. Besides, they possess specific receptors for carbohydrate components, as well as for the Fc region of IgG and for complement C_3, allowing them to differentiate the opsonized matter. They also possess lysosomal enzymes, although in much lower amounts than sinus endothelial cells.

In a very complete study, Poon et al. [125] proposed an algorithm to infer how nanoparticles can be eliminated in vivo (**Figure 4**). Most of the nanoparticles with diameters smaller than the glomerular filtration size limit (~5.5 nm) are eliminated by the kidneys and leave the body through the urine [123] although fecal elimination of small nanoparticles is also observed [125]. Biodegradable nanocarriers or nanoparticles larger than 5.5 nm can be decomposed [126, 127] or metabolized [128] and can be returned to the systemic circulation. Most non-biodegradable nanoparticles larger than 5.5 nm are retained long-term in Kupffer cells [129]. If the nanoparticles can evade Kupffer cells or if Kupffer cells are incapacitated, the nanoparticles can undergo hepatobiliary clearance. Similar to the glomerular filtration size limit, the authors proposed that there is a filtration size limit in hepatic sinusoidal endothelium. Nanoparticles larger than the fenestra of sinusoidal endothelium in the liver have restricted access to hepatocytes, whereas nanoparticles smaller than the

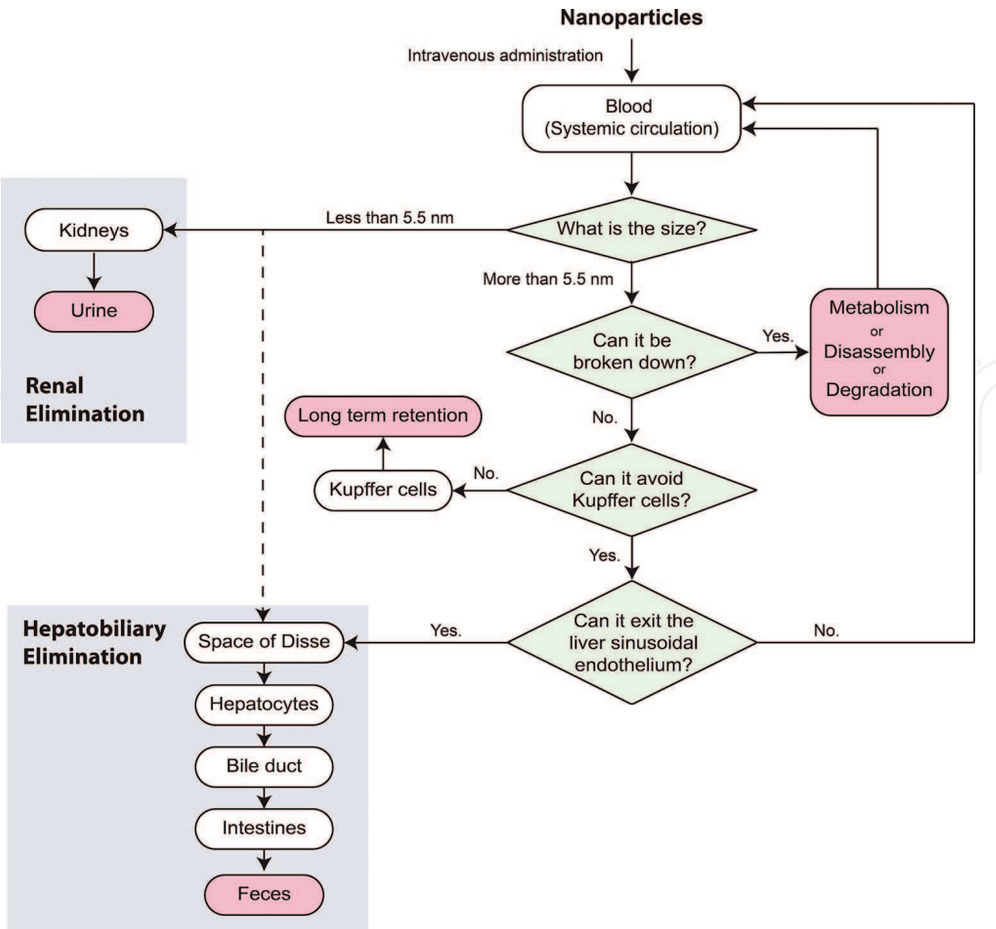

Figure 4.
Flow diagram for removal of nanoparticles in vivo. Reprinted with permission from reference [125]. Copyright 2019 American Chemical Society.

