

## Review article

## Microspheres for targeted drug delivery- A review on recent applications

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## ABSTRACT

Microspheres are one of the most explored drug carriers for site-specific delivery of cargoes in a controlled and sustained manner. They are biocompatible, biodegradable, and are capable of surface modifications. A variety of polymers have been explored which can encapsulate drugs with different physicochemical properties. All these properties, together with the different strategies makes them a deserving candidate for targeting to various sites of the body, including lungs, liver, bone, brain, colon, eye, and other malignant tissues in different parts of the body. They are also capable of targeting the macrophages and to cellular organelles like mitochondria. The objective of this review is to highlight the applications of microspheres in the field of targeted drug delivery system.

## 1. Introduction

The constraints associated with conventional dosage forms and classical oral drug delivery systems is leading the pharmaceutical community towards a new era of drug delivery systems i.e., Novel Drug Delivery Systems (NDDS). The concept of targeted drug delivery, indeed, as a subset of NDDS is being investigated substantially nowadays. However, the concept of targeting is not new to the drug delivery domain. It dates back to 1906, when sir Paul Ehrlich, postulated the concept of 'magic bullet' and laid down the foundation of a new paradigm in the field of drug delivery [1]. Thenceforth, the concept has been evolving continuously, with newer and innovative approaches adding on to the existing knowledge.

Targeting refers to the selective accumulation of cargo in organs, tissues, cells or intracellular structures by systemic or local drug delivery [2]. The preferential accumulation of the drugs at the targeted site spares the rest of the healthy tissues of the body and increases the therapeutic index of the drug, thus improving the overall treatment outcome [3]. Targeting a drug delivery system, either passively or by specific means requires the use of carriers such as nanoparticles, liposomes, micellar systems, microspheres etc [4].

The growing number of studies in the recent years, illustrating the potential use of microspheres as drug delivery carriers for targeted delivery has attracted the attention of researchers across the globe.

Microspheres are free-flowing particles ranging between 1  $\mu\text{m}$  and 1000 $\mu\text{m}$  and are capable of delivering the therapeutics with a satisfactory sustained release/controlled release profile [5]. They are matrix particles in which the actives are homogeneously distributed in the polymeric network. They are capable of encapsulating small molecules, proteins/peptides and nucleic acids [6]. The high translational efficiency and clinical success rate compared to nanoparticles give them an upper-hand over nanoparticulate drug delivery systems [7]. They provide several advantages over conventional dosage forms like enhanced solubility of poorly soluble drugs, protection of drugs from enzymatic and photolytic degradation, decreased dosing frequency, improved bioavailability, providing controlled release profile, reduction in dose and drug toxicities, etc [8]. They can be manufactured by various techniques including solvent evaporation [9,10], spray drying [11,12], phase separation [13] and polymerization [14].

The currently marketed microsphere formulations are available as long-acting injectable depots which provide controlled release of the encapsulated drug over a specific period of time. Most of these formulations contain hormonal analogues as the encapsulated drugs [15]. Apart from hormones, several other drugs acting on central nervous system and some opioid antagonists are also available as microsphere formulations for several applications [16]. Unfortunately, microspheres for targeted delivery of the drugs are not available in the market till date. However, a lot of research is currently in progress where these carriers

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are being explored for their applications in Targeted Drug Delivery System (TDDS). Indeed, several ongoing clinical trials on microspheres encapsulating anticancer drugs like doxorubicin (DOX) and irinotecan for colon cancer, rectal cancer and hepatocellular carcinoma are the proofs which showcase the potential of microspheres to be used in targeting drugs to desired locations [17,18].

