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Radiolabeled liposomes and lipoproteins as lipidic nanoparticles for imaging and therapy

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ABSTRACT

Radiolabeled lipidic nanoparticles, particularly liposomes and lipoproteins, are of great interest as agents for imaging and therapy, due not only to their peculiar physicochemical and biological properties, but also to their great versatility and the ability to manipulate them to obtain the desired properties. This review provides an overview of radionuclide labeling strategies for preparing diagnostic and therapeutic nanoparticles based on liposomes and lipoproteins that have been developed to date, as well as the main quality control methods and *in vivo* applications.

1. Introduction

Nanoparticles are defined as *those particles whose diameters are smaller than a few hundred nm*. Such dimensions are similar to those of many biological macromolecules, and thus, these particles are very useful for diagnosis and therapy. Their pharmacokinetic behavior and biodistribution is different from those of small molecules (< 1 nm) and microparticles (> 1 μm), which makes their applications with regard to different clinical objectives possible. Nanoparticle have been used since the middle of the last century. However, despite their potential benefits, routine clinical use is less than expected in comparison to the large number of studies that have been carried out.

The first radionuclide-labeled nanoparticles that were used for diagnostic imaging were nanocolloids (^{99m}Tc-S colloid, ^{68}Ga-ferric oxide, ^{113m}In-ferric hydroxide), whose use began in the 1950s (Berezin, 2015). The discovery of liposomes in 1965 by Bangham et al. (Bangham et al., 1965) and the development of protein labeling methods (Lane and Richardson, 2011) launched the development of radiolabeled nanoparticles for clinical use since 1971 (Gregoriadis and Ryman, 1971). Today, there is a wide range of radiolabeled nanoparticles for diagnostic, therapeutic or theragnostic purposes (therapeutic and diagnostic properties in the same compound). These are grouped into radiolabeled metallic, polymeric and lipidic nanoparticles. In this work, we will focus on the main methods of radiolabeling, quality control and *in vivo* biodistribution of lipidic nanoparticles, which include liposomes and lipoproteins.

2. Radiolabeled liposomes

A liposome is an artificial spherical vesicle with one or more

Abbreviations: SPECT, single-photon emission tomography; keV, kilo electron-volt; PET, positron emission tomography; MeV, mega electron-volt; ^{18}F-FCP, carboplatin derivative labeled with ^{18}F; 8HQ, 8-hydroxyquinoline; 8HQS, 8-hydroxyquinoline sulfate; 2HQ, 2-hydroxyquinoline; HMPAO, hexamethylene-propyleneamine oxime; BMEDA, N,N-bis (2-mercaptoethyl)-N'',N''-diethyl-ethylenediamine; DTPA, diethylenetriamine pentaacetic acid; NTA, nitrile acetic acid; GSH, glutathione; DOTA, 1,4,7,10-tetraazacyclotetradecane-1,4,7,10 tetra acetic acid; DF, deferoxamine; BAT, 6-[p-(bromoacetamido) benzyl]-1,4,8,11-tetraazacyl chlortetradecane-*N',N'',N'''*-tetraacetic acid; HYNIC, hydrazinonicotinic acid; PEG, polyethylene glycol; TLC, thin-layer chromatography; ITLC, instant thin-layer chromatography; HPLC, high-performance liquid chromatography; DLS, dynamic light scattering; TEM, Transmission Electron Microscopy; SEC, Size exclusion chromatography; DSC, Differential scanning calorimetry; ICP-AS, Atomic emission spectroscopy with coupled inductive plasma; SPE, solid phase extraction; LD_{50}, lethal dose-50; ERS, endothelial reticulum system

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concentric lipid bilayers that enclose an internal aqueous volume. Lipid bilayers form spontaneously by dispersing phospholipids, which are neutral or charged, in water and are stabilized by hydrophobic interactions and Van de Waals forces. The preparation methods are diverse, which allows for obtaining different compositions and sizes, ranging from 50 to 1000 nm, although the most stable are those with sizes ranging from 90 to 250 nm (Phillips et al., 2009). For medical use, the recommended range is between 50 and 500 nm (Bozzuto and Molinari, 2015).

The main application of liposomes is drug delivery (Bozzuto and Molinari, 2015; Gregoriadis et al., 1974; Gregoriadis and Ryman, 1971; Lamichhane et al., 2018, 2017; Man et al., 2019; Maurer et al., 2001), although they have also been used in gene therapy (Lamichhane et al., 2018; Maurer et al., 2001). The need to visualize liposome distribution *in vivo* and to observe the sites of accumulation of the transported drug boosted efforts in liposome radiolabeling (Berezin, 2015). The first radionuclide-labeled liposomes (labeled with ^{131}I) were obtained in 1971 by Gregoriadis and Ryman (Gregoriadis and Ryman, 1971) and were first used in humans in 1974 (Gregoriadis et al., 1974). Labeling was performed by trapping ^{131}I-albumin inside the liposome. Later, other researchers labeled phosphatidylcholine and cholesterol liposomes with ^{99m}Tc with the aim of tumor detection (Richardson et al., 1977). However, the *in vivo* stability of these first radiolabeled liposomes was poor. Not long after, liposomes labeled with ^{111}In and ^{67}Ga (HWANG et al., 1982) were also obtained.

Physicochemical characteristics of liposomes (size, lipid composition, surface charge and bilayer fluidity, among others) are responsible for their versatility, which has enabled the development of different radionuclide labeling methods for the preparation of radiopharmaceuticals to be used in diagnosis and therapy (Ehlerding et al., 2016; Goins et al., 2010; Hwang et al., 1982; Richardson et al., 1977). A radiopharmaceutical is *any substance that, in its chemical composition, has a radioactive atom and that, due to its pharmaceutical form, quantity, and quality of radiation, can be administered to patients for the purpose of diagnosis or therapy* (Secretaría de Salud, 2017). Nuclear medicine employs radiopharmaceuticals in SPECT and PET imaging techniques for diagnostic purposes. Liposomes, as radiopharmaceuticals for SPECT image, are labeled with γ-emitting radionuclides, with energies between 100 and 500 keV (^{99m}Tc, ^{111}In, ^{67}Ga), while those used in PET are labeled with β^+ emitters, with maximum energies ranging from several hundred keV to MeV (^{123}I, ^{18}F, ^{68}Ga, ^{64}Cu, ^{52}Mn, ^{89}Zr). For therapy purposes, liposomes have been labeled with α and β^- emitters such as ^{90}Y,^{166}Ho, ^{213}Bi and ^{225}Ac. Other radionuclides with β- and γ emissions, such as ^{131}I, ^{177}Lu (Ehlerding et al., 2016) and ^{186,188}Re (Goins et al., 2010; Medina et al., 2007) have also been used for therapy and therapy/imaging purposes (theragnostic applications).

Liposome labeling can be carried out in the inner aqueous core or on the lipid membrane (inside or on the surface). In both cases, different strategies are used. The decision to label in one place or another depends on the purposes of the study, the characteristics of the liposomes to be labeled, the radionuclide, the chelators to be used and the molecules that are intended to be trapped within the liposome.

2.1. Strategies for liposome labeling in the inner aqueous core

The introduction of the radionuclide into the aqueous interior of the liposome can be done by passive encapsulation during its preparation or by diffusion through the bilayer once the liposome is obtained.

The passive encapsulation method during liposome preparation (Fig. 1A) has been used to label liposomes with ^{18}F (Lamichhane et al., 2017; Man et al., 2019; Marik et al., 2007; Oku et al., 2011), ^{67}Ga (HWANG et al., 1982), ^{99m}Tc (Andreopoulos and Kasi, 1997; Caride and Zaret, 1977; Gregoriadis et al., 1974; Oku et al., 1993), ^{111}In (Essien and Hwang, 1988; HWANG et al., 1982; Lamichhane et al., 2017; Man et al., 2019), ^{131}I (Man et al., 2019), but it presents disadvantages, since it is laborious, requires preparing the liposomes every time the labeling is performed, and yields are low (generally < 10%, although in some cases, a yield of up to 30% has been reported), which constitutes an inconvenience for its clinical application. Currently, the method is scarcely used, although good results have been reported with regard to the encapsulation of the carboplatin derivative ^{18}F-FCP (Lamichhane et al., 2017).

The diffusion through the liposome bilayer is a labeling method that uses liposomes previously prepared (Figs. 1B and C). The radionuclide is first introduced into the aqueous nucleus of the liposome with the help of an ionophore by forming an ionophore-radionuclide complex. Some ionophores, such as the molecule A23187, are added during the preparation of the liposome so that they are part of the lipid bilayer (Fig. 1B) (Jensen and Bunch, 2007; Kubo et al., 1993; Man et al., 2019; Mauk and Gamble, 1979). Other ionophores bind directly to the radionuclide and help it pass through the bilayer (Fig. 1C). The ionophores most used are: free-form 8-hydroxyquinoline (8HQ) or sulfated 8-hydroxyquinoline (8HQS), 2-hydroxyquinoline (2HQ), hexamethylene-propyleneamine oxime (HMPAO). N,N-bis(2-mercaptoethyl)-N'',N''-diethyl-ethylenediamine (BMEDA), acetyl acetone and tropolone (Bao et al., 2003; Boerman et al., 2000; Corvo et al., 2000; Essien and Hwang, 1988; Gabizon and Papahadjopoulos, 1988; Goins et al., 1996; Henriksen et al., 2015; HWANG et al., 1982; Medina et al., 2007; Petersen et al., 2011, 2016; Phillips et al., 1992; Woodle, 1993)

Once the ionophore-radionuclide complex reaches the aqueous nucleus of the liposome, the radionuclide is transferred to a previously incorporated hydrophilic chelator, which has a greater affinity for the radionuclide than the ionophore (Fig. 1C). Since the hydrophilic chelator cannot pass through the lipid bilayer, the radionuclide is trapped in the aqueous nucleus of the liposome. Among pre-incorporated hydrophilic chelators, diethylenetriamine pentaacetic acid (DTPA) (Man et al., 2019), nitrile acetic acid (NTA) (Gabizon et al., 1988; HWANG et al., 1982; Mauk and Gamble, 1979) glutathione (GSH) (Bao et al., 2003; Boerman et al., 1997; Goins et al., 1996; Medina et al., 2007; Phillips et al., 1992), 1,4,7,10-tetraazacyclotetradecane-1,4,7,10 tetra acetic acid (DOTA) (Henriksen et al., 2015; Petersen et al., 2011) and deferoxamine (DF) (Gabizon et al., 1988; Woodle, 1993) have been used.