fenestrae have better access through the fenestra to enter the perisinusoidal space. In general, nanoparticles must escape these barriers established by non-parenchymal cells in the liver before they have the potential to enter the perisinusoidal space and interact with hepatocytes for elimination. Once the nanoparticles successfully interact with them, they can transit to enter the bile ducts. Finally, the nanoparticles enter the intestine and are eliminated from the body through the feces.

7. Conclusions

The academy and industry have made extraordinary advances in a wide variety of areas due to the development of nanotechnology and the control of structures at the nanoscopic levels. Particularly in the field of medicine, nanotechnology has the potential to generate a significant impact on human health, being able to improve the diagnosis, prevention and treatment of diseases. In this field, nanotechnology seeks to encapsulate drugs and/or tracer compounds in nanoparticles to increase their efficiency by allowing direct delivery to target tissues, while they reduce their toxicity avoiding accumulation and the consequent side effects in healthy tissues. The encapsulation of drugs also allows their controlled release, thus avoiding maximum levels of highly harmful or subtherapeutic concentrations. Moreover, nanoparticles are of great value in the transport of drugs with low solubility in water, which turns out to be the major problem when introducing new drugs to the market because it limits their bioavailability in the body. A wide variety of materials can be used for the preparation of nanoparticles depending on the intended function of the system.

Acknowledgements

This work has been partially supported by the European Commission (FEDER/ERDF) and the Spanish Ministry of Science and Innovation (Refs. CTQ2017-87708-R and PID2020-113081RB-I00) through the Spanish State Research Agency, and the research support program of the Seneca Foundation of Science and Technology of Murcia, Spain (Ref. 20977/PI/18). Marta G. Fuster acknowledges support from Spanish MINECO (FPI grant, PRE2018-086441). Mercedes G. Montalbán acknowledges support from the University of Murcia and Santander Bank through the research project (Ref. RG2020-002UM) associated with her postdoctoral contract.

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References

[1] L Zhang, FX Gu, JM Chan, AZ Wang RL and OF. Nanoparticles in Medicine: Therapeutic Applications and Developments. Educ Policy Anal Arch. 2007;8(5):761-769.

[2] van der Meel R, Sulheim E, Shi Y, Kiessling F, Mulder WJM, Lammers T. Smart cancer nanomedicine. Nat Nanotechnol. 2019 Nov;14(11): 1007-1017.

[3] Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. Nat Publ Gr. 2010;7:653-664.

[4] Dogra P, Butner JD, Ruiz Ramírez J, Chuang Y, Noureddine A, Jeffrey Brinker C, et al. A mathematical model to predict nanomedicine pharmacokinetics and tumor delivery. Comput Struct Biotechnol J. 2020 Jan;18:518-531.

[5] Schaming D, Remita H. Nanotechnology: from the ancient time to nowadays. Found Chem. 2015;17(3): 187-205.

[6] Reibold M, Paufler P, Levin AA, Kochmann W, Pätzke N, Meyer DC. Carbon nanotubes in an ancient Damascus saber. Nature. 2006 Nov;444(7117):286-286.

[7] Feynman RP. There's plenty of room at the bottom. Gilbert ed. H, editor. New York: Reinhold Publishing Corp; 1961.

[8] Taniguchi N. On the basic concept of nanotechnology. Proceeding ICPE. 1974;

[9] Faraday M. Experimental relations of gold (and other metals) to light. Philos Trans R Soc London. 1857 Dec;147(0): 145-181.

[10] Mie G. Beiträge zur Optik trüber Medien, speziell kolloidaler Metallösungen. Ann Phys. 1908;330(3): 377-445.

[11] Richard Zsigmondy. Properties of colloids. Nobel Lect. 1926;1:1.