Microspheres can be targeted to the desired location by active or passive targeting strategies. Passive targeting is based upon the size and general surface properties of the microspheres such as degree of hydrophobicity, surface charge, and non-specific adhesion, which directs them towards the particular organ. Additionally, the approach confers special properties to the carriers which aid them to cross physiological barriers like the specialized epithelia and Blood Brain Barrier (BBB) [19]. On the other hand, active targeting is mostly associated with ligand-receptor recognition, referred to as secondary active targeting. Apart from this, targeting to cell organelles i.e. tertiary targeting, and to different organs of the body i.e. primary targeting is also accomplished by microspheres [20,21]. Indeed, microspheres can be tailored to target a specific site by a combination of more than one targeting approaches too [22]. The choice of the targeting approach depends upon the intended area of human body, since each human body part has a different physiology. Size based targeting is mainly a cornerstone approach for lung targeting and liver targeting, as the drug cargo has to reach and get retained in the smallest capillary network of the lungs and in the arteries surrounding the tumours in the liver [23,24]. Surface modifications either by linking the microspheres with targeting moieties or by coating them with materials with special properties like acid resistant or mucoadhesive polymers can be used for targeting almost all the organs including lungs, brain, colon, eyes, tumours, etc. [19,25–28]. Apart from the normally explored targeting strategies, very specific approach lies in bone targeting, where inorganic materials are used to deliver the drugs to the bony tissues [29].

The currently available reviews concerning microspheres as a drug delivery system mainly focuses on its properties, methods of preparation and applications for controlled delivery. However, to the best of our knowledge, there is a lack of literature which specifically highlights the applications of microspheres in the field of targeted delivery. Therefore, this review mainly focuses on the recent applications of microspheres for targeting to various organs such as lungs, brain, liver, colon, bone, etc. The main objective of the review was to understand and highlight the recent advances in the formulation of microspheres for targeted delivery. Various databases such as PubMed, Scopus, ScienceDirect, Web of Science, etc were explored to strive for a diverse literature assembling heterogeneous scientific data which can further pave a path and facilitate research in the area of targeted drug delivery using microspheres.

## 2. Applications of microspheres for targeting various sites

### 2.1. Microspheres for lung targeting

The respiratory system, and mainly the lungs are one of the most critical and vital organ systems involved in supplying oxygen to the body, removing the metabolic wastes, and maintaining the pH balance of the body [30]. According to a recently published report based on the data obtained from the 'Global burden of Diseases, Injuries and Risk factor studies, 2017', Chronic Respiratory Diseases (CRDs) are a leading cause of morbidity worldwide, adding on to the socio-economic burden. These CRDs include asthma, Chronic Obstructive Pulmonary Disease (COPD), interstitial lung disease and pneumoconiosis [31]. Besides these, most of the cancer related deaths in the world are due to the lung cancer [32]. However, the currently available conventional treatment modalities are unable to treat these disorders to the extent that is expected, and therefore the targeted approach is being scrutinized these days.

Microspheres, either by intravenous route or by inhalation, have been explored as a suitable carrier for drug delivery to target the lungs

by virtue of its size (passive targeting) and also by surface modifications (active targeting). Passive targeting is achieved by fabricating the particles within size range of 5–10  $\mu\text{m}$ , so that the capillary network of the lungs withholds the particles following intravenous administration. It is because the size of the capillaries in the lungs are smaller than the size of the injected microparticles [33]. Considering this, several studies have been reported where researchers have tried to deliver a plethora of therapeutics targeted to the lungs for the treatment of various diseases.

Size controlled microspheres selectively accumulate in the lungs and thereby improve the treatment efficacy, while decreasing the side-effects observed with conventional formulations. This has been proven in studies reported by several researchers. For example, Sangi et al. formulated intravenous 5-Fluorouracil (5-FU) gelatin microspheres (5–15  $\mu\text{m}$ ) using a Nano Spray Dryer. Such a formulation is expected to decrease the toxic effects of the chemotherapeutic drug by passively targeting the lungs and thereby, decreasing the dose. The results of the study indicated that the drug concentration in the lungs was remarkably higher than the other tissues and the formulation released 99.1% of the encapsulated drug over a period of 12 h, unlike the conventional 5-FU injection which released 89% of the drug in 30 min, suggesting that the formulation may have the capability of providing sustained release [23]. Size based lung targeting of biodegradable PLGA microspheres with dipalmitoyl phosphatidyl choline (DPPC) as an additional component has been proven effective in reaching the deep alveoli, as reported by Kotta Sabna et al., where they developed Vitamin E TPGS stabilized dexamethasone loaded DPPC and PLGA microspheres [34].