The diffusion strategy with which ^{52}Mn, ^{64}Cu, ^{67,68}Ga, ^{89}Zr, ^{99m}Tc, ^{111}In, ^{177}Lu, ^{186}Re and ^{188}Re have been labeled is one of the most frequently used for liposome labeling, since the labeling yields are high (usually > 90%), in addition to good *in vivo* stability, as the radionuclide is not exposed to blood components.

2.2. Strategies for liposome labeling on the membrane

Another possible strategy is the liposomal membrane labeling known as *surface labeling*. It can be achieved within the membrane (forming part of the bilayer, as shown in Fig. 2 A–C) or on top of it (Fig. 2D). Different methods have also been used in this type of labeling; some authors have performed labeling directly on the membrane, but without clarifying the exact site where the radionuclide binds (Man et al., 2019). For labeling within the membrane, a lipophilic chelator with high affinity for the radionuclide is used. In this case, the chelator binds to the bilayer phospholipids before or during liposome preparation. Then, the radionuclide is added during or after liposome preparation (Fig. 2A and B).

Among the chelators used are DTPA and its derivatives (Ahkong and Tilcock, 1992; Man et al., 2019; Tilcock et al., 1994), 6-[p-(bromoacetamido) benzyl]-1,4,8,11-tetraazacyl chlortetradecane-*N',N'',N'''*-tetraacetic acid (BAT) (Seo et al., 2008), hydrazinonicotinic acid (HYNIC) (Laverman et al., 1999), iminothiolane (Varga et al., 2017), DOTA (Man et al., 2019), DF (Lobatto et al., 2020; Perez-Medina et al., 2014; Seo et al., 2015) and biotin (biotin ligand conjugated to the phospholipid head region) (Medina et al., 2004a; Medina et al., 2006). The radionuclide can be free or coupled to a molecule, as in the case of the ^{99m}Tc-tricarbonyl complex used by Varga et al. (Varga et al., 2017), and the

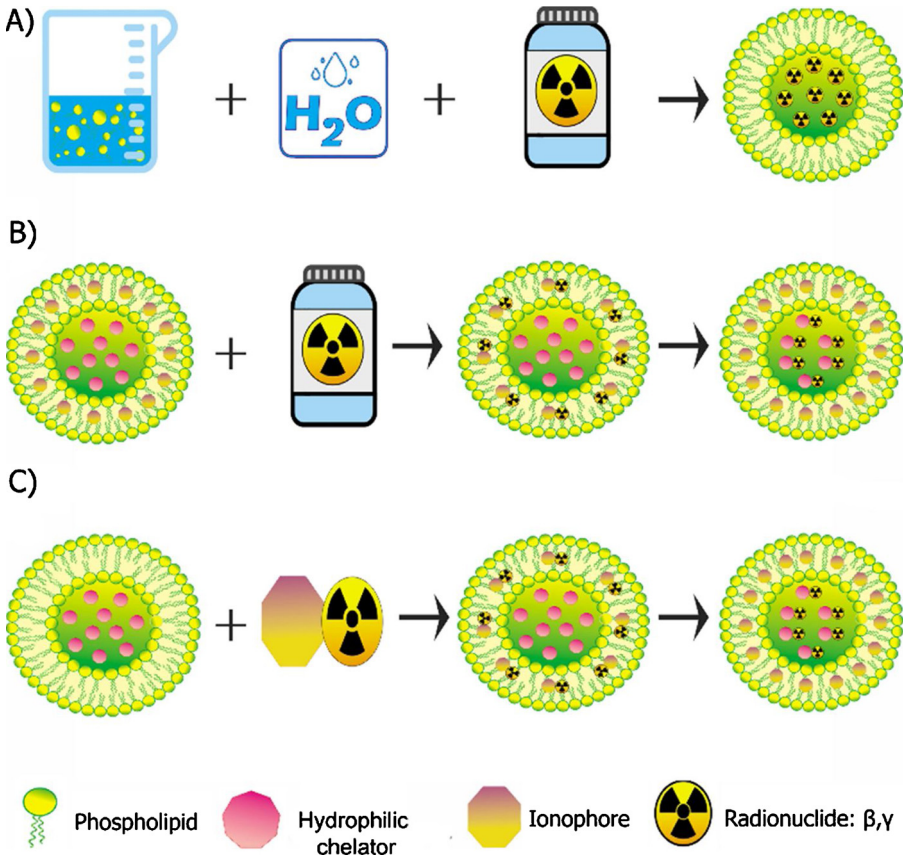

Fig. 1. Strategies for liposome labeling in the inner aqueous core.

bifunctional chelator used by Seo et al. (Seo et al., 2011). Another possibility is to prepare a hydrophobic complex between the chelator and the radionuclide, such as ^{99m}Tc-BMEDA or ^{99m}Tc-HYNIC-dodecyl amide (Isaac-Olivé et al., 2019), which passively enters into the previously-prepared liposome membrane (Fig. 2C).

Surface labeling can also be performed by attaching the free or chelated radionuclide to a ligand with affinity for the surrounding medium of the membrane, such as the polyethylene glycol (PEG) that covers the lipid layer in the PEGylated liposomes (Fig. 2D) (Man et al., 2019; Maurer et al., 2001). Surface labeling provides high labeling yields, but the *in vivo* stability of the radiolabeled liposome is lower than when the radionuclide is trapped in the aqueous nucleus, since the radionuclide finds it easier to attach to the blood components, which affects the biodistribution of the liposome radiopharmaceutical. In these cases, *in vivo* stability depends on the stability constant of the chelator -radionuclide complex. On the other hand, membrane labeling modifies the original liposome to a greater degree than when it is labeled in the inner core.

The methods that employ previously prepared liposomes are more convenient, since they are less time-consuming, achieve high specific activities, adequate *in vivo* stability and the cost is lower. An additional advantage is that radiolabeling can be performed immediately before use. Table 1 summarizes the radionuclides employed in the liposome-labeling methods described above. The last column groups membrane-labeling methods, either on or within the membrane. These were not separated, since most of the time, published works do not specify the location of the radionuclide.

In Table 1, it can be seen that ^{99m}Tc, ^{111}In and ^{64}Cu are the most commonly used radionuclides for imaging with liposomes. In recent years, ^{89}Zr (Lamichhane et al., 2018; Man et al., 2019; Seo et al., 2015), 67,68Ga (Gabizon et al., 1988; Helbok et al., 2010; HWANG et al., 1982; Woodle, 1993) and ^{123,124}I (Man et al., 2019; Mougin-Degraef et al., 2007; Srivatsan and Chen, 2014) have also been used.

2.3. Characterization of radiolabeled liposomes

After the preparation of radiolabeled liposomes and before *in vivo* studies, characterization and quality control is required. Radiolabeled compounds need to be of high quality because if this quality is not guaranteed, an incorrect imaging or an undue dose of radiation is obtained. In order to secure a useful study, it is necessary to control the quality of the prepared liposome, that of the radionuclide before labeling, and that of the final radiolabeled product. This final radiolabeled product must be pure in the chemical, radiochemical and radionuclidic sense. It must also be sterile, non-toxic and under the limits of bacterial endotoxin content.

In order to achieve chemical purity, it is necessary to control the prepared liposome before labeling. Chemical impurities negatively influence labeling efficiency, toxicity, stability, biodistribution and pharmacokinetics of the product (INTERNATIONAL ATOMIC ENERGY AGENCY, 2018; The International Pharmacopoeia, 2013). Physicochemical controls carried out to prepare highly-purified and stable liposomes include: the evaluation of organoleptic characteristics, particle size and distribution, net surface charge, membrane transition temperature, osmolality, *in vitro* stability, composition and quantification of lipids and their degradation products, quantification of the pre-incorporated hydrophilic chelator, sterility, bacterial endotoxin content, biodistribution, pharmacokinetics and acute toxicity (Food and Drug Administration, 2018). Many of these controls are performed only during the design and development stage, while others are carried out on all batches that are prepared. Table 2 summarizes the analytical techniques frequently employed for such controls.