[12] Drexler KE. Molecular engineering: An approach to the development of general capabilities for molecular manipulation. Proc Natl Acad Sci U S A. 1981;78(9 I):5275-8.

[13] Matsumura Y, Maeda H. A New Concept for Macromolecular Therapeutics in Cancer Chemotherapy: Mechanism of Tumoritropic Accumulation of Proteins and the Antitumor Agent Smancs. Cancer Res. 1986;46(8):6387-6392.

[14] Youn YS, Bae YH. Perspectives on the past, present, and future of cancer nanomedicine. Adv Drug Deliv Rev. 2018;

[15] Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date. Pharm Res. 2016;33(10): 2373-2387.

[16] Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nat Rev. Drug Discov. 2021;20(2):101-124.

[17] Global Industry Analysts I. Nanotechnology in Drug Delivery - Global Market Trajectory & Analytics. 2020.

[18] Jain PK, ElSayed IH, El-Sayed MA. Au nanoparticles target cancer. Nano Today. 2007;2(1):18-29.

[19] Anderson SD, Gwenin V V., Gwenin CD. Magnetic Functionalized Nanoparticles for Biomedical, Drug Delivery and Imaging Applications. Nanoscale Res Lett. 2019 Dec;14(1):1-16.

[20] Sharma A, Goyal AK, Rath G. Recent advances in metal nanoparticles

in cancer therapy. J Drug Target. 2018 Sep;26(8):617-632.

[21] Malekzad H, Sahandi Zangabad P, Mirshekari H, Karimi M, Hamblin MR. Noble metal nanoparticles in biosensors: recent studies and applications. Nanotechnol Rev. 2017 Jun;6(3): 301-329.

[22] O'Neal DP, Hirsch LR, Halas NJ, Payne JD, West JL. Photo-thermal tumor ablation in mice using near infrared-absorbing nanoparticles. Cancer Lett. 2004;209(2):171-176.

[23] Vines JB, Yoon J-H, Ryu N-E, Lim D-J, Park H. Gold Nanoparticles for Photothermal Cancer Therapy. Front Chem. 2019 Apr;7(APR):167.

[24] Jain PK, Huang X, El-Sayed IH, El-Sayed MA. Noble metals on the nanoscale: Optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. Acc Chem Res. 2008;41(12): 1578-1586.

[25] Cao X, Ye Y, Liu S. Gold nanoparticle-based signal amplification for biosensing. Anal Biochem. 2011;417(1):1-16.

[26] Libutti SK, Paciotti GF, Byrnes AA, Alexander HR, Gannon WE, Walker M, et al. Phase I and pharmacokinetic studies of CYT-6091, a novel PEGylated colloidal gold-rhTNF nanomedicine. Clin Cancer Res. 2010;16(24):6139-6149.

[27] Coyne DW. Ferumoxytol for treatment of iron deficiency anemia in patients with chronic kidney disease. Expert Opin Pharmacother. 2009 Oct;10(15):2563-2568.

[28] Abo-zeid Y, Ismail NS, McLean GR, Hamdy NM. A molecular docking study repurposes FDA approved iron oxide nanoparticles to treat and control COVID-19 infection. Eur J Pharm Sci. 2020;153(April):105465.

[29] Schrand AM, Rahman MF, Hussain SM, Schlager JJ, Smith DA, Syed AF. Metal-based nanoparticles and their toxicity assessment. Wiley Interdiscip Rev. Nanomedicine Nanobiotechnology. 2010 Sep;2(5): 544-568.

[30] Park S, Lee YK, Jung M, Kim KH, Chung N, Ahn EK, et al. Cellular toxicity of various inhalable metal nanoparticles on human alveolar epithelial cells. In: Inhalation Toxicology. Taylor & Francis; 2007. p. 59-65.

[31] Sengupta J, Ghosh S, Datta P, Gomes A, Gomes A. Physiologically Important Metal Nanoparticles and Their Toxicity. J Nanosci Nanotechnol. 2014 Jan;14(1):990-1006.

[32] Yildirimer L, Thanh NTK, Loizidou M, Seifalian AM. Toxicological considerations of clinically applicable nanoparticles. Nano Today. 2011 Dec;6(6):585-607.