Similar to 5-FU, several other chemotherapeutic drugs like docetaxel, doxorubicin, carboplatin, etc. have also been encapsulated in microspheres for targeting to lungs [35–37]. Other than anticancer drugs, some antibiotics and anti-asthmatics can also be delivered to target the lungs for treating infections and asthma respectively [38–41].

In addition to monotherapy, microspheres have been evaluated for their potential for co-delivery of two drugs, providing a sustained release at the targeted site to combat the problem of drug resistance in Non Small Cell Lung Cancer (NSCLC). The combination of afatinib (AFT) [oral] and paclitaxel (PTX) [intravenous] has shown positive clinical outcomes in the treatment of NSCLC. However, the different administration intervals and routes lead to poor patient compliance. To overcome this issue, a novel formulation was designed where afatinib containing Solid Lipid Nanoparticles (SLN) were encapsulated in porous PLGA microspheres (pMS) containing paclitaxel. The quick release of PTX and prolonged release of AFT imitated the intravenous and oral administration of the two drugs respectively. The formulation exhibited 96 h of two stage release and was able to achieve high drug concentration in lungs. The *in-vitro* cytotoxicity assay revealed a significant decrease in the cell vitality of H1975 cells and PC9/G cells compared to the blank [Fig. 1(A), left panel]. The tissue distribution studies revealed to have the highest concentration of AFT and PTX being present in the lung tissue, suggesting that the system was successfully targeted to the desired location [Fig. 1(A), right panel] [42].

Apart from small molecules, microspheres are also able to encapsulate protein and peptide drugs for targeting to lungs. This was evidenced by a study conducted by Liu et al. on construction and evaluation of nanoporous ion-responsive targeted drug delivery system (PIRTDDS) to enhance the drug availability of Human Recombinant Interferon Alpha-2b (rhINF $\alpha$ -2b) to the lungs. rhINF $\alpha$ -2b, which has a broad spectrum antitumour, antiviral and immune enhancement activity, was passively targeted to the lungs and showed prolonged release. These chitosan rhINF $\alpha$ -2b Carboxymethyl Nanoporous Microspheres (CS- rhINF $\alpha$ -2b-CCPM) had a size distribution between 5 and 15  $\mu\text{m}$  and the nanopores were 300–400 nm in diameter. The microspheres, fabricated using an electrostatic self-assembly technique could release the drug based on the mechanism of ion-exchange. The positively charged protein combines with negatively charged carboxymethyl chitosan ions in the microspheres in a condition when the isoelectric point of rhINF $\alpha$ -2b is more than the *in-vitro* pH. The physiological environment of human body