Radiochemical impurities are originated in the labeling process (due to unwanted content of ionophore and chelators or incomplete

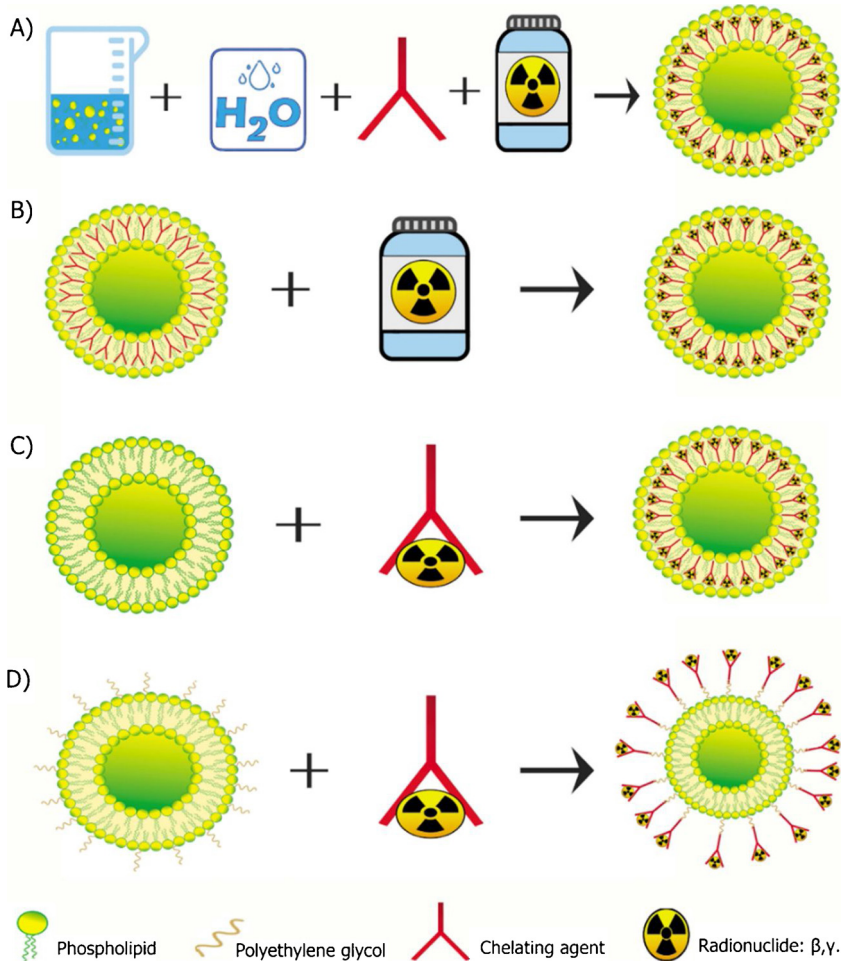

Fig. 2. Strategies of liposome labeling on the membrane.

chelation of the radionuclide), by radiolysis during storage, by changes in temperature, pH and light, among other factors (Food and Drug Administration, 2018; Petersen et al., 2012). It is important that once the liposome has been labeled, any non-adhered radioactive tracer is removed. For this purpose, centrifugation, column chromatography, ion exchange and prolonged dialysis have been used (Caride, 1990). Radiochemical purity is usually determined by chromatography (thin-layer chromatography [TLC], instant TLC [ITLC] and high-performance liquid chromatography [HPLC]) (INTERNATIONAL ATOMIC ENERGY AGENCY, 2018; The International Pharmacopoeia, 2013). Additionally, the identity of the radionuclide is verified by measuring its half-life and by nuclear spectrometry (γ or β) (INTERNATIONAL ATOMIC ENERGY AGENCY, 2018; The International Pharmacopoeia, 2013).

After the liposome has been radiolabeled, it is necessary to evaluate the *in vivo* study, appearance, identity of the radiolabeled molecule, pH, osmolality, isotonicity, total activity, radiochemical purity, stability, sterility and bacterial endotoxin content in the solution to be injected (INTERNATIONAL ATOMIC ENERGY AGENCY, 2018).

Appearance is measured by means of visual inspection (INTERNATIONAL ATOMIC ENERGY AGENCY, 2018; The International Pharmacopoeia, 2013). HPLC, SEC and ITLC can be used to determine the identity of the radiolabeled molecule, using the unlabeled liposome as a control (INTERNATIONAL ATOMIC ENERGY AGENCY, 2018; Petersen et al., 2012; The International Pharmacopoeia, 2013). pH is determined with a potentiometer or with pH strips; osmolality by osmometry and NaCl equivalency (INTERNATIONAL ATOMIC ENERGY AGENCY, 2018; The International Pharmacopoeia, 2013); isotonicity by conductimetry; total activity and radioactive concentration, through the use of a dose calibrator; radiochemical purity by TLC, ITLC, HPLC, SEC, solid phase extraction (SPE) or paper electrophoresis (Andreopoulos and Kasi, 1997; Biltonen and Lichtenberg, 1993; Duan et al., 2016; INTERNATIONAL ATOMIC ENERGY AGENCY, 2018; Lappin and Garner, 2003; Laverman et al., 1999; Oku et al., 1993; Seo et al., 2008; The International Pharmacopoeia, 2013; Varga et al., 2017).

2.4. *In vivo* behavior of radiolabeled liposomes

The in vivo behavior of radiolabeled liposomes is evaluated from biodistribution studies. This method should be performed with rodents (mice between 25–30 g and rats between 200–300 g), through nuclear imaging techniques (scintigraphy, SPECT or PET) and/or *ex vivo* organ activity quantification (Andreopoulos and Kasi, 1997; Gabizon and Papahadjopoulos, 1988; Helbok et al., 2010; INTERNATIONAL ATOMIC ENERGY AGENCY, 2018; Laverman et al., 1999; Mougin-Degraef et al., 2007; Oku et al., 1993; Petersen et al., 2016; Varga et al., 2017). Although not fully extrapolable to humans, these tests provide a glimpse of the *in vivo* behavior of the products. For the pharmacokinetic and pharmacodynamic tests carried out in recent years, sub-pharmacological doses or micro-doses are generally accepted for liposomes labeled with positronic radionuclides (ICH, M3(R2); ICH, E., 2009; Lappin and Garner, 2003; Petersen et al., 2012). This approach allows human trials to be carried out at the stage called *Phase 0*, in order to use fewer animals in toxicology tests, shorten research times and reduce costs (Lappin and Garner, 2003). Also, acute toxicity is determined by LD_{50} (Food and Drug Administration, 2018).

Table 1
Main radionuclides and methods of liposome radiolabeling for imaging, therapy and theragnostic applications.

Radio-nuclide	Labeling site				
	In the aqueous core				On the membrane
	During liposome preparation		After liposome preparation		Inside or on the bilayer
	Free	Binding to a molecule (chelator)	Ionophore or chelator for bilayer incorporation	Ionophore binding to the radionuclide in order to assist it	
^{18}F		(Emmetiere et al., 2013; Lamichhane et al., 2017)			(Marik et al., 2007)
^{52}Mn				(Jensen et al., 2018)	(Jensen et al., 2018)
^{64}Cu				(Bunney et al., 2017; Jensen et al., 2018; Petersen et al., 2016, 2011)	(Jensen et al., 2018; Lee et al., 2016; Seo et al., 2008)
67,68Ga	(HWANG et al., 1982)		(Gabizon et al., 1988; HWANG et al., 1982; Woodle, 1993)		(Helbok et al., 2010)
^{89}Zr				(Li et al., 2017; Man et al., 2019)	(Lamichhane et al., 2018; Lobatto et al., 2020; Perez-Medina et al., 2014; Seo et al., 2015)
^{99m}Tc	(Andreopoulos and Kasi, 1997)	(Caride and Zaret, 1977; Gregoriadis et al., 1974; Oku et al., 1993)		(Boerman et al., 1997; Goins et al., 2010; Petersen et al., 2016; Phillips et al., 1992)	(Ahkong and Tilcock, 1992; Arrieta et al., 2014, 2012; Bao et al., 2004; Goto et al., 1989; Helbok et al., 2010; Isaac-Olivé et al., 2019; Laverman et al., 1999; Oku et al., 1993; RICHARDSON et al., 1977; Seo et al., 2008; Tilcock et al., 1994; Varga et al., 2017)
^{111}In	(Essien and Hwang, 1988; HWANG et al., 1982)	(Lamichhane et al., 2017; Man et al., 2019)	(Jensen and Bunch, 2007; Kubo et al., 1993; Man et al., 2019; Mauk and Gamble, 1979)	(Essien and Hwang, 1988; HWANG et al., 1982)	(Helbok et al., 2010; Medina et al., 2004a; Mougin-Degraef et al., 2007)
$^{131, 124}$I		(Man et al., 2019)			(Mougin-Degraef et al., 2007; Srivatsan and Chen, 2014)
^{177}Lu				(Bunney et al., 2017)	(Helbok et al., 2010)
^{188}Re				(Bao et al., 2003)	(Medina et al., 2007)
^{159}Gd		(Soares et al., 2011)			
^{90}Y			(Utkhede et al., 1994)		
^{166}Ho					(Zielhuis et al., 2006)
^{213}Bi			(Lingappa et al., 2010)		
^{225}Ac		(Sofou et al., 2004)			

Table 2
Physicochemical characterization of prepared liposomes.