[33] Teleanu D, Chircov C, Grumezescu A, Volceanov A, Teleanu R. Impact of Nanoparticles on Brain Health: An Up to Date Overview. J Clin Med. 2018 Nov;7(12):490.

[34] Bozzuto G, Molinari A. Liposomes as nanomedical devices. Int J Nanomedicine. 2015;10:975-999.

[35] Bangham AD, Horne RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. J Mol Biol. 1964 Jan;8(5):660-IN10.

[36] Cattel L, Ceruti M, Dosio F. From conventional to stealth liposomes: A new frontier in cancer chemotherapy. J Chemother. 2004;16(SUPPL. 4): 94-97.

[37] Torchilin V. Tumor delivery of macromolecular drugs based on the EPR

effect. Adv Drug Deliv Rev. 2011;63(3):131-135.

[38] Zamboni WC. Concept and Clinical Evaluation of Carrier-Mediated Anticancer Agents. Oncologist. 2008 Mar;13(3):248-260.

[39] Juliano RL, Stamp D. Pharmacokinetics of liposome-encapsulated anti-tumor drugs. Biochem Pharmacol. 1978 Jan;27(1):21-27.

[40] Daemen T, Hofstede G, Ten Kate MT, Bakker-Woudenberg IAJM, Scherphof GL. Liposomal doxorubicin-induced toxicity: Depletion and impairment of phagocytic activity of liver macrophages. Int J Cancer. 1995 May;61(5):716-721.

[41] Junghanns JUAH, Müller RH. Nanocrystal technology, drug delivery and clinical applications. Int J Nanomedicine. 2008;3(3):295-309.

[42] Sato M, Sambito MA, Aslani A, Kalkhoran NM, Slamovich EB, Webster TJ. Increased osteoblast functions on undoped and yttrium-doped nanocrystalline hydroxyapatite coatings on titanium. Biomaterials. 2006 Apr;27(11):2358-2369.

[43] Shegokar R, Müller RH. Nanocrystals: Industrially feasible multifunctional formulation technology for poorly soluble actives. Int J Pharm. 2010;399(1-2):129-139.

[44] Jawahar N, Meyyanathan S. Polymeric nanoparticles for drug delivery and targeting: A comprehensive review. Int J Heal Allied Sci. 2012;1(4):217.

[45] Montalbán M, Coburn J, Lozano-Pérez A, Cenis J, Víllora G, Kaplan D. Production of Curcumin-Loaded Silk Fibroin Nanoparticles for Cancer Therapy. Nanomaterials [Internet]. 2018 Feb 24 [cited 2018 Apr 27];8(2):126. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29495296

[46] Nagaich U. Polymeric nanocapsules: An emerging drug delivery system. J Adv Pharm Technol Res. 2018;9(3):65.

[47] Zabihi F, Xin N, Jia J, Chen T, Zhao Y. High yield and high loading preparation of curcumin-PLGA nanoparticles using a modified supercritical antisolvent technique. Ind Eng Chem Res. 2014;53(15):6569-6574.

[48] Teixeira M, Pedro M, Nascimento MSJ, Pinto MMM, Barbosa CM. Development and characterization of PLGA nanoparticles containing 1,3-dihydroxy-2-methylxanthone with improved antitumor activity on a human breast cancer cell line. Pharm Dev Technol. 2019 Oct;24(9):1104-1114.

[49] Zhang Z, Tsai P-C, Ramezanli T, Michniak-Kohn BB. Polymeric nanoparticles-based topical delivery systems for the treatment of dermatological diseases. Wiley Interdiscip Rev. Nanomedicine Nanobiotechnology. 2013 May;5(3):205-218.

[50] Zhang Z, Wang J, Chen C. Near-infrared light-mediated nanoplatforms for cancer thermo-chemotherapy and optical imaging. Adv Mater. 2013 Jul;25(28):3869-3880.

[51] Ren H, Huang X. Polyacrylate nanoparticles: toxicity or new nanomedicine? Eur Respir J. 2010 Jul;36(1):218-221.

[52] El-Ansary A, Faddah LM. Nanoparticles as biochemical sensors. Nanotechnol Sci Appl. 2010;3(1):65-76.