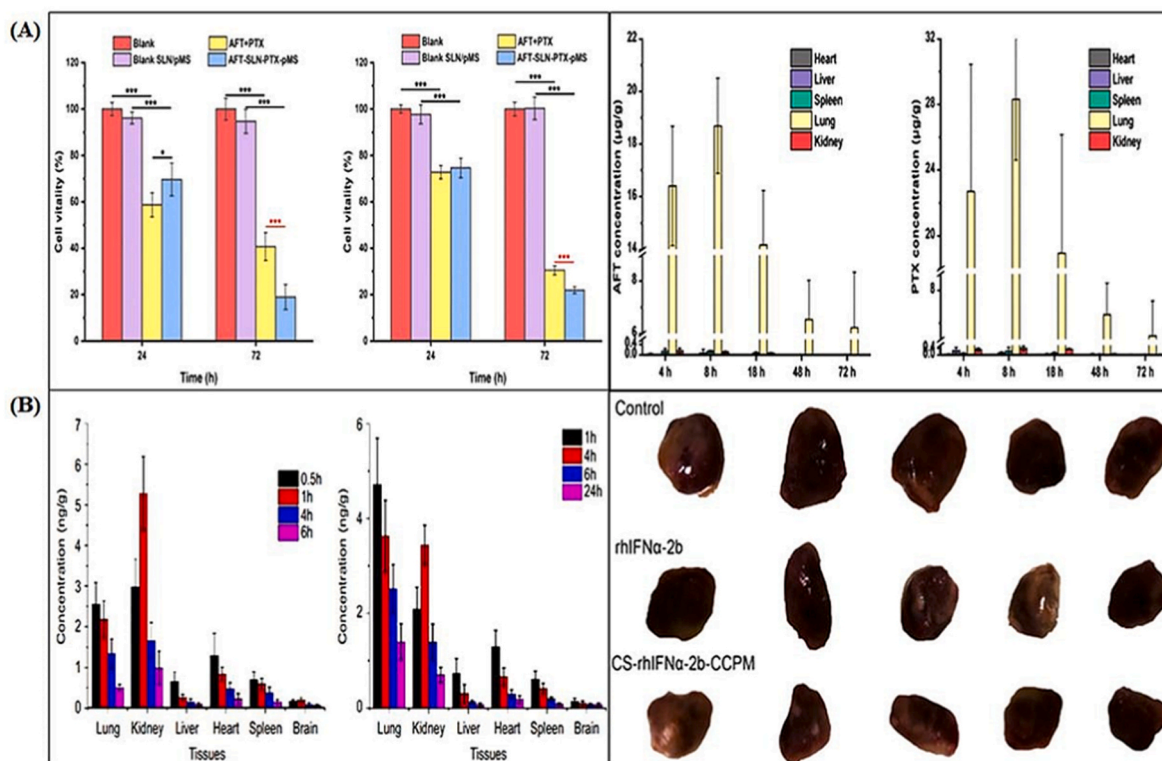


**Fig. 1.** Lung targeting. (A) The in-vitro cytotoxicity assay of H1975 and PC9/G cells after treatment with AFT and PTX solution, the release medium from SLN/pMS (without AFT and PTX) or AFT-SLN-PTX-pMS at 1 and 3 days, showing a significant decrease in cell vitality on treatment with AFT-SLN-PTX-pMS; mean  $\pm$  S.D.,  $n = 6$ . (\*\* $p < 0.001$  and \* $p < 0.05$  (\*\* $p < 0.001$  and \* $p < 0.05$ ) [left panel], Reprinted with permission from Ref. [42]; (B) Tissue distribution diagram of rhIFN $\alpha$ -2b stock solution and rhIFN $\alpha$ -2b nanoporous microspheres respectively after administration via tail vein injection (left panel), International Journal of Nanomedicine 2019 14 5339–5353 ‘Originally published by and used with permission from Dove Medical Press Ltd. [43]; Tumour appearance (right panel), International Journal of Nanomedicine 2019 14 5339–5353 ‘Originally published by and used with permission from Dove Medical Press Ltd [43].

consists of positive ions (such as  $\text{Na}^+$ ) which plays an important role in rhIFN $\alpha$ -2b release from the nanoporous microspheres by ion-exchange mechanism, so that the drug can showcase its biological activity. Tissue distribution study carried out to confirm the targeting achieved by the formulation demonstrated highest concentration of the drugs reaching the lungs [Fig. 1(B), left panel]. The results revealed that the novel targeted formulation improved the stability of the drug *in-vivo*, increased the encapsulation efficiency, and had better antitumour activity as compared to the stock, which was evident from the significant decrease in the volume of tumours treated with (CS- rhIFN $\alpha$ -2b-CCPM) [Fig. 1(B), right panel] [43].

Inhalable polymeric microspheres have been used for targeting the lungs locally by some surface modifications. A recently reported study by A. Sharma et al. demonstrated the development of inhalable polymeric microspheres of N-acetyl cysteine- Poly-lactide-co-glycolide- mucous penetrating host defence peptide (NAC/PLGA-MPP) synergized with mucous penetrating and biofilm disrupting properties for the treatment of tuberculosis (TB). It was hypothesized that the mucous penetrating properties of NAC, biofilm disrupting properties of the Host Defence Peptide-IDR 1018 (Innate Defence Regulator-1018) and size controlled targeted delivery to the lungs possibly can reduce the treatment duration of the TB therapy to 1.5 months, which would otherwise last for 6–9 months with the commonly used standard TB therapy. The value-added formulation could be advantageous for fighting against TB-superbugs when used as a supplement to the existing directly-observed therapy, short-course (DOTS) therapy. The hypothesis was positively supported by the findings of the study where a significant reduction was observed in the mycobacterial burden in a dose-dependent manner in the lungs of the mice as compared to the untreated animals and placebo-treated controls [Fig. 2(A)] [25].