Parameter	Analytic technique	References
Particle size and distribution. Polydispersity index.	Photonic correlation spectroscopy / dynamic light scattering (DLS) / quasi-elastic scattering	(Bozzuto and Molinari, 2015; Duan et al., 2016; Gabizon et al., 1988; Goins et al., 2010; Helbok et al., 2010; Laverman et al., 1999; Li et al., 2017; Love et al., 1989; Mougin-Degraef et al., 2007; Oku et al., 1993; Tilcock et al., 1994; Varga et al., 2017)
	Doppler laser velocimetry	(Nogueira et al., 2015)
	Analysis of the particle trajectory	
	Transmission electron microscopy (TEM)	(Bozzuto and Molinari, 2015; Li et al., 2017; Petersen et al., 2016; Varga et al., 2017)
	Size exclusion chromatography (SEC)	(Bozzuto and Molinari, 2015; Li et al., 2017)
Surface net charge	Z potential	(Duan et al., 2016; Lamichhane et al., 2018; Li et al., 2017; Nogueira et al., 2015; Oku et al., 1993; Oswald et al., 2016; Petersen et al., 2016; Varga et al., 2017)
Membrane transition temperature	Differential scanning calorimetry (DSC)	(Biltonen and Lichtenberg, 1993)
Composition	High performance liquid chromatography (HPLC)	(Clogston and Patri, 2011; Duan et al., 2016; Goins et al., 2010; Oswald et al., 2016; Yoshino et al., 2012)
	Size exclusion chromatography (SEC)	(Bozzuto and Molinari, 2015; Goins et al., 2010 Petersen et al., 2016)
	Atomic emission spectroscopy with coupled inductive plasma (ICP-AS)	(Petersen et al., 2016)
	Colorimetry	(Andreopoulos and Kasi, 1997; Oswald et al., 2016; Varga et al., 2017)
Organoleptic characteristics	Visual inspection	(Food and Drug Administration, 2018; INTERNATIONAL ATOMIC ENERGY AGENCY, 2018; The International Pharmacopoeia, 2013)
in vitro stability	Placing liposomes in various solutions and assessing degradation	(Laverman et al., 1999; Mougin-Degraef et al., 2007; Petersen et al., 2016; Tilcock et al., 1994; Varga et al., 2017)

It is well-known that the biodistribution of radiolabeled liposomes is subject to many factors, such as size, concentration and surface charge of the liposome, lipid composition and permeability of the bilayer, preparation technique and total administered dose. These variables influence the interactions of the liposomes with plasma components (proteins, macromolecules), the endothelial reticulum system (ERS), extravasation patterns and rapid clearance to the spleen and liver (Boerman et al., 2000, 1997; Gabizon and Papahadjopoulos, 1988; Gaddy et al., 2015; Jensen and Bunch, 2007; Love et al., 1989; Maurer et al., 2001; Medina et al., 2004a; Petersen et al., 2012).

Regarding *size*, liposomes of the same composition and different size have a different behavior. The larger ones clear up faster than the smaller ones. It has also been shown that size influences the type of cell in which liposomes are captured; the larger ones are captured exclusively by Kupffer cells, while the smaller ones are captured by hepatocytes and tumors, particularly those of ~ 100 nm (Boerman et al., 2000, 1997; Bozzuto and Molinari, 2015; Caride, 1990; Gregoriadis et al., 1974; Laverman et al., 1999; Love et al., 1989; Maurer et al., 2001; Nogueira et al., 2015; Oku et al., 1993). The size of the liposomes also depends on extravasation and diffusion in the tissues, in addition to renal excretion (Boerman et al., 2000; Bozzuto and Molinari, 2015; Nogueira et al., 2015).

Superficial charge and charge density are also important factors in the stability, biodistribution, pharmacokinetics and cellular affinity of the radiolabeled liposomes (Boerman et al., 2000; Bozzuto and Molinari, 2015; Love et al., 1989; Maurer et al., 2001; Nogueira et al., 2015). A high electrostatic charge favors the interaction of liposomes with biomolecules and cells (Boerman et al., 2000); negatively-charged liposomes clear up faster than neutral and positively-charged ones (Bao et al., 2003; Love et al., 1989; Medina et al., 2004a; Richardson et al., 1977). On the other hand, positively-charged liposomes bind more easily to blood proteins and nucleic acids, compared to neutral ones, due to electrostatic interactions with these types of molecules; they are also better-captured by tumors and are more stable in the tumor microenvironment (Boerman et al., 2000, 1997; Bozzuto and Molinari, 2015; Nogueira et al., 2015). Negatively-charged liposomes are trapped in ischemic tissues and are better transporters of DNA (Bozzuto and Molinari, 2015; Medina et al., 2004a). Neutral liposomes tend to aggregate and do not activate the complement (Bozzuto and Molinari, 2015).

The length of the chain, the unsaturation, the charge and the stability of the bilayer lipids are factors encompassed by the term *lipid composition*. They determine the permeability of the layer to the agents of interest and have an influence on the liposome's pharmacokinetics and *in vivo* stability (Andreopoulos and Kasi, 1997; Boerman et al., 2000, 1997; Nogueira et al., 2015). Those formed by saturated lipids are less permeable than those formed by unsaturated lipids. The addition of cholesterol and PEG to the bilayer (among other compounds) considerably increases the time in which the liposomes remain in circulation (Boerman et al., 2000; Caride, 1990; Helbok et al., 2010; Love et al., 1989; Nogueira et al., 2015; Oswald et al., 2016; Petersen et al., 2016).

In particular, PEG (PEGylated liposomes) helps to increase blood circulation time and decreases the uptake by the ERS (Boerman et al., 2000, 1997; Bozzuto and Molinari, 2015; Helbok et al., 2010; Medina et al., 2004a; Nogueira et al., 2015; Varga et al., 2017; Yoshino et al., 2012), which has been one of the main obstacles to the use of liposomes as agents for imaging with radionuclides. It has been shown that the addition of a PEG bilayer also reduces the influence of the size, composition and dose of lipids on the biodistribution of liposomes (Allen et al., 1991; Boerman et al., 2000). PEG stabilizes liposomes from the steric point of view, helps to form a hydrophilic surface around the liposome that interferes with its interaction with plasma proteins, and reduces uptake by the ERS (Boerman et al., 2000; Bozzuto and Molinari, 2015; Medina et al., 2004a). Its content is directly proportional to the liposome circulation time; the higher the concentration of PEG, the greater the circulation time (Boerman et al., 2000; Bozzuto and Molinari, 2015; Li et al., 2017; Medina et al., 2004a). However, recent reports have shown that successive injections of PEGylated liposomes induce a significant immune response in experimental animals (Nogueira et al., 2015). The addition of PEG also allows the insertion of chelates into the surface of the membrane in order to bind radionuclides (Man et al., 2019).

The addition of cholesterol reduces the permeability of hydrophilic molecules across the membrane and improves its stability both *in vitro* and *in vivo*. Cholesterol decreases the interaction of the lipid layer with blood proteins, such as albumin, m-transferrin and immunoglobulins; many of which responsible for phospholipid loss and membrane destabilization (Boerman et al., 2000; Love et al., 1989; Nogueira et al., 2015; Oku et al., 1993).

In order to reduce the high uptake (> 70%) of the labeled liposomes by the ERS and prolong the circulation time, making changes in the size, composition and charge of the liposome (Ahkong and Tilcock, 1992; Allen et al., 1991; Goins et al., 1996; Goto et al., 1989; Lee et al.,

2016; Love et al., 1989; Medina et al., 2004b; Oku et al., 1993; Seo et al., 2011; Yoshino et al., 2012), ERS blockade (Caride, 1990), binding to monoclonal antibodies (immunoliposomes) or surface ligands with affinity for certain cells (Boerman et al., 2000; Caride, 1990; Medina et al., 2004a; Mougin-Degraef et al., 2007; Petersen et al., 2012) and the preparation of pH-sensitive liposomes (Bozzuto and Molinari, 2015; Duan et al., 2016; Goins et al., 2010; Maurer et al., 2001; Nogueira et al., 2015), among other strategies, have been used. The use of one or the other depends on the objectives of the study.

2.5. Applications of radiolabeled liposomes for imaging and therapy

Radiolabeled liposomes have been used as diagnostic nanoparticles in preclinical studies to evaluate the *in vivo* behavior of the payload drugs, in therapy monitoring, and in theragnostic applications. As diagnostic nano-radiopharmaceuticals, they have been used primarily to visualize tumors (Boerman et al., 2000; Caride, 1990; Goins, 2008; Jensen and Bunch, 2007; Kubo et al., 1993; Man et al., 2019; Oku et al., 2011, 1993; Petersen et al., 2012, 2011; Richardson et al., 1977; Seo et al., 2015; Srivatsan and Chen, 2014), inflammatory and infectious processes (Andreopoulos and Kasi, 1997; Boerman et al., 1997; Caride, 1990; Goins, 2008; Love et al., 1989; Seo et al., 2015), ERS imaging (Allen et al., 1991; Goins, 2008; Lee et al., 2016) and imaging of cardiovascular diseases (Caride and Zaret, 1977; Gaddy et al., 2015; Goins, 2008; Lobatto et al., 2020; Stendahl and Sinusas, 2015). In order to visualize tumors, molecules of different types such as peptides, antibodies, enzyme inhibitors, or known radiopharmaceuticals, such as ^{18}F-FDG and ^{18}F-FDP (Lamichhane et al., 2018; Man et al., 2019; Marik et al., 2007; Medina et al., 2004b), ^{99m}Tc-DISIDA (Medina et al., 2004b), ^{99m}Tc-MIBI (Medina et al., 2004b), ^{99m}Tc-HMPAO (Goins, 2008; Man et al., 2019; Medina et al., 2004a), ^{111}In-bisphosphonates (Goins, 2008), ^{111}In-IgG (Goins, 2008), ^{111}In-NTA (Goins, 2008), ^{99m}Tc-HAS (Man et al., 2019), ^{99m}Tc-DTPA (Goins, 2008; Man et al., 2019), ^{99m}Tc-streptokinase (Lamichhane et al., 2018; Man et al., 2019; Marik et al., 2007;), ^{99m}Tc-BMEDA (Arrieta et al., 2014, 2012; Bao et al., 2004; Isaac-Olivé et al., 2019), ^{99m}Tc-Biotin (Medina et al., 2004a, 2006), are inserted.

