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Design of Hollow Nanocapsules and their Applications in Biomedical Imaging

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Abstract *Different nanocarrier systems owing to their nano size, increased surface area and unique electrical and magnetic properties are employed for biomedical applications. Among the various nanocarrier systems, hollow nanocapsules are one of the most interesting carrier systems for thier use in bioimaging and biomedical applications. The unique properties of hollow nanocapsules make them suitable for use in bioimaging. In this article hollow nanocpsules have been classified into four classes based on thier composition: (i) liposomes, (ii) polmersomes and other polymeric hollow nanocapsules, (iii) metallic hollow nanocapsules and (iv) others. The formation and design of these hollow nanocapsules and their diagnostic and therapeutic applications using different agents/imaging probes and imaging modalities is discussed briefly. Hollow nanocapsules have revolutionized the field of biomedical imaging.*

Key Words: Biomedical Applications, Bioimaging, Hollow Nanocapsules, Imaging Probes, Nanocarriers

List of Abbreviations

CT	Computed Tomography
MRI	Magnetic Resonance Imaging
FLI	Fluoresence Imaging
PET	Positron Emission Tomography
USI	Ultrasound Imaging
PAI	Photoacoustic Imaging
DOTA	Dodecane Tetraacetic Acid
Lip-Mel	Melanin Based Liposomes
I.V.	Intravenous
HPNs	Hollow Polymeric Nanocapsules
SNCs	Silica Nanocapsules
QDs	Quantum Dots
PLGA	Poly (Lactic-Co-Glycolic Acid)
BPLP	Biodegradable and Photoluminescent Polyester
SPIONs	Supramagnetic Iron Oxide Nanoparticles

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Introduction

Measuring, characterizing, and visualizing the biological processes in living systems at cellular or molecular level
is termed as molecular imaging. The imaging technique utilized to envision the biological process instantaneously with little obstruction in biological processes is bioimaging (Dergunov & Pinkhassik, 2014). Different types of bioimaging techniques like ultrasound, computed tomography (CT), fluoresence imaging, magnetic resonance imaging (MRI) and positron emission tomography (PET) using ultrasound, X-rays, fluorescence, magnetic resonance and positron respectively, as a light source, are available for diagnosis of different diseases(Rani, Sethi, & Singh, 2019).

The basic concept of bioimaging is based on acheiving high signal strength using small quantity of molecular probe. Biomedical imaging can be applied for imaging of multiple processes occuring at the same time, measuring interactions of proteins, quantification of various metabolites and ions, determining the disease prognosis, visulaizing the effect of drug at target site, tracing the therapy producing 3-D images(Silindir, Erdoğan, Özer, & Maia, 2012).

Various imaging probes for different imaging modalities have been developed but the major issues encountered with imaging is the delivery of imaging probes across the biological barriers and establishing highly sensitive imaging tools with good resolution. Bionanotechnology has led to the development of variety of nanostructures addressing these issues. Recently, various bionanomaterials have been developed which serve as excellent candidates for imaging probe delivery to the target site with improved efficacy and safety enhancing the quality of imaging techniques owing to their unique properties like small size, high surface area, optical emission properties and unique electrical and magnetic behaviour(Rani et al., 2019; Silindir et al., 2012).

Different nanomaterials like nanoparticles, carbon nanotubes, fullerenes, graphene and quantum dots have been used in biomedical imaging producing high quality images due to their unique characteristics and aided in better disease detection, therapeutic response estimation and drug delivery to target site(Xia et al., 2019). Among these nanomaterials are the hollow nanocapsules.

Hollow nanocapsules are nanosized capsules, interior of these nanocapsules is a hollow space, as indicated by the name, which can be filled with any substance, a therapeutic agent or an imaging probe. The important characteristic of nanocapsules is the compatibility of the material introduced into the hollow space and the core of nanocapsule. Moreover, the nanocapsules safegaurd the entrapped material from external biological surroundings and the biological environment from the harmful effects of the incorporated material. Thus, hollow nanocapsules have various applictions like in drug delivery, therapeutic applications, diagnostic applications etc. Hollow nanocapsules also find applications in biomedical imaging due to their distinctive properties. They are potential carriers for various imaging probes offering minimal adverse effects and optimal efficacy and safety and are therefore, used widely in biomedical imaging (Dergunov & Pinkhassik, 2014).