[53] Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, McCullough J. The big picture on nanomedicine: The state of

investigational and approved nanomedicine products. Nanomedicine Nanotechnology, Biol Med. 2013;9(1):1-14.

[54] Pham DT, Tiyaboonchai W. Fibroin nanoparticles: a promising drug delivery system. Drug Deliv. 2020;27(1):431-448.

[55] Lammers T, Ferrari M. The success of nanomedicine. Nano Today. 2020 Apr;31:100853.

[56] Chen H, Zhen Z, Todd T, Chu PK, Xie J. Nanoparticles for improving cancer diagnosis. Vol. 74, Materials Science and Engineering R: Reports. Elsevier Ltd.; 2013. p. 35-69.

[57] Maeda H, Matsumura Y. Tumoritropic and lymphotropic principles of macromolecular drugs. Crit Rev. Ther Drug Carrier Syst. 1989;6(3):193-210.

[58] Maeda H, Bharate GY, Daruwalla J. Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect. Eur J Pharm Biopharm. 2009;71(3):409-419.

[59] Greish K, Fang J, Inutsuka T, Nagamitsu A, Maeda H. Macromolecular Therapeutics. Clin Pharmacokinet. 2003;42(13):1089-1105.

[60] Maeda H. Towards a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity. Adv Drug Deliv Rev. 2015;91:3-6.

[61] Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. Adv Drug Deliv Rev. 2013;65(1):71-79.

[62] Jokerst J V., Lobovkina T, Zare RN, Gambhir SS. Nanoparticle PEGylation for imaging and therapy. Vol. 6, Nanomedicine. NIH Public Access; 2011. p. 715-728.

[63] Yuan F, Dellian M, Fukumura D, Leunig M, Berk DA, Jain RK, et al. Vascular Permeability in a Human Tumor Xenograft: Molecular Size Dependence and Cutoff Size. Cancer Res. 1995;55(17):3752-3756.

[64] Smith BR, Kempen P, Bouley D, Xu A, Liu Z, Dai H, et al. Shape Matters: Intravital Microscopy Reveals Surprising Geometrical Dependence for Nanoparticles in Tumor Models of Extravasation. 2013;12(7):3369-3377.

[65] Iwai K, Maeda H, Konno T. Use of Oily Contrast Medium for Selective Drug Targeting to Tumor: Enhanced Therapeutic Effect and X-Ray Image. Cancer Res. 1984;44(5):2115-2121.

[66] Danhier F. To exploit the tumor microenvironment: Since the EPR effect fails in the clinic, what is the future of nanomedicine? J Control Release. 2016;244:108-121.

[67] Nichols JW, Bae YH. Odyssey of a cancer nanoparticle: From injection site to site of action. Nano Today. 2012;7(6):606-618.

[68] Carter CL, Allen C, Henson DE. Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. Cancer. 1989;63(181).

[69] Gillies RJ, Schornack PA, Secomb TW, Raghunand N. Causes and Effects of Heterogeneous Perfusion in Tumors. Neoplasia. 1999;1(3):197-207.

[70] Jain RK. Transport of Molecules in the Tumor Interstitium: A Review. Cancer Res. 1987;47(12):3039-3051.

[71] Fang J, Islam R, Islam W, Yin H, Subr V, Etrych T, et al. Augmentation of EPR Effect and Efficacy of Anticancer Nanomedicine by Carbon Monoxide

Generating Agents. Pharmaceutics. 2019;11(7):343.

[72] Tahara Y, Yoshikawa T, Sato H, Mori Y, Zahangir MH, Kishimura A, et al. Encapsulation of a nitric oxide donor into a liposome to boost the enhanced permeation and retention (EPR) effect. Medchemcomm. 2017;8(2):415-421.

[73] Islam W, Fang J, Imamura T, Etrych T, Subr V, Ulbrich K, et al. Augmentation of the enhanced permeability and retention effect with nitric oxide–generating agents improves the therapeutic effects of nanomedicines. Mol Cancer Ther. 2018;17(12):2643-2653.

[74] Nagamitsu A, Greish K, Maeda H. Elevating blood pressure as a strategy to increase tumor-targeted delivery of macromolecular drug SMANCS: Cases of advanced solid tumors. Jpn J Clin Oncol. 2009;39(11):756-766.