Although this work is focused on the diagnostic and therapeutic use of radiolabeled liposomes, it is not possible to mention liposomes without mentioning its main application in the delivery of non-radioactive therapeutic agents. The methods for inserting therapeutic drugs into liposomes (with or without radionuclides) vary, depending on factors such as the composition, size, shape, liposome charge, the nature of the drug to be transported, the bilayer properties and the type of study to be performed (Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Maurer et al., 2001; Sercombe et al., 2015; van der Geest et al., 2016). In particular, the composition of the lipid layer, its transition temperature, the drug-loading method and the site of action are essential in the design of liposomes as drug carriers.

Different methods are used to insert drugs into liposomes. The insertion site, as described above, may be the aqueous core or the membrane; hydrophilic drugs are introduced into the aqueous core. In general, ionophores (Bozzuto and Molinari, 2015) or pH-sensitive liposomes (Bozzuto and Molinari, 2015; Duan et al., 2016; Maurer et al., 2001) are used, among other methods. Lipophilic drugs are inserted into the membrane and into the interstitial space of the bilayer, while a wide range of molecules, such as proteins, DNA, antibodies and conventional radiopharmaceuticals, can be inserted onto the membrane surface. Very diverse methods have been used for these liposomes, depending on the established objectives (Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Maurer et al., 2001; Sercombe et al., 2015).

Despite all the advantages previously described for liposomes, there are only a few liposomal formulations that have been approved for use in humans, and none of them are radiolabeled. These products that have obtained approval for commercialization can be seen in Table 3. Those that are applied in cancer therapy take the lead: (1) doxorubicin (Bozzuto and Molinari, 2015; Çağdaş et al., 2014; Goins, 2008; Lamichhane et al., 2018; Man et al., 2019; Maurer et al., 2001; Medina et al., 2004b; Pattni et al., 2015; Petersen et al., 2012; Same et al., 2016; Sercombe et al., 2015; van der Geest et al., 2016), daunorubicin (Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Maurer et al., 2001; Medina et al., 2004b; Pattni et al., 2015; Sercombe et al., 2015), and vincristine (Medina et al., 2004b; Pattni et al., 2015; Sercombe et al., 2015; van der Geest et al., 2016), against different types of cancer; (2) the antifungal amphotericin B (Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Maurer et al., 2001; Medina et al., 2004b; Pattni et al., 2015; Sercombe et al., 2015); (3) Inflexal Bern V vaccines, against influenza (Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Maurer et al., 2001; Pattni et al., 2015; Sercombe et al., 2015) and Epaxal Bern 10, against hepatitis (Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Maurer et al., 2001; Pattni et al., 2015; Sercombe et al., 2015); (4) verteporfin, against molecular degeneration (Sercombe et al., 2015), (5) cytrabine, against meningitis (Bozzuto and Molinari, 2015; Pattni et al., 2015; Sercombe et al., 2015) and (6) morphine sulfate, against pain (Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Man et al., 2019; Maurer et al., 2001; Medina et al., 2004b; Pattni et al., 2015; Sercombe et al., 2015; van der Geest et al., 2016). Of these commercially-approved drug transport liposomes, some of them, such as the doxorubicin liposome formulation, have been radiolabeled with ^{99m}Tc for clinical use (Arrieta et al., 2014, 2012).

Considering a large number of liposomal drugs with anti-cancer, antibiotic, vaccine and anti-inflammatories payloads are in preclinical and clinical stages, radiolabeling of these liposomal formulations to obtain multimodal systems is a developing field. The liposomal drugs under development are described in the extensive reviews carried out by Bozzuto (Bozzuto and Molinari, 2015), Man (Man et al., 2019), Lamichhane (Lamichhane et al., 2018), Maurer (Maurer et al., 2001), Sercombe (Sercombe et al., 2015), Van der Geest (van der Geest et al., 2016) and Pattni (Pattni et al., 2015). The most commonly-used radionuclides to visualize the behavior of drugs transported by liposomes are ^{64}Cu, ^{68}Ga ^{89}Zr, ^{99m}Tc and ^{111}In (Lamichhane et al., 2018; Man et al., 2019; Maurer et al., 2001; Petersen et al., 2012; Sercombe et al., 2015; van der Geest et al., 2016). Liposomes labeled with radionuclides can be used not only for the detection and staging of the cancer, but also for monitoring treatment response, adjusting doses and assessing patient susceptibility to treatment (Goins, 2008; Lamichhane et al., 2018; Maurer et al., 2001; Medina et al., 2004a; Petersen et al., 2012).

The possibility of labeling nanoparticles with radionuclides for imaging and therapy simultaneously resulted in the emergence of theragnosis, an interdisciplinary field that combines the properties of diagnosis and therapy and is evolving as a contributor to personalized medicine (Duan et al., 2016; Same et al., 2016). Within the different nanoparticles used for theragnostic imaging, liposomes have drawn much attention due to the aforementioned physical and biological properties. Fig. 3 shows the general strategies that could be implemented to prepare theragnostic liposomes. Among them, are those that carry therapeutics drugs (chemotherapeutic agents or biological material) and are labeled with a radionuclide for diagnosis (SPECT/PET) (Fig. 3a), those that are dually-labeled with a radionuclide for imaging and a radionuclide for therapy (example: ^{64}Cu/^{177}Lu) (Fig. 3b), and those that are labeled with a therapeutic radionuclide that also emits γ radiation, detectable by SPECT, such as ^{131}I, ^{177}Lu or ^{186,188}Re (direct theragnostic agents) (Fig. 3c).

The preparation of theragnostic liposomes includes all aspects already described. The lipids that form the bilayer of the liposomes for theragnostic imaging are usually in a gel state. Cholesterol and/or PEG is added to the bilayer to enhance the liposomal properties. The drugs and genetic material that are carried by these theragnostic liposomes can be used in different therapy modalities (Bozzuto and Molinari, 2015; Choi et al., 2016).

Table 3
Commercially-approved drug transport liposomes.

Drug	Application	Approval	Reference
Doxorubicin	Ovarian and breast cancer	1995	(Bozzuto and Molinari, 2015; Çağdaş et al., 2014; Goins, 2008; Lamichhane et al., 2018; Man et al., 2019; Maurer et al., 2001; Medina et al., 2004b; Pattni et al., 2015; Petersen et al., 2012; Same et al., 2016; Sercombe et al., 2015; van der Geest et al., 2016)
Daunorubicin	HIV-associated Kaposi sarcoma	1996	(Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Maurer et al., 2001; Medina et al., 2004b; Pattni et al., 2015; Sercombe et al., 2015)
Verteporfin	Molecular degeneration	2000	(Pattni et al., 2015; Sercombe et al., 2015)
Vincristine	Non-Hodgkin lymphoma	2012	(Medina et al., 2004b; Pattni et al., 2015; Sercombe et al., 2015; van der Geest et al., 2016)
Amphotericin B	Antifungal	1997	(Bozzuto and Molinari, 2015; Çağdaş et al., 2014; Lamichhane et al., 2018; Maurer et al., 2001; Medina et al., 2004b; Pattni et al., 2015; Sercombe et al., 2015)
Epaxal	Hepatitis vaccine		(Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Maurer et al., 2001; Pattni et al., 2015; Sercombe et al., 2015)
Inflexal V	Influenza vaccine		(Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Maurer et al., 2001; Pattni et al., 2015; Sercombe et al., 2015)
Cytarabine	Meningitis		(Bozzuto and Molinari, 2015; Pattni et al., 2015; Sercombe et al., 2015)
Bortezomib	Myeloma		(Sercombe et al., 2015)
Morphine sulfate	Pain	2004	(Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Man et al., 2019; Maurer et al., 2001; Medina et al., 2004b Pattni et al., 2015; Sercombe et al., 2015; van der Geest et al., 2016)

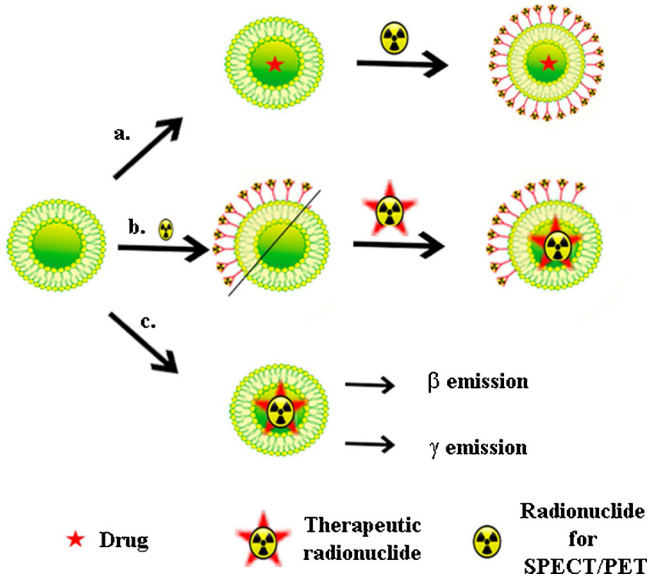

Fig. 3. General schemes for labeling theragnostic liposomes.

The radionuclides most used with theragnostic liposomes (Fig. 3 and Table 1) are 67,68Ga, ^{99m}Tc, ^{111}In and ^{64}Cu; these radionuclides have been the most used for SPECT or PET imaging. ^{131}I, ^{177}Lu, and ^{186,188}Re are used as direct theragnostic agents because their β radiation is suitable for therapy, although the γ radiation that is emitted does not produce a sharp SPECT image. It is worth mentioning that dual labeling with ^{64}Cu/^{177}Lu has also been employed (Ehlerding et al., 2016).