Types of Hollow Nanocapsules

Based on the composition and mechanism of formation, hollow nanocapsules are classified as follows,

- Liposomes
- Polymersomes and other polymeric hollow nanocapsules
- Metallic hollow nanocapsules
- Others (Jang, Yim, & Hwang, 2014)

Liposomes

Spherical vesicles composed of phospholipid bilayers are termed as liposomes (Akbarzadeh et al., 2013). These lipid-based vesicles are biocompatible, non-immunogenic, non-toxic and have longer blood circulation which allows them to act as a good carrier for the imaging probes used in different imaging modalities and improving their quality. Liposomes with an imaging probe can be manufactured by various methods used for liposome formation. The imaging probe can be introduced into liposome either during its formation or after the formation of liposome i.e. passive loading or active loading of probe respectively (Xia et al., 2019).

Passive Loading

It involves the introduction of the probes into the aqueous or lipid phase, based on the hydrophilicity of the probes, during liposome synthesis. Most commonly employed methods are thin fim hydration and ethanol injection method. For loading a hydrophobic probe like IR7803 using thin film

hydration method involves the introduction of the hydrophobic probe , into the organic phase followed by the removal of organic phase forming a thin lipid film with a hydrophobic probe. Then the dried lipid film is hydrated by the aqueous phase forming multilamelar liposomes which on purification yield unilamellar liposomes with a probe (Figure 1(i)). Ethanol injection method can also be used for loading hydrophobic probes (Figure 1(ii)). In this method, liposomes with hydrophobic probes are formed by direct introduction of ethanol containing lipids and hydrophobic probes into the aqueous phase drop wise. Hydrophilic probes like small molecules (ICG22) can also be loaded using the same methods i.e. thin film hydration and ethanol injection method. The procedure for loading hydrophilic probes using these methods is same as that for hydrophobic probes except the hydrphilic probe is introduced into the aqueous phase rather than the organic phase (Figure 2 (i) and (ii)). Liposomes can carry both types of probes simultaneously by incorporating the probes into the respective phase based upon their hydrophilicity. In some cases, the probes can be conjugated chemically with the lipids and introduced as hydrophobic probes into liposomes (Xia et al., 2019).

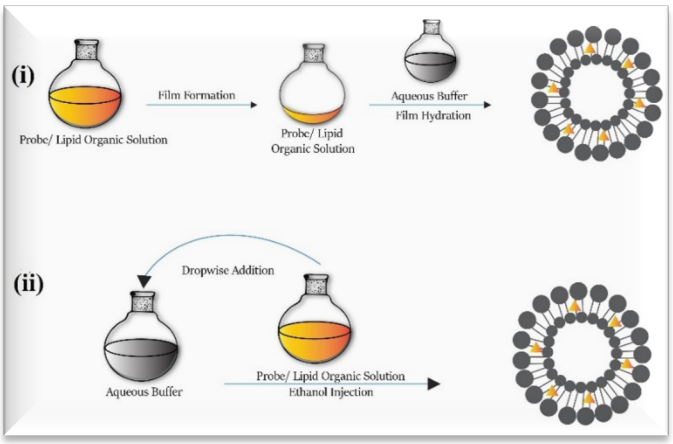

Figure 1: Passive Loading of Hydrophobic Probe by Thin film Hydration Method (i) and Ethanol Injection Method (ii)

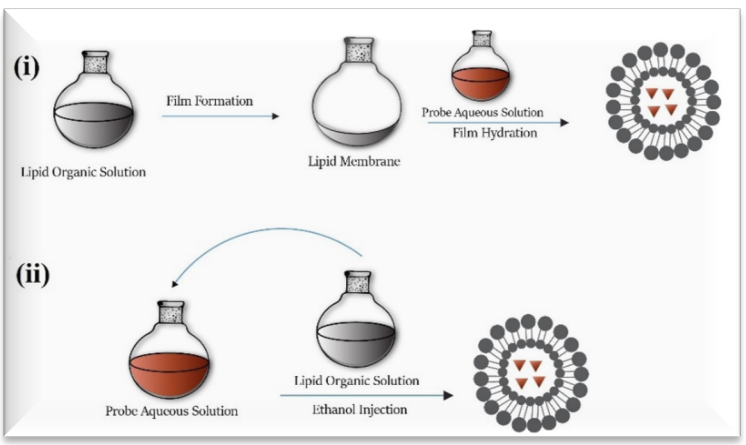

Figure 2: Passive Loading of Hydrophilic Probe by Thin film Hydration Method (i) and Ethanol Injection Method (ii)