[75] Balasubramanian V, Domanskyi A, Renko JM, Sarparanta M, Wang CF, Correia A, et al. Engineered antibody-functionalized porous silicon nanoparticles for therapeutic targeting of pro-survival pathway in endogenous neuroblasts after stroke. Biomaterials. 2020 Jan;227:119556.

[76] Bian X, Wu P, Sha H, Qian H, Wang Q, Cheng L, et al. Anti-EGFR-iRGD recombinant protein conjugated silk fibroin nanoparticles for enhanced tumor targeting and antitumor efficiency. Onco Targets Ther. 2016;9:3153-3162.

[77] Heo DN, Yang DH, Moon HJ, Lee JB, Bae MS, Lee SC, et al. Gold nanoparticles surface-functionalized with paclitaxel drug and biotin receptor as theranostic agents for cancer therapy. Biomaterials. 2012;33(3):856-866.

[78] Bhattacharya D, Das M, Mishra D, Banerjee I, Sahu SK, Maiti TK, et al. Folate receptor targeted, carboxymethyl chitosan functionalized iron oxide nanoparticles: A novel ultradispersed nanoconjugates for bimodal imaging. Nanoscale. 2011 Apr;3(4):1653-1662.

[79] Lammers T, Kiessling F, Hennink WE, Storm G. Drug targeting to tumors: Principles, pitfalls and (pre-) clinical progress. J Control Release. 2012;161(2):175-187.

[80] Danhier F, Feron O, Préat V. To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J Control Release. 2010;148(2):135-146.

[81] Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, et al. Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. Cancer Res. 2006;66(13): 6732-6740.

[82] Jiménez Blanco JL, Benito JM, Ortiz Mellet C, García Fernández JM. Molecular nanoparticle-based gene delivery systems. J Drug Deliv Sci Technol. 2017;42:18-37.

[83] Wong J, Brugger A, Khare A, Chaubal M, Papadopoulos P, Rabinow B, et al. Suspensions for intravenous (IV) injection: A review of development, preclinical and clinical aspects. Adv Drug Deliv Rev. 2008;60(8):939-954.

[84] Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins: Basic science and product development. J Pharm Pharmacol. 2010;62(11):1607-1621.

[85] Morgan S, Grootendorst P, Lexchin J, Cunningham C, Greyson D. The cost of drug development: A systematic review. Health Policy (New York). 2011;100(1):4-17.

[86] Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC,

Munos BH, Lindborg SR, et al. How to improve RD productivity: The pharmaceutical industry's grand challenge. Nat Rev. Drug Discov. 2010;9(3):203-214.

[87] Services H. Waiver of In-vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. US Dep Heal Hum Serv Food Drug Adm Cent Drug Eval Res. 2017;3-5.

[88] Kalepu S, Nekkanti V. Insoluble drug delivery strategies: Review of recent advances and business prospects. Acta Pharm Sin B. 2015;5(5):442-453.

[89] Müller RH, Jacobs C, Kayser O. Nanosuspensions as particulate drug formulations in therapy: Rationale for development and what we can expect for the future. Adv Drug Deliv Rev. 2001;47(1):3-19.

[90] Xie M-B, Li Y, Zhao Z, Chen A-Z, Li J-S, Hu J-Y, et al. Solubility enhancement of curcumin via supercritical CO2 based silk fibroin carrier. J Supercrit Fluids [Internet]. 2015 Aug [cited 2016 Apr 26];103:1-9. Available from: http://www.sciencedirect.com/science/article/pii/S0896844615001679

[91] Fuster MG, Carissimi G, Montalbán MG, Víllora G. Improving Anticancer Therapy with Naringenin-Loaded Silk Fibroin Nanoparticles. Nanomater (Basel, Switzerland). 2020 Apr;10(4).

[92] Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric systems for controlled drug release. Chem Rev. 1999;99(11):3181-3198.

[93] Kost J, Horbett TA, Ratner BD, Singh M. Glucose-sensitive membranes containing glucose oxidase: Activity, swelling, and permeability studies. J Biomed Mater Res. 1985 Nov;19(9): 1117-1133.