2.6. Remarks on liposomes

Of all the drug-transporting nanoparticles, liposomes have been the most studied. The possibility of controlling its size, shape, charge and composition in order to target a specific tissue, with established accumulation and elimination times, allows molecules of different physicochemical and biological properties to be coupled both in the aqueous core and in the bilayer or on the surface of it; thus they are currently considered the best drug carriers (Bozzuto and Molinari, 2015; Man et al., 2019). Drugs of varied nature (anticancer, antibiotics, hormones, vaccines, peptides, antibodies, nucleic acids) have been inserted into the liposomes (Bozzuto and Molinari, 2015; Gaddy et al., 2015; Lamichhane et al., 2018; Maurer et al., 2001; Mougin-Degraef et al., 2007; Sercombe et al., 2015; van der Geest et al., 2016) that are applied in a wide variety of clinical situations; particularly in cancer therapy, inflammation and infection. Since they are composed of biological molecules, their toxicity is low and they are biodegradable (Bozzuto and Molinari, 2015; Lamichhane et al., 2018; Man et al., 2019; Maurer et al., 2001; Medina et al., 2004a; Sercombe et al., 2015).

Liposomes are not agents with specific recognition. Its natural accumulation in tumors is mediated by EPR. However, molecules for specific recognition can be conjugated to their surface; a reason for why they are still a very attractive option. Whenever liposomes are used as transport systems for therapeutic agents, there will be a need to radiolabel them. With the development of new radionuclide production methods, there will be new alternatives and applications of nano-systems for imaging and therapy based on radiolabeled liposomes.

3. Radiolabeled lipoproteins

3.1. Lipoproteins

Lipoproteins are supramolecular complexes of lipids and proteins with different sizes and densities. These complexes have a hydrophobic core formed by triglycerides and cholesterol esters, covered by phospholipids and free cholesterol. On the phospholipid monolayer, apolipoproteins are interspersed, which participate in particle stabilization and in the specific recognition of cell surface receptors (Almer et al., 2015; Chaudhary et al., 2019).

Due to their specific recognition, high compatibility, stability and safety, they have become bionic transporters that are used for diagnosis and therapy. Unlike liposomes, they can circulate for an extended period of time in the bloodstream without being captured by the ERS or recognized by the immune system (Lacko et al., 2015; Mooberry et al., 2016; Raut et al., 2018a, 2018b; Sabnis et al., 2017). Their small size allows them to reach the tumors and bind to tumor receptors easily (Amin and Amin, 2018; Bozóky, 2003; Isaac-Olivé et al., 2019; Jasanada et al., 1996; Lees and Lees, 1991; Ng et al., 2011; Ponty et al., 1993).

According to their size, composition and density, lipoproteins are divided into four main types: (1) chylomicrons (CM), (2) very low-density lipoproteins (VLDL), (3) low-density lipoproteins (LDL) and (4) high-density lipoproteins (HDL). The composition and specific function varies from one type to another (Bozóky, 2003; Bricarello et al., 2011; Ng et al., 2011). There is a fifth type, an intermediate-density lipoprotein (IDL), which is a product of the metabolism of VLDL and has a very short lifespan because it transforms rapidly into LDL (Bozóky, 2003; Bricarello et al., 2011).

Lipoproteins have been acquired by their isolation from human or

animal plasma; they have been prepared from natural and recombinant proteins, and natural and synthetic lipids, and they have been synthesized from their chemical components (reconstituted lipoproteins). If the surface is modified with the idea of redirecting them, then they are called *modified lipoproteins*. Recombinant, reconstituted and modified lipoproteins are not exactly the same as the endogenous type, but resembles the behavior of their counterpart; therefore, they are biomimetic nanocarriers called *lipoprotein-like nanoparticles* (Amin and Amin, 2018; Raut et al., 2018a, 2018b; Rensen et al., 2001).

In principle, any lipoprotein-like nanoparticle can be used as a transporter of substances for diagnosis and therapy; however, LDL and HDL have been the most used. A variety of agents, including radionuclides, have been inserted for the preclinical detection of various pathologies, such as cancer, atherosclerosis and adrenocortical dysfunction (Amin and Amin, 2018; Bozóky, 2003; Bricarello et al., 2011; Davies et al., 2004; Huettinger et al., 1984; Isaac-Olivé et al., 2019; Lees et al., 1988, 1985; Ng et al., 2011; Pérez-Medina et al., 2016; Perez-Medina et al., 2015; Ponty et al., 1993; Rosen et al., 1990). Radiolabeling lipoproteins different to LDL or HDL is less common. Huettinger (Huettinger et al., 1984) used ^{123}I-VLDL for the visualization of the hepatic LDL receptor in mice and recently, Paulus (Paulus et al., 2019) employed ^{18}F-CM for the imaging of the brown adipose tissue. The choice of which lipoprotein is radiolabeled and the radiolabeling site depends on the characteristics of the molecule or ion to be inserted, the composition of the lipoprotein and the purpose of the study.

3.1.1. LDL function

Native LDL is a quasi-spherical particle whose diameter range is between 19 and 25 nm. It is the main transporter of plasma cholesterol to tissues (Bozóky, 2003). An inverse relationship between the size of the lipoprotein and its ability to cross the endothelial barrier and enter the arteries has been found (Hill et al., 2010). Hence, LDL is the main atherogenic lipoprotein; particularly, the oxidized LDL form (Ishino et al., 2008; Iuliano et al., 1996; Shaish et al., 2001). LDL accumulates inside atherosclerotic plaques and plays an important role in plaque rupture and thrombus formation (Bozóky, 2003; Bricarello et al., 2011; Davies et al., 2004; Rosen et al., 1990; Shaish et al., 2001; Sobal et al., 2006).

Apo-B100 of the LDL interacts with the specific receptors of LDL (LDL-R), located on the cell surface of many tissues, including the liver. (Huettinger et al., 1984; Moerlein et al., 1991; Vallabhajosula et al., 1988). This lipoprotein is internalized in cells by receptor-mediated endocytosis due to ionic interactions between the highly-cationic binding sites of the receptor and the anionic binding groups of the cell surface (Almer et al., 2015; Hill et al., 2010). Once inside the cell, LDL breaks down into its protein and lipid components, while excess free cholesterol is re-esterified by acyl-CoA-cholesterol acyltransferase (ACAT) for intracellular storage (Carvajal, 2014). LDL-R is recycled and returns to the cell surface to participate in another receptor-ligand interaction (Carvajal, 2014). Since many tumors overexpress LDL-R, LDL can be captured by them. In addition to the mechanism of interaction with LDL-R, LDL can be captured and degraded by the macrophages of the ERS (Huettinger et al., 1984; Vallabhajosula et al., 1988). The internalization of LDL in tumor cells, mediated by the overexpression of the LDL-R, makes the LDL-R a molecular target and LDL a suitable specific-recognition particle for transporting imaging and therapeutic agents.

3.1.2. HDL function

Native HDL ranges from 7 to 13 nm in size. It is responsible for the reverse transport of cholesterol from tissues to the liver, where it is incorporated through a specific Apo-A1 receptor called *SR-B1* (Bozóky, 2003; Isaac-Olivé et al., 2019). Due to this function, HDL has atheroprotective properties, since it promotes the flow of cholesterol from the macrophages of the atherosclerotic plaques to the liver for excretion (Jung et al., 2014; Murphy et al., 2009; Pérez-Medina et al., 2016). It has been reported that, in addition to cholesterol, it carries other substances; thus, it has a multifaceted role in intercellular communication (Kuai et al., 2016), as well as anti-inflammatory and anti-oxidative properties (Frias et al., 2007; Murphy et al., 2009).

The specific HDL receptor, SR-B1, is present in the hepatocyte plasma membrane (Lacko et al., 2015; Mooberry et al., 2016; Raut et al., 2018b, 2018a; Sabnis et al., 2017). However, unlike LDL, when HDL interacts with SR-B1, it does not internalize or break down in the cell, but rather returns to the bloodstream in order to acquire more cholesterol (Rensen et al., 2001). Like LDL, the HDL content is lower in patients with certain types of cancer, apparently due to an increase in the expression of SR-B1 receptors in some types of cancer cells, such as uterine, breast, and some lung cancer lines that produce a decrease in HDL blood levels (Isaac-Olivé et al., 2019; Ng et al., 2011; Pérez-Medina et al., 2016), which is a good way to diagnose tumors by imaging, and to direct therapies.

Unlike LDL, which, from its formation to degradation, has a single morphology, HDL has more than one. Native HDL biosynthesis has been well-explained by McMahon (McMahon et al., 2015) and Resen (Rensen et al., 2001). This begins with the secretion of Apo-A1 in the hepatocyte of the liver and enterocyte of the small intestine. This Apo-A1 is poor in lipids and begins to trap phospholipids and free cholesterol, forming a discoidal structure less than 8 nm in size, called *nascent HDL*. The captured free cholesterol is esterified by the action of the enzyme lechitin cholesteril acyltransferase, and gains hydrophobicity. Consequently, the esterified cholesterol lodges in the core of the HDL, increasing its size and changing its morphology to a spherical shape. This spherical-shaped HDL is known as *mature HDL* and reaches a size of 13 nm. Mature HDL selectively delivers the esterified cholesterol to the hepatocyte *via* the SR-B1 receptor.

The SR-B1 receptor is overexpressed in several cancer cell lines and is considered a molecular target. The recognition of the SR-B1 receptor by HDL is through interactions of the SR-B1 with the Apo-A1 apoprotein. The SR-B1 receptor recognizes both nascent (discoidal) HDL and mature (spherical) HDL and thus, these are excellent transporters of imaging and therapeutic agents. The difference in the hydrophobic properties of the nascent and mature HDL cores allows the transport of imaging and therapeutic agents with different hydrophobic or hydrophilic properties.