Active Loading

It involves the incorporation of a probe after the formation of liposomes. In order to load hydrophobic probes actively, hydrophobic chelators are required that are introduced into liposomes by passive laoding. After the introduction of chelator, the solution of hydrophobic probe is mixed with the dispersion of

chelator loaded liposomes resulting in incorporation of the probe into liposomes due to strong interaction with the chelator (Figure 3). For example, porphyrin-phospholipid (chelator) loaded liposomal dispersion when mixed with 64CuCl2 solution results in active loading of copper 64. For active loading of hydrophilic probes, the hydrophilic chelators like DOTA and others are used for incorporating the probes into aqueous phase (Figure 3) (Xia et al., 2019)

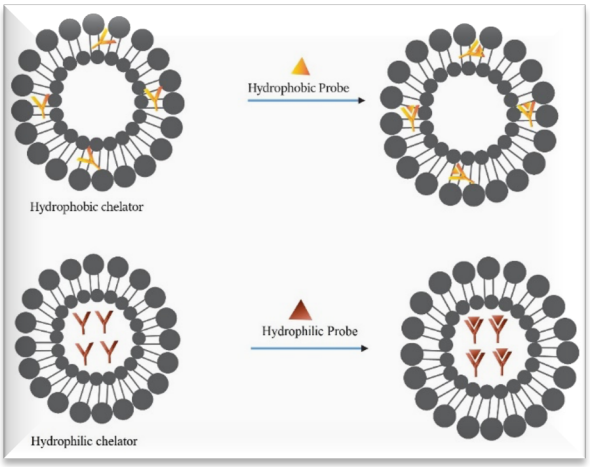

Figure 3: Active Loading of Hydrophobic and Hydrophilic Probes

Applications in Bioimaging

Liposomes based probes for different imaging modalities have been developed. Liposomes based probes for ultrasound imaging (USI), positron emission tomography (PET) and computed tomography (CT) are used for estimation of therapeutic response and disease detection. For surgical guidance fluoresence imaging (FLI) probes are suitable and drug delivery to target site can be achieved by using liposome based magnetic resonance imaging (MRI) probes.

In a study, the sustained action of ferriliposomes was evaluated using MRI of a mouse. The ferriliposomes were injected intraperitoneally and MR images were obtained at 1hour and 48 hours after injection and magnetic field was applied for 1 hour to the tumour (indicated by white arrows in figure 5. The images at 1h and 48h showed darker area at tumour site which assured the efficient targeted delivery of ferriliposomes.

In some cases, two imaging probes of different imaging modalities or a single probe with properties to be used for two different imaging modalities can be loaded in liposomes forming bimodal imaging liposome-based probes. If more than two imaging probes are loaded in liposomes, then multimodal imaging probes loaded liposomes are formed. These bimodal or multimodal imaging probes exhibit high resolution and sensitivity for detection of diseases as compared to monomodal probes for imaging. Melanin has been observed to show bimodal imaging characteristics, showing MR and photoacoustic signals. In a study, liposomes containing melanin (lip-mel) were prepared and used for imaging MDA-MB-231 cancer (human breast cancer epithelial cell lines) using photoacoustic imaging and MRI techniques before and after an I.V injection of lip-mel at different time intervals i.e. 1hour, 4hours and 24 hours. The images with lip-mel showed the progressive accumulation of melanin in the tumour (Figure 6) (Xia et al., 2019).

Polymersomes and other Hollow Polymeric Nanocapsules (HPNs)

Hollow capsules formed by the self-assembly of amphiphilic block copolymers are referred as polymersomes. They provide enhanced stability, longer blood circulation, increased penetration in tissues and targeted delivery. Polymersomes and other HPNs made up of different polymers can be used for imaging applications as a delivery system for various imaging ligands that can either be incorporated inside the polymer shell or attached to the surface of polmeric shell(Deng, Gigliobianco,

Censi, & Di Martino, 2020; Leong, Teo, Aakalu, Yang, & Kong, 2018).

Polymeric Nanocapsule Formulation

Generally, the basic process for formulation of nanocapsules is the preparation of a hollow cavity using a template which is then sacrificed after the formation of the polmer shell. Metals, inorganic materials or micelles or emulsions can be used as templates. The removal of these templates can be carried out by various processes like dissolution, evaporation, solvent etching or calcination after the formation of polmeric shell (Meier, 2000). In recent years polymer based nanocapsule preparation methods have been developed by scientist. Mainly three basic methods have been developed for the preparation of these nanocapsules. These include interfacial deposition methods, nano-emulsion method & layer by layer method. Advancements in these methods are only to improve the applications of drug targeting and delivery. Nanocapsules formed by these methods have greater potential to contribute towards the loading of drug or probe either by active or passive loading (Deng et al., 2020).