Microspheres have been proven efficacious in tertiary targeting also. This has been illustrated in a study, where sialic acid (SA) modified PEG-PLGA Curcumin-TPP microspheres were prepared for the treatment of Acute Lung Injury (ALI), which is characterized by inflammation in the lung tissues. The study was based upon the fact that the production of reactive oxygen species (ROS) played an essential role in the development of the disease and mitochondria are the main organelles affected due to excess ROS in the cells. Therefore, the covalent conjugation of a mitochondrial peptide-TPP with curcumin could selectively target the mitochondria. Also, the surface modification of the microspheres with SA allowed their interaction with an integrin, E selectin which is over-expressed on inflamed tissues; thus, the microspheres were able to target the lungs, they being inflamed due to the pathology of the disease, [Fig. 2(B)]. The results indicated that SA/PEG-PLGA/MS were able to selectively target the mitochondria and showed increased cellular uptake in inflammatory cells [Fig. 2(C)]. The formulation also demonstrated to have better antioxidant activity and ability to inhibit cell apoptosis by reducing ROS generation [44].

## 2.2. Brain targeting

The ‘Global Burden of Diseases, Injuries and Risk factor studies (GBD), 2016’ showed that neurological disorders like Alzheimer’s disease, Parkinson’s disease, dementias, multiple sclerosis, idiopathic epilepsy, and migraine are the major source of disability and second major source of deaths across the world [45]. The treatment of such Central Nervous System (CNS) disorders is quite challenging because of the presence of physiological barriers like the blood brain barrier (BBB), which is responsible for restricting the entry of almost all the drugs and foreign molecules in the brain [46]. Several approaches have been tried