[94] Volpatti LR, Matranga MA, Cortinas AB, Delcassian D, Daniel KB, Langer R, et al. Glucose-Responsive Nanoparticles for Rapid and Extended Self-Regulated Insulin Delivery. ACS Nano. 2020;14(1):488-497.

[95] Chen M, Shao Z, Chen X. Paclitaxel-loaded silk fibroin nanospheres. J Biomed Mater Res - Part A. 2012;100 A(1):203-10.

[96] Jones RT. Blood Flow. Annu Rev. Fluid Mech. 1969 Jan;1(1):223-244.

[97] Moghimi SM, Patel HM. Serum-mediated recognition of liposomes by phagocytic cells of the reticuloendothelial system - The concept of tissue specificity. Adv Drug Deliv Rev. 1998;32(1-2):45-60.

[98] Tenzer S, Docter D, Kuharev J, Musyanovych A, Fetz V, Hecht R, et al. Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. Nat Nanotechnol. 2013;8(10):772-781.

[99] Salvati A, Pitek AS, Monopoli MP, Prapainop K, Bombelli FB, Hristov DR, et al. Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. Nat Nanotechnol. 2013;8(2):137-143.

[100] Milton Harris J, Chess RB. Effect of pegylation on pharmaceuticals. Nat Rev. Drug Discov. 2003;2(3):214-221.

[101] Totten JD, Wongpinyochit T, Carrola J, Duarte IF, Seib FP. PEGylation-Dependent Metabolic Rewiring of Macrophages with Silk Fibroin Nanoparticles. ACS Appl Mater Interfaces. 2019;11(16):14515-14525.

[102] Rodriguez PL, Harada T, Christian DA, Pantano DA, Tsai RK, Discher DE. Minimal "Self" Peptides That Inhibit Phagocytic Clearance and Enhance Delivery of Nanoparticles.

Science (80-). 2013 Feb;339(6122): 971-5.

[103] Wernig G, Chen SY, Cui L, Van Neste C, Tsai JM, Kambham N, et al. Unifying mechanism for different fibrotic diseases. Proc Natl Acad Sci U S A. 2017;114(18):4757-4762.

[104] Hu C-MJCMJ, Zhang LL, Aryal S, Cheung C, Fang RH, Zhang LL. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. Proc Natl Acad Sci U S A. 2011;108(27):10980-10985.

[105] Parodi A, Quattrocchi N, Van De Ven AL, Chiappini C, Evangelopoulos M, Martinez JO, et al. Synthetic nanoparticles functionalized with biomimetic leukocyte membranes possess cell-like functions. Nat Nanotechnol. 2013;8(1):61-68.

[106] Nikitin MP, Zelepukin I V., Shipunova VO, Sokolov IL, Deyev SM, Nikitin PI. Enhancement of the blood-circulation time and performance of nanomedicines via the forced clearance of erythrocytes. Nat Biomed Eng. 2020;4(7):717-731.

[107] Catto V, Farè S, Cattaneo I, Figliuzzi M, Alessandrino A, Freddi G, et al. Small diameter electrospun silk fibroin vascular grafts: Mechanical properties, in vitro biodegradability, and in vivo biocompatibility. Mater Sci Eng C. 2015;54:101-111.

[108] Tan M, Liu W, Liu F, Zhang W, Gao H, Cheng J, et al. Silk fibroin-coated nanoagents for acidic lysosome targeting by a functional preservation strategy in cancer chemotherapy. Theranostics. 2019;9(4):961-973.

[109] Meng F, Cheng R, Deng C, Zhong Z. Intracellular drug release nanosystems. Mater Today. 2012;15(10):436-442.

[110] Zhao Z, Li Y, Xie M Bin. Silk fibroin-based nanoparticles for drug delivery. Int J Mol Sci. 2015;16(3): 4880-4903.

[111] Jordan A, Scholz R, Wust P, Fähling H, Krause J, Wlodarczyk W, et al. Effects of Magnetic Fluid Hyperthermia (MFH) on C3H mammary carcinoma in vivo. Int J Hyperth. 1997;13(6):587-605.