Interfacial Deposition Method

Interfacial deposition method was first described in 1989 by Fessi et al. for the fabrication of nanocapsules(Fessi, Puisieux, Devissaguet, Ammoury, & Benita, 1989). This method requires both the aqueous and organic phase. Organic phase is obtained by mixing the hydrophobic part (oil) into organic solvents and then adding it into other phase by a needle. Polymeric material that forms a film is dissolved in one of the phases depending upon its hydrophilic or hydrophobic nature (Figure 4). Stability of the nanocapsules can be enhanced by incorporating one or more surfactants. . Finally removal of the organic solvent by evaporation or diffusion results in the formation of nanocapsules. The key characteristics of resultant nanocapsules formed are typically dependent upon the amount of polymer, volume ratio between the two phases and method employed for injecting the organic phase. Interfacial deposition method is widely used due to its simplicity,less energy expenditure and its applications for diverse payloads(Ferranti, Marchais, Chabenat, Orecchioni, & Lafont, 1999).

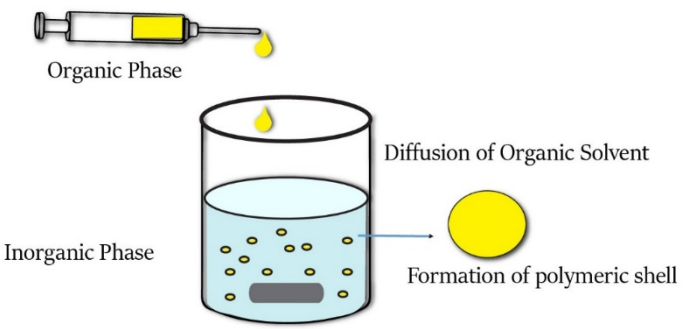

Figure 4: Formation of Hollow Nanocapsules by Interfacial Deposition Method

Nano-Emulsion Template Method

Advancements with time resulted in launch of new energetic instruments for the formation of nanoemulsion for fabrication of nanocapsules. Nano-emulsion template method uses high energy tools for the preparation of nanocapsules by using different strategies including diffusion/evaporation or monomers coacervation. Generally, nanoemulsion is formed by emulsification of either aqueous phase in organic phase or organic phase in aqueous phase using a surfactant provided with continuous energy like homogenization or sonication. A stable nanoemulsion is formed by reduction in interfacial tension acheived by self-assembly of surfactants at the interface of two phases. Film-forming polymer, active agent or other components can be dispersed or dissolved in either of the two phases depending on the nanocapsules (Anton, Benoit, & Saulnier, 2008).
Different strategies which are used for this method are explained below

Emulsion–Diffusion/Evaporation Method

Emulsion-diffusion/evaporation method is one of the most common methods employed for fabrication of polymeric nanocapsules through nanoemulsion. In this method organic phase is emulsified into aqueous

phase followed by the removal of the organic solvent by evaporation or diffusion into the external phase(Figure 5 I and II). Nanocapsules are generated by the blend of two phenomena- interfacial phenomena and precipitation of polymer during evaporation or diffusion of organic solvent(Quintanar-Guerrero, Allémann, Doelker, & Fessi, 1998). Polymers employed in this method should be soluble in organic solvents like ethanol, ethyl acetate and acetone that are miscible with water so that organic solvent is diffused into water and easily removed (Esmaeili & Niknam, 2014).

Emulsion–Coacervation Method

Emulsion-coacervation method differs from emulsion–diffusion/evaporation method in a way that in this process polymer shell is formed by chemical cross-linking or physical coacervation rather than diffusion or evaporation(Figure 5 III) (Ma, Feng, Ye, Wang, & Fan, 2001). This method is fundamentally used for monomers/polymers having cross-linking functional groups or polyectectrolytes for the formation of nanocapsules. It has been observed that chemical cross-linking may result in stable nanocapsules as compared to physical coacervation (Steinmacher et al., 2017). A thin polymeric shell is formed with increased amount of cross-linker which prevents the leakage of hydrophilic agent into the aqueous phase. Nanocapsules can also be formed by free radical polymerization. Generally, a template core is obtained by synthesizing a polymeric shell made of amphiphilic copolymer having same monomeric units. Free radical polymerization occurs as the monomer is added with initiator and cross-linker resulting in the formation of polymeric shell. Hollow structures are then obtained by removing the amphiphilic copolymer via hydrolysis (Ishizuka, Utama, Kim, Stenzel, & Zetterlund, 2015).