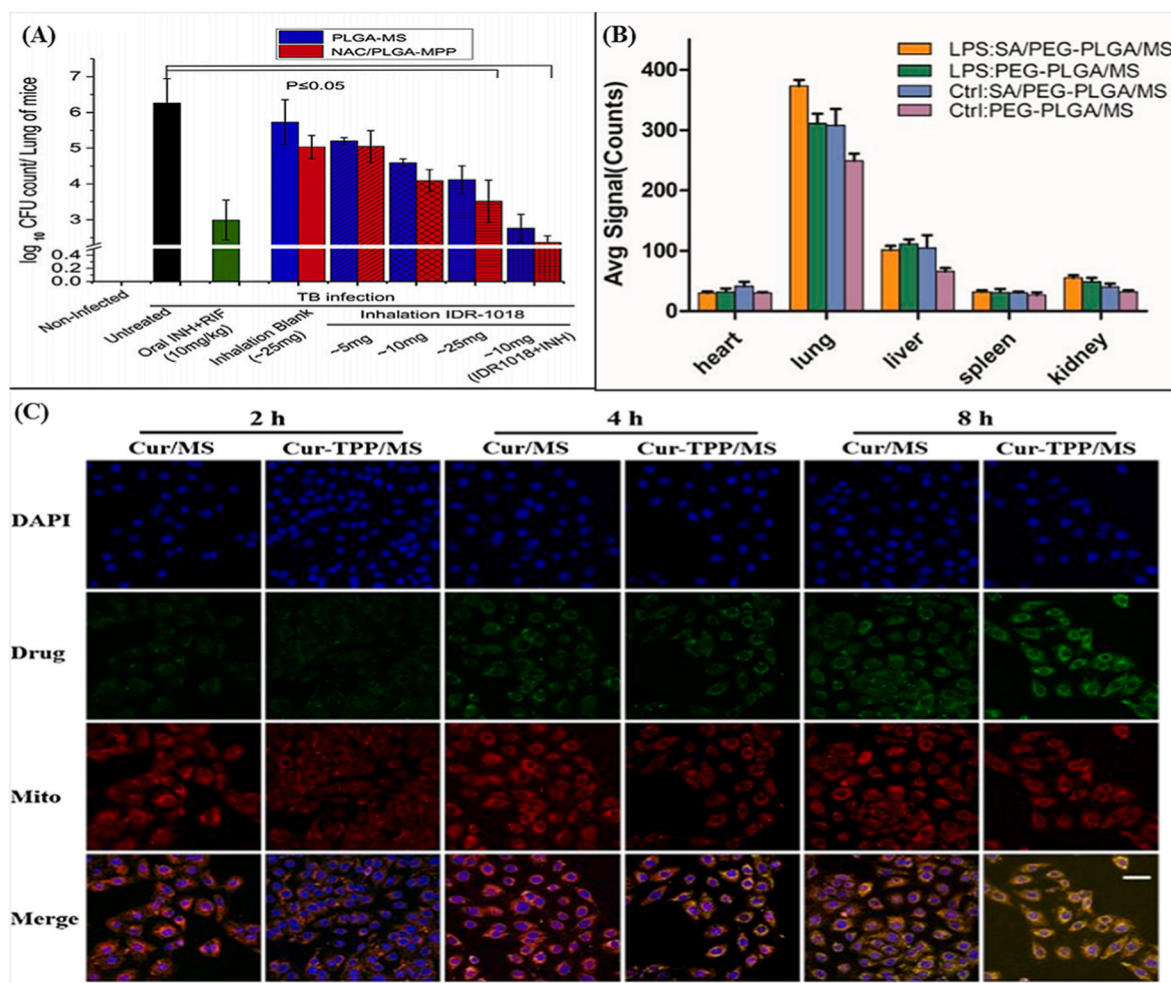


**Fig. 2.** Lung targeting. (A) Counting of CFU in M.Tb infected mice, Reprinted with permission from Ref. [25]; (B) Biodistribution study diagram showing the quantitative analysis of fluorescence intensity in various organs of ALI mice treated with ICG-labeled microspheres., Reprinted with permission from Ref. [44]; (C) *In-vitro* study on HUVEC cells showing selective targeting to mitochondria: The co-localization of Cu-TPP/MS and Mito-Tracker Red is depicted by yellow area of overlapping image., Reprinted with permission from Ref. [44]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

by researchers for targeting to the brain, which includes physiological transport mechanisms such as adsorption mediated transcytosis [47], inhibition of the active efflux pumps [48], receptor mediated transport mechanism [49], cell mediated endocytosis [50] and the use of peptide vectors [51]. Besides this, Various approaches for drug delivery like microspheres, biodegradable wafers [52] and colloidal drug carrier systems [53,54]; and an alternative nose to brain route have been studied extensively. It seems that most of the research in the field of brain targeting is more inclined towards nano formulations, they being excellent carriers which can traverse through the BBB due to their nanometer size and surface functionalization. However, for nose to brain delivery, microspheres are being extensively investigated.

Microspheres, in the form of dry powder formulations, are a favourable drug delivery system for nose to brain targeting, offering numerous advantages like delayed mucociliary clearance, enhanced drug concentration at the site of deposition and superior stability profile [55]. The olfactory region of the nose is the best site to potentiate direct nose to brain delivery [56]. Considering this fact, A. Hafner et al. formulated melatonin mucoadhesive microspheres using pectin and hypromellose as polymers. The optimized formulation showed the increased dissolution rate and total entrapment of melatonin in a ratio of 1:3, pectin to hypromellose compared to pure melatonin powder. Inspiratory airflow rate and addition of lactose monohydrate as an inert