[112] Dianzani C, Zara GP, Maina G, Pettazzoni P, Pizzimenti S, Rossi F, et al. Drug delivery nanoparticles in skin cancers. Biomed Res Int. 2014;2014(July).

[113] Kim SY, Naskar D, Kundu SC, Bishop DP, Doble PA, Boddy A V., et al. Formulation of Biologically-Inspired Silk-Based Drug Carriers for Pulmonary Delivery Targeted for Lung Cancer. Sci Rep. 2015;5(April):1-13.

[114] Paranjpe M, Müller-Goymann CC. Nanoparticle-mediated pulmonary drug delivery: A review. Int J Mol Sci. 2014;15(4):5852-5873.

[115] ZhuGe DL, Wang LF, Chen R, Li XZ, Huang ZW, Yao Q, et al. Cross-linked nanoparticles of silk fibroin with proanthocyanidins as a promising vehicle of indocyanine green for photo-thermal therapy of glioma. Artif Cells, Nanomedicine Biotechnol. 2019;47(1):4293-4304.

[116] Zhan S, Paik A, Onyeabor F, Ding B, Prabhu S, Wang J. Oral Bioavailability Evaluation of Celastrol-Encapsulated Silk Fibroin Nanoparticles Using an Optimized LC–MS/MS Method. Molecules. 2020 Jul;25(15):3422.

[117] Brooks AE. The potential of silk and silk-like proteins as natural mucoadhesive biopolymers for controlled drug delivery. Front Chem. 2015;3(NOV):1-8.

[118] Gaul R, Ramsey JM, Heise A, Cryan SA, Greene CM. Nanotechnology

approaches to pulmonary drug delivery: Targeted delivery of small molecule and gene-based therapeutics to the lung. Design of Nanostructures for Versatile Therapeutic Applications. Elsevier Inc.; 2018. 221-253 p.

[119] Takeuchi I, Shimamura Y, Kakami Y, Kameda T, Hattori K, Miura S, et al. Transdermal delivery of 40-nm silk fibroin nanoparticles. Colloids Surfaces B Biointerfaces. 2019 Mar;175(November 2018):564-568.

[120] Deen WM, Lazzara MJ, Myers BD. Structural determinants of glomerular permeability. Am J Physiol - Ren Physiol. 2001;281(4 50-4).

[121] M. Ohlson, J. Sörensson BH. A gel-membrane model of glomerular charge and size selectivity in series. Am J Physiol. 2001;280:396-405.

[122] Longmire M, Choyke PL, Kobayashi H. Clearance Properties of Nano-sized Particles and Molecules as Imaging Agents: Considerations and Caveats. Nanomedicine. 2012;3(5):703-717.

[123] Soo Choi H, Liu W, Misra P, Tanaka E, Zimmer JP, Itty Ipe B, et al. Renal clearance of quantum dots. Nat Biotechnol. 2007;25(10):1165-1170.

[124] Kuntz E, Kuntz H-D. Hepatology Principles and Practices. Vol. 53, Journal of Chemical Information and Modeling. 2013. 1689-1699 p.

[125] Poon W, Zhang YN, Ouyang B, Kingston BR, Wu JLY, Wilhelm S, et al. Elimination Pathways of Nanoparticles. ACS Nano. 2019;13(5):5785-5798.

[126] Hwang KJ, Luk KFS, Beaumier PL. Hepatic uptake and degradation of unilamellar sphingomyelin/cholesterol liposomes: A kinetic study. Proc Natl Acad Sci U S A. 1980;77(7 II):4030-4.

[127] Mohammad AK, Reineke JJ. Quantitative detection of PLGA nanoparticle degradation in tissues following intravenous administration. Mol Pharm. 2013;10(6):2183-2189.

[128] Lu Y, Chen SC. Micro and nano-fabrication of biodegradable polymers for drug delivery. Adv Drug Deliv Rev. 2004;56(11):1621-1633.

[129] Sadauskas E, Wallin H, Stoltenberg M, Vogel U, Doering P, Larsen A, et al. Kupffer cells are central in the removal of nanoparticles from the organism. Part Fiber Toxicol. 2007;4(May 2014).