Double Emulsion Method

Double emulsion method is established on the fundamental basis of emulsion coacervation and emulsion diffusion/evaporation methods. The nanoemulsion formed from these two methods can be further emusified into a third phase to form double emulsion or emulsions of emulsions. Double emulsions can be oil in water in oil (O/W/O) or water in oil in water (W/O/W) type depending upon the sequence of the phases. An important aspect to be considered in double emulsion formation is the choice of surfactants to be used for stability of emulsion (Ashjari, Khoee, & Mahdavian, 2012). Evaporation, diffusion, coacervation alone or in combination can be employed for the formation of polymeric shell. Both hydrophilic and hydrophobic substances can be encapsulated simultaneously into the nanocapsules fabricated by double emulsion method (Deng et al., 2020).

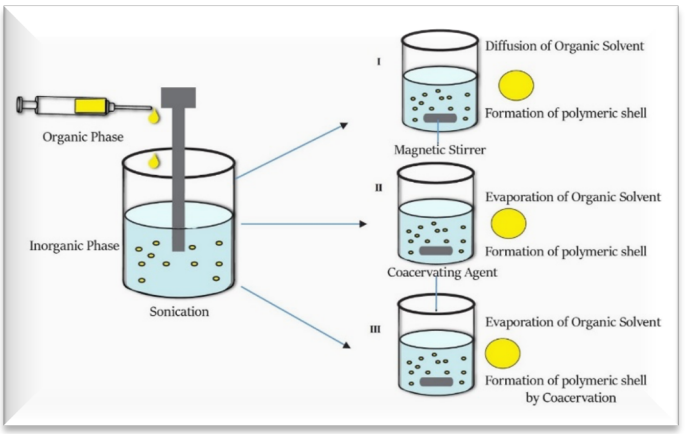

Figure 5: Formation of Nanocapsules by Nanoemulsion Method. (I) and (II) Emulsion Diffusion/Evaporation Method, (III) Emulsion-Coacervation Method

Layer-by-Layer Method

One of the most promising strategy for formation of multilayered nanocapsules is layer by layer (LBL) method. Nanocapsules are formed by the deposition of polyanioins and polycations on hydrophilic core by electrostatic attractions followed by template core

sacrification (Figure 6) (Cuomo et al., 2015). Hydrophilic substances are loaded in LBL polymer based nanocapsules by diffusion of the substance after the formation of nanocapsules while, hydrophobic subtances are loaded by hydrphobic effect or electrostatic interactions. These multilayer nanocapsules can carry various payloads to different sites simultaneously (Ledo et al., 2019). Diversified drug release patterns can be acheived in various pH environment by employing LBL polymer based nanocapsules. Thus, LBL method is useful for formulation of nanocapsules for oral drug delivery to regulate the drug release in gastrointestinal tract (Yiping Zhang et al., 2017).

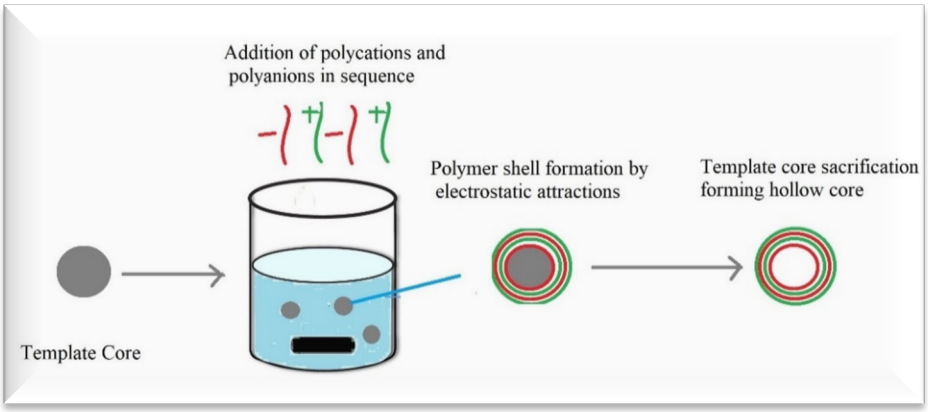

Figure 6: Formation of Nanocapsules using Layer by Layer (LbL) Method.