carrier and were identified as a key parameters responsible for determining the deposition of microspheres within the nasal cavity [Fig. 3 (A)] [57]. A similar study was performed by E. Gavini et al. where an antimigraine drug, zolmitriptan (Zol) was formulated as a microsphere formulation using chitosan glutamate (CG213) and HPMC as polymers [58]. The study concluded that the CG213 microparticles showed better efficacy in terms of Zol uptake. As shown in [Fig. 3(B)], the Zol concentration at 1 h after nasal administration of CG213 Zol microparticles was equivalent to the concentration of Zol given by IV route, suggesting that drug administration by nasal route can provide rapid relief in migraine. Such results were justified by the capability of chitosan to temporarily open the tight junctions of nasal mucosal tissues, thereby enhancing the drug permeation [59,60] as well as its targeting ability to efficiently target the brain, as evidenced by previous research [61,62]. The nasal formulation was found to promote the central uptake of Zol by opening the tight junctions of the mucosal tissues and thus reduced the drug tone at peripheral level, providing an advantage over intravenous administration. Similar studies have been reported for the encapsulation of various drugs such as methotrexate, clonazepam, etc for brain targeting via intranasal route [63,64].

Apart from nose to brain approach, intravenous route has been evaluated for brain targeting by Mohanraj et al. where they tried to explore the potential of poly (butylene succinate) (PBSu) polymer for

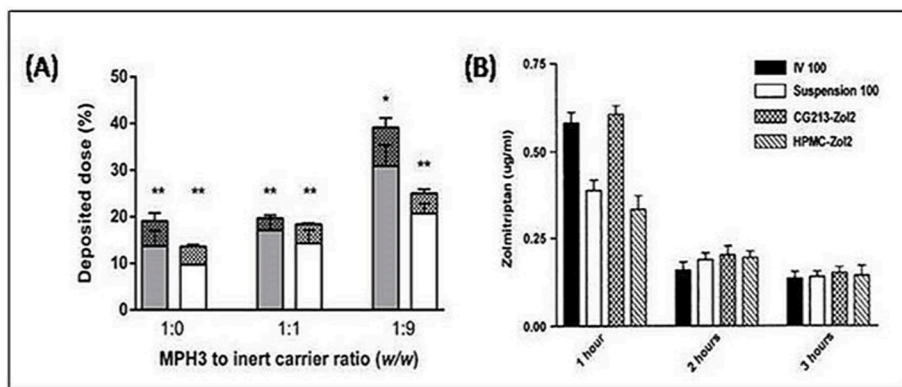


**Fig. 3.** Brain targeting. (A) Turbinate (no pattern fill) and olfactory (pattern fill) deposition of melatonin-loaded pectin/hypromellose microspheres (pectin/hypromellose 1:3, w/w; MPH3) blended with lactose monohydrate at ratio of 1:0, 1:1 and 1:9, w/w, upon nasal administration under inspiratory flow rate of 0 L/min (■) and 20 L/min (□), Reprinted with permission from Ref. [57]; (B) The concentration of Zol in CSF of rats after administering 100 µg dose by intravenous infusion or by nasal route. The nasal formulations consisted of powders of CG213-Zol or HPMC-Zol and a 5 mg/ml suspension., Reprinted with permission from Ref. [110].

fabricating levo dopa loaded microspheres for the management of Parkinson's disease. The non-toxic degradation products, namely 1,4-butane diol and succinic acid gives the polymer an advantage of being biocompatible in nature. Moreover, the presence of large amount of succinate dehydrogenase enzyme in the brain is responsible for the conversion of succinate to form fumarate as an intermediate product in the TCA (tricarboxylic acid) cycle. These reasons are a good justification for selecting PBSu polymer for drug delivery across the BBB. The microspheres were conjugated with transferrin so that they can traverse the BBB by transferrin receptor mediated endocytosis. The results concluded that levodopa was successfully encapsulated in smooth microspheres [19].

### 2.3. Liver targeting

Liver diseases are responsible for approximately two million deaths per year, one million deaths due to the viral hepatitis and Hepatocellular Carcinoma (HCC) and one million deaths due to complications of cirrhosis [65]. These sobering numbers highlight the solemnity of the hurdles existing in the currently available therapies. Various efforts have been made by researchers across the world in the areas of novel drug delivery approaches such as nanoparticles, liposomes, and microspheres which can improve the therapeutic outcomes.