3.2. Strategies for labeling lipoproteins

3.2.1. Labeling on the lipoprotein surface

Depending mostly on the purpose of the study, radionuclides can be inserted into lipoproteins in both the surface membrane and the inner core. Two routes are used to insert radionuclides into the surface membrane of lipoproteins: (1) covalent modification of phospholipids or surface apolipoprotein or (2) non-covalent intercalation in phospholipids of an amphiphilic agents. The structure of this agent is partially “buried” on the surface of the lipoprotein, leaving the hydrophilic part exposed to the aqueous environment (Ng et al., 2011).

For covalent modification (Fig. 4a), the molecule or ion to be inserted is conjugated either to the lysine, arginine, tyrosine or cysteine residues of the apolipoprotein, or to the main phospholipid of the surface membrane (Amin and Amin, 2018; Atsma et al., 1993; Chang et al., 1993; Huettinger et al., 1984; Isaacsohn et al., 1986; Lees et al., 1985; Moerlein et al., 1991; Ng et al., 2011; Pérez-Medina et al., 2016; Shaish et al., 2001; Sobal et al., 2006; Tietge et al., 2004; Vallabhajosula et al., 1988). For this modification, various methodologies are used, such as the reduction of the disulfide bridges of a protein (Sobal et al., 2006) or the synthesis of lipoprotein nanoparticles with phospholipids that subsequently bind to a molecule, which can then be attached to the radionuclide or to the imaging agent of interest (Moerlein et al., 1991; Shaish et al., 2001). This method has been used to insert radionuclides such as ^{59}Fe (Jung et al., 2014), ^{68}Ga (Moerlein et al., 1991), ^{99m}Tc (Atsma et al., 1993; Bozóky, 2003; Lees et al., 1988,

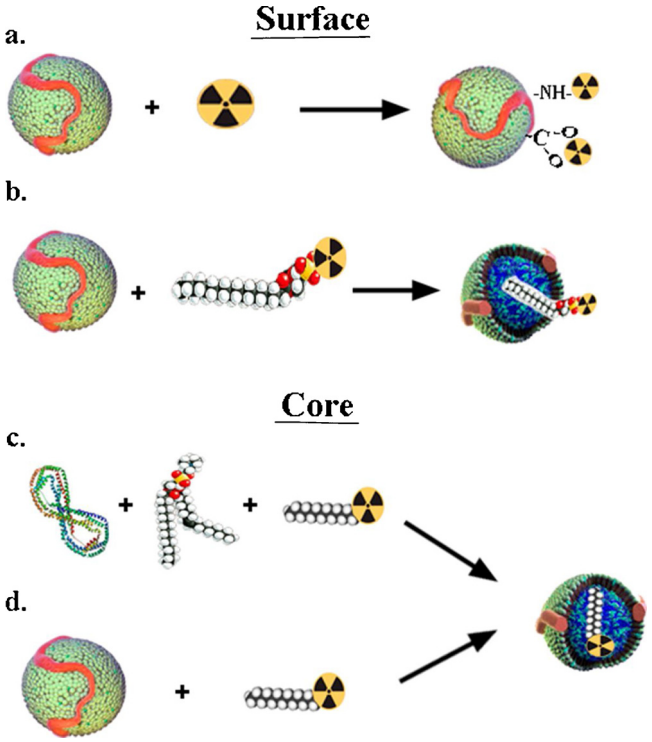

Fig. 4. Strategies for lipoprotein labeling in the membrane and core. The lipoprotein represented in the figure is a generic one, which can be considered CM, VLDL, LDL, discoidal HDL or mature HDL.

1985; Ponty et al., 1993; Sobal et al., 2006; Vallabhajosula et al., 1988), ^{111}In (Chang et al., 1993; Moerlein et al., 1991; Rosen et al., 1990; Tietge et al., 2004) or ^{123,125,131}I (Chang et al., 1993; Huettinger et al., 1984; Lees et al., 1985; Ponty et al., 1993; Shaish et al., 2001; Terpstra et al., 1989; Vallabhajosula et al., 1988) into VLDL or LDL.

For labeling with ^{99m}Tc, $^{99m}TcO_4^-$ obtained from the ^{99}Mo-^{99m}Tc generator, is reduced with sodium dithionite (Atsma et al., 1993; Bozóky, 2003; Isaacsohn et al., 1986; Lees et al., 1985; Ponty et al., 1993; Sobal et al., 2006; Vallabhajosula et al., 1988) or with $SnCl_2$ in the presence of sodium borohydride (Atsma et al., 1993; Sobal et al., 2006). Iodine radionuclide labeling (^{123}I, ^{125}I, ^{131}I) is performed using iodine monochloride (Chang et al., 1993; Huettinger et al., 1984; Lees et al., 1985; Ponty et al., 1993; Shaish et al., 2001; Vallabhajosula et al., 1988) and iodogen (Shaish et al., 2001). In the case of ^{68}Ga and ^{111}In, diethylenetriamine pentaacetic acid (DTPA) or its bicyclic anhydride has been used as a bifunctional chelator (Moerlein et al., 1991; Rosen et al., 1990), while labeling with ^{89}Zr has been carried out by incorporating DF to the lysine residues of the apolipoprotein of the HDL (Pérez-Medina et al., 2016). The disadvantage of the covalent modification method is that the modifications cannot be considerable, since the *in vivo* behavior of the labeled lipoprotein may be affected (Bricarello et al., 2011).

For non-covalent intercalation (Fig. 4b), molecules, such as fatty acids, have been used. They are modified with chelators, which enables the binding of a radionuclide to the phospholipids by means of weak interactions. Radionuclides, such as ^{99m}Tc (Ponty et al., 1993), ^{125}I (Shaish et al., 2001; Xiao et al., 1999) and ^{131}I (Vallabhajosula et al., 1988), have been intercalated in this manner.

3.2.2. Labeling in the inner core

The inner hydrophobic core of lipoproteins is ideal for inserting poorly-soluble drugs or imaging agents. The insertion into the inner core can be performed *via* two methods: (1) loading the drug during the synthesis of the lipoprotein (Lacko et al., 2015; Mooberry et al., 2016; Raut et al., 2018a, 2018b; Sabnis et al., 2017), as represented in Fig. 4c,

Table 4
Labeling of lipoproteins with radionuclides.

Lipoprotein/ Radionuclide	Labeling site/method						
	Membrane/ Covalent modification			Membrane/ Non-covalent insertion	Within the core/lipid exchange		
	VLDL	LDL	HDL	LDL	CM	LDL	HDL
^{59}Fe							(Jung et al., 2014)
^{68}Ga		(Moerlein et al., 1991)					
^{89}Zr			(Perez-Medina et al., 2015; Pérez-Medina et al., 2016; Senders et al., 2020)				(Senders et al., 2020)
^{99m}Tc		(Atsma et al., 1993; Bozóky, 2003; Isaacsohn et al., 1986; Ishino et al., 2008; Iuliano et al., 1996; Lees et al., 1988, 1985; Sobal et al., 2006; Vallabhajosula et al., 1988; Vallabhajosula and Goldsmith, 1990) (Chang et al., 1993; Jasanada et al., 1996; Moerlein et al., 1991; Rosen et al., 1990; Tietge et al., 2004)		(Ponty et al., 1993)			(Isaac-Olivé et al., 2019)
^{111}In				(Jasanada et al., 1996)			
^{123,125,131}I	(Huettinger et al., 1984)	(Atsma et al., 1993; Chang et al., 1993; Lees et al., 1985; Moerlein et al., 1991; Ponty et al., 1993; Shaish et al., 2001; Vallabhajosula et al., 1988)		(Shaish et al., 2001; Xiao et al., 1999)		(Terpstra et al., 1989)	(Terpstra et al., 1989)
^{18}F					(Paulus et al., 2019)		

or (2) loading the lipoprotein core with hydrophobic molecules after lipoprotein preparation (Almer et al., 2015; Isaac-Olivé et al., 2019), as represented in Fig. 4d. Depending on the hydrophilicity/hydrophobicity of the drug to be loaded, the lipoprotein is modified by reconstitution or recombination with a polar solvent or surfactant, and then the desired agent is introduced (Ng et al., 2011; Terpstra et al., 1989; Xiao et al., 1999). The loading phase of the lipoprotein core has been used to introduce different therapeutic drugs (Ng et al., 2011) and, to a lesser extent, for HDL labeling with ^{99m}Tc (Isaac-Olivé et al., 2019) and ^{59}Fe (Jung et al., 2014).

Both labeling on the membrane and the inner core have advantages and disadvantages. Labeling in the inner core or on membrane phospholipids is the most favorable because the labeled product is more stable *in vivo*. In the case of therapeutic agents, toxicity and side effects are reduced (Almer et al., 2015). However, unlike liposomes, which have been labeled with various radionuclides, lipoproteins and lipoprotein-like nanoparticles have been labeled with a small number of them. Table 4 shows the radionuclides currently used for the labeling of these native and biomimetic nanoparticles, as well as the different strategies and methods employed for each one of them. Among the lipoproteins and lipoprotein-like nanoparticles, LDL has been the most radiolabeled, followed by HDL and, to a lesser degree, VLDL and CM.