Application in Imaging

Polymersomes and other polymeric nanocapsules have been widely used for bioimaging applications. For example, in a study porous polmersomes encapsulating gadolinium (imaging probe) were prepared and used to visulaize kidney and bladder of a mouse using MRI before and after an I.V. injection of the formulation. The images after the injection of formulation showed better contrast (Figure 3.4)(Leong et al., 2018).Multifunctional oligomers conjugated poly (lactic-co-glycolic acid) PLGA nanocapsules incorporating SPIONs for imaging and targeting the tumor by magnetic resonance were developed by Wang and co-workers. These magnetic nanocapsules exhibited excellent in vivo and in vitro biocompatibility, stability and targeted drug delivery in addition to enhanced bioimaging properties (Wang et al., 2020).

Recently Yajie Zhang and his co-workers deveopled poly (lactic-co-glycolic acid) PLGA and BPLP (biodegradable and photoluminescent polyester) based nanocapsules with bimodal imaging probes incorporating SPIONs for imaging and targeting the tumor by magnetic resonance (Yajie Zhang, García-Gabilondo, Rosell, & Roig, 2020). In another study Yajie Zhang and his co-workers deveopled PLGA and BPLP based nanocapsules with multiple (a MRI, a PET and two FLI) imaging probes for theranostic purpose. These PLGA based nanocapsules demonstrated magnificent biosafety and imaging potential rendering them suitable for theranostic purpose(Yajie Zhang, García-Gabilondo, Grayston, et al., 2020).

Metallic Hollow Nanocapsules

Hollow nanocapsules can also be formed using different metals owing to the nano range properties and novelty in functionality of metals (Jang et al., 2014). In most of the case the metals that are used include gold, silver, bismuth, ytterbium or gadolinium. These high atomic weight metals can show better contrast as compared to conventional ones like iodine (Sadeghian, Akhlaghi, & Mesbahi, 2020).

Gold based nano hollow capsules are used for many therapeutic purposes and bioimaging like a Ce6 (a photosensitizer with anticancer activity) loaded gold nanocapsule, a vesicular assembly of gold nanoparticles, was developed for cancer treatment and imaging. These Ce6 loaded gold nanocapsules showed better contrast in-vivo fluorescent images and photoacoustic images of the tumour(Jang et al., 2014).

Polydopamine based gold nanospheres as USI contrast agent and photothermal therapy have suggested a new avenue for the treatment of cancers. These nanospheres were prepared with varing amount of gold nanoparticles and ultrasound images were obtained using these nanospheres. Intensified

ultrasound images were produced with increased amount of gold naoparticles which suggested that these polydopamine based gold nanospheres can serve as an excellent contrast agent for ultrasound imaging (Shang et al., 2020).

Silica-Based Nanocapsules (SNCs)

SNCs have been pursued for bioimaging applications by incorporation of varied sorts of fluorescent probes (e.g., organic dyes, conjugated polymers, and QDs) and magnetic nanocrystals (e.g., iron oxide, and manganese oxide)
SNCs are formulated by just three simple steps including,

- Formation of a template that is in nano range
- Development of silica-based shell on nano template
- Removal or etching of template (Figure 13) (Yu Zhang, Hsu, Ren, Li, & Wang, 2015)

Silica based nanocapsules labelled with nile red have been employed in a study to obtain FLI of prostate cancer cells. The study showed the better contrasting properties and penetration of SNCs. In another study, PEGylated silica based nanocapsules incorporating iron oxide were prepared and used for visualizing the tumor site (Yu Zhang et al., 2015). Doxorubicin loaded silica-based iron oxide nanocapsules were prepared by Yu Chen et al. These nanocapsules showed high loading efficiency for drug and better contrast magnetic resonance images demonstrating bioimaging and therapeutic functionalities of the nanocarrier system (Chen et al., 2010).

Conclusion

Hollow nanocapsules have revolutionalized the field of bioimaging due to their outstanding properties. A brief review of advances in hollow nanocapsules in biomedical applications is disscussed in this paper. The structure of nanocapsule allows the incorporation of multiple agents for targeted delivery, bioimaging and therapeutic purpose. Targeted delivery and biocompatibility can be acheived by surface modification of hollow nanocapsules. These advantages make hollow nanocapsules a promising platform for the development of different bimodal and multimodal biomedical devices.

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