3.3. Quality control of radiolabeled lipoproteins

A radiolabeled lipoprotein is a radiopharmaceutical and, as in the case of radiolabeled liposomes, any radiopharmaceutical that is intended for *in vivo* use ultimately requires quality control. The criteria and methods for quality control of radiolabeled lipoproteins are the same as those mentioned for liposomes. The physicochemical characterization is performed in the same manner as that of the liposomes and is summarized in Table 2. In the case of composition, total proteins are determined by the Lowry method, (Chang et al., 1993; Lees et al., 1985; Ponty et al., 1993; Rosen et al., 1990; Terpstra et al., 1989) Biuret (Terpstra et al., 1989) or with the bicinchoninic acid (BCA) test kit (Isaac-Olivé et al., 2019; Sobal et al., 2006). Phospholipids, cholesterol and triglycerides are determined by means of an enzymatic reaction (Bozóky, 2003; Isaac-Olivé et al., 2019; Sobal et al., 2006; Terpstra et al., 1989).

3.4. Applications of labeled lipoproteins

The targeting mechanism of LDL and HDL, mediated by their respective receptors, is the main reason for enhancing the cellular uptake, unlike liposomes, and is also the basis to use them as nanocarriers for therapeutic drugs (Almer et al., 2015; Bricarello et al., 2011; Kuai et al., 2016; Murphy et al., 2009) and contrast agents for magnetic resonance imaging (MRI) (Almer et al., 2015; Amin and Amin, 2018; Bricarello et al., 2011; Jung et al., 2014; Sabnis et al., 2017), CT (Almer et al., 2015), fluorescence imaging (Almer et al., 2015) and theragnostic imaging (Almer et al., 2015; Ng et al., 2011). Their hydrophobic core allows the incorporation of poorly-soluble molecules, as well as the bioconjugation to different types of molecules (Ng et al., 2011).

LDL has been labeled with ^{99m}Tc, ^{111}In, and ^{123}I for the visualization of atherosclerotic plaques (Atsma et al., 1993; Chang et al., 1993; Davies et al., 2004; Ishino et al., 2008; Iuliano et al., 1996; Lees et al., 1988; Rosen et al., 1990; Shaish et al., 2001; Xiao et al., 1999), tumors (Almer et al., 2015; Bozóky, 2003; Jasanada et al., 1996; Ponty et al., 1993; Zheng et al., 2005), the adrenal cortex (Huettinger et al., 1984; Isaacsohn et al., 1986; Lees et al., 1985) and the liver (Huettinger et al., 1984), among other applications.

The ^{99m}Tc-labeled HDL has been used to visualize sites that overexpress the SR-B1 receptor, such as liver and malignant tumors (Isaac-Olivé et al., 2019). It has been labeled with ^{59}Fe for preclinical studies of pharmacokinetics and biodistribution of superparamagnetic iron hydroxide for MRI (Jung et al., 2014). Labeling with ^{89}Zr (Perez-Medina et al., 2015; Pérez-Medina et al., 2016) and ^{125}I (Shaish et al., 2001) has been used to visualize arteriosclerotic lesions and tumor-associated macrophages. The advantage of HDL is that, depending on the hydrophobicity / hydrophilicity of the agent to be carried, they can be prepared nascent (discoidal) (Perez-Medina et al., 2015; Pérez-Medina et al., 2016) or mature (spherical) (Isaac-Olivé et al., 2019; Lacko et al., 2002). Both types of HDL have been radiolabeled; Pérez-Medina (2015, 2016) has radiolabeled the discoidal type with ^{89}Zr (Perez-Medina et al., 2015; Pérez-Medina et al., 2016), while Isaac-Olivé (2019) has radiolabeled the spherical variant with ^{99m}Tc (Isaac-Olivé et al., 2019). These nanoparticles show different drug-loading capacity and tumor-specific targeting (Kannan Mutharasan et al., 2016; Lacko et al., 2007); therefore, they offer different options for different applications.

Depending on HDL composition and the route of administration, it accumulates in kidneys (Isaac-Olivé et al., 2019; Pérez-Medina et al., 2016), liver (Isaac-Olivé et al., 2019; Jung et al., 2014; Pérez-Medina et al., 2016), spleen (Isaac-Olivé et al., 2019; Jung et al., 2014), heart (Shaish et al., 2001), aorta (Murphy et al., 2009; Shaish et al., 2001), lungs (Isaac-Olivé et al., 2019; Shaish et al., 2001) and atherosclerotic plaques (Jung et al., 2014; Murphy et al., 2009; Pérez-Medina et al., 2016; Shaish et al., 2001), with a pharmacokinetic behavior superior to that of LDL.

Although the advantages of imaging techniques such as SPECT and PET are very well-known, and the possibility of lipoproteins to reach the tumors better than liposomes is also known, there are fewer works on radiolabeled lipoproteins than liposomes. Lipoproteins are very attractive as drug carriers (Almer et al., 2015; Murphy et al., 2009), therefore radiolabeling them is an excellent option for obtaining theragnostic nanoparticles. The preparation of theragnostic lipoproteins is similar to that for liposomes, as represented in Fig. 3. It also has the possibility of linking specific molecules, which allows the design of many different nanoparticles, while keeping the specific recognition for cell receptors such as SR-B1 and LDL-R. The over-expression of these receptors in cancer cells makes lipoproteins excellent specific-recognition agents.

3.5. Remarks on lipoproteins

Lipoprotein-like nanoparticles resemble the native, endogenous lipoproteins; for this reason, their immunogenicity is low, their recognition by SR-B1 and LDL-R is high and their participation in metabolic processes is similar to that of their native counterparts. These characteristics, together with the ability to internalize the compounds that are carried, make them ideal systems for the transport of imaging and therapy agents. Their chemical structure allows for various radiolabeling strategies and methods. Radiolabeling of these lipoprotein-like nanoparticles will soon represent a necessity for the development of drug transport and delivering systems, since radiolabeling automatically converts them into theragnostic systems, which are of great importance for monitoring the response to treatments.

4. Conclusion and future perspectives

Due to their versatility, biocompatibility, low toxicity, among other notable properties, lipid nanoparticles (liposomes and lipoproteins) have proven to be excellent transporters of agents for imaging and therapy. Radiolabeling strategies with imaging radionuclides (γ or β^+ emitters) improve their clinical potential. They could also be imaging tools, or useful for identifying patients amenable to targeted therapy. Combining both the specific drug-delivery capability of these nanoparticles and their potential as radiopharmaceuticals for molecular imaging, they may also be used in the development of new theragnostic nanoplatforms.

Lipid nanoparticles can be manipulated to vary their architecture and physicochemical properties. They have been labeled with

radionuclides; the labeling strategies depend mostly on the aim of the study. If the aim is to know the *in vivo* distribution and to observe the accumulation sites of the liposomes or lipoproteins, membrane (surface) labeling is suggested, but if the determination of the accumulation and / or pharmacokinetics of the encapsulated molecule is required, it is preferable to encapsulate the radionuclide (free or chelated) within the inner core.

Liposomes have been radiolabeled with many of the imaging radionuclides available, being ^{99m}Tc, ^{111}In and ^{64}Cu the most used. They have also been labeled with therapeutic radionuclides for oncological applications, such as ^{90}Y, ^{213}Bi and ^{225}Ac. Even though only a few formulations have been evaluated in humans in comparison with the large number of preclinical studies conducted. The reasons why radiolabeled liposomes have not reached a clinical stage in research, are not yet clear.

Although lipoproteins have better specific recognition, cell uptake and superior physiological properties than liposomes, they have been radiolabeled much less and no clinical study has been approved or carried out as of yet. LDL has been radiolabeled since the 80 s and is the most-radiolabeled lipoprotein. HDL was radiolabeled on very few occasions before 2014; from that year until today, the number of works, involving radiolabeling, has been slowly increasing. VLDL was radiolabeled in 1984 and CM in 2019. Most of the radionuclides employed for lipoprotein radiolabeling are for imaging. This point, along with their capacity to carry other agents, including drugs, makes them excellent theragnostic nanoplatforms.

Except for ^{131}I, therapeutic radionuclides have not been transported by lipoproteins yet. In the near future, the efficacy of lipoproteins for the transport of therapeutic radionuclides (α and β- emitters) should be evaluated. Given the various type of lipoproteins and various chemical forms that may be encapsulated, a large field in therapeutic radiopharmaceuticals, based on lipoproteins, is waiting to be explored.

Usually, the quality control of radiolabeled lipidic nanoparticles (liposomes and lipoproteins), at a preclinical level, includes *in vitro* serum stability, pharmacokinetics and/or biodistribution studies and *in vivo* imaging. However, for the application of radiolabeled lipidic nanoparticles in humans, the guarantee of the compliance with the requirements imposed by Good Manufacturing Practices is obligatory; thus, it is necessary to design simple production processes with specific quality control. This could be another reason why these nanoparticles have not reached the clinical stage yet.

The requirements for preclinical studies related to toxicology, long-term stability and *in vivo* behavior of radiolabeled lipid nanoparticles, including dosimetry, are in many cases, more stringent than for traditional drugs. These problems are joined by those related to intellectual property, which often extend the start-up periods of commercialization. Greater interaction between scientists, the industry and health services are essential to accelerate the stages of escalation of production processes in order to introduce these lipid nanoparticles into daily clinical practice.

Soon, radiolabeling liposomes and lipoproteins that carry a therapeutic drug will be modified or functionalized to prepare multimodal and multimeric therapeutic systems that combine radiotherapy/chemotherapy/photo-dynamic therapy/thermotherapy/biological therapy and will even react to an exogenous stimuli (a magnetic field, ultrasound waves, an electric field, a temperature change) or endogenous stimuli (pH change, enzyme transformation, temperature and redox reactions). These systems are expected to be more effective in the treatment of disease and should boost personalized medicine. The development of such systems requires platforms where different moieties and agents (including radionuclides) may be transported at once; lipidic nanoparticles such as liposomes and lipoproteins seem to be ideal candidates for this purpose.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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