Microspheres, as drug delivery carriers have got important applications in one of the most commonly used techniques, called Transarterial Embolization (TAE) for the treatment of unresectable primary and metastatic liver tumours [66,67]. It is a minimally invasive technique, where the microspheres are delivered to the tumour through the catheter. The particles get lodged in the small arteries surrounding the tumour tissues, thus restricting the blood supply. An advancement to TAE is Transarterial Chemoembolization (TACE) [68], where a chemotherapeutic agent is loaded into microspheres, and Transarterial Radioembolization (TARE) [69], where a radioactive compound (usually Yttrium<sup>90</sup>) is loaded, to further improve the treatment efficacy. A survey conducted by Society of Interventional Radiology revealed an important fact that for chemoembolization with microspheres stating the desired size range as 300–500 µm (47%), followed by 100–300 µm (26%) and 500–700 µm (8%) [24].

Several studies have been reported in the literature using these techniques, with or without modifications [24,69,70]. One such reported study by Liang Y-Jun and his team shows the synergistic combination of chemoembolization and magnetic hyperthermia to treat HCC. They studied PTX loaded magnetic PLGA microspheres to achieve chemoembolization and hyperthermia to target hepatocellular carcinoma cells. It was proposed that the magnetic navigation embolization technique delivers magnetic microspheres to the desired tissues via application of external magnetic field can overcome the limitations, such as proximal embolization, associated with the currently used TAE technique. The size-controlled microspheres prepared using rotating

membrane emulsification technique showed excellent results with HePG2 cells and HL-7702 cells in terms of performance and biosafety. Moreover, it was identified from the thermal transition character of PLGA that heating accelerates the drug release efficiently, and it was proven that the system caused irreversible thermal damage to the tumour tissues [70].

In another study, an attempt was made to overcome the RES system and to deliver an effective amount of dose to the liver tumours, by Mao, Jingsong et al. who developed microwave (MW) sensitized system of (mPEG-PLGA@ZrO<sub>2</sub>@[DOX + ILS]) drug loaded microspheres. This MW sensitized drug delivery system consisted of DOX loaded into the hollow cavity of ZrO<sub>2</sub> nanoparticles and further the nanoparticles and ILS (Microwave sensitive ionic liquid) were encapsulated in PEG-PLGA microspheres. The smart system offered several advantages like the selective administration of (mPEG-PLGA@ZrO<sub>2</sub>@[DOX + ILS]) to the blood supplying arteries, microcapsular deshielding by MW irradiation and betterment of nanocarriers uptake and DOX because of MW non-thermal effect. The findings highlighted the possibility of temperature sensitive controlled release of the drug based upon the characteristics of the materials used for fabricating the microspheres. The ionic liquid present in these microspheres had an impressionable effect in MW ablation and was able to increase the ablation area by 30.6% compared to control group. The novel microspheres selectively embolized the tumour vessels and caused tumour ischemia and necrosis. Also, it increased the tumour cell toxicity of DOX and decreased its cardiotoxicity [71].

In addition to the applications of microspheres in TAE, studies have been reported where covalent conjugation of microspheres with receptor recognizing moieties has successfully targeted the drug to the desired site. F. Van Dijk et al. formulated a subcutaneous microsphere formulation of rho-kinase inhibitor for PDGFβ receptor targeting. The upregulation of rho-kinase activity in liver cirrhosis leads to portal hypertension. Systemic administration of a rho-kinase inhibitor is associated with side effects of systemic hypotension. Therefore, an attempt was made to achieve liver specific delivery by targeting PDGFβ receptors that are overexpressed in liver cirrhosis, on the key pathogenic cells, i.e., myofibroblasts. The carrier, encapsulating the rho-kinase inhibitor, Y27632 consisted of mouse serum albumin (MSA) covalently coupled with several PDGFβ receptors recognizing moieties. The combined targeting-sustained released approach demonstrated that the microspheres were pharmacologically active at the target site in contrast to the ineffective equimolar quantities of the free form of the drug [72].

### 2.4. Colon targeting

The prevalence of local colon diseases has increased substantially in the past few decades across the world [73]. According to GLOBOCAN 2018 data, colon cancer is the third most frequently occurring cancer in the world and comprises of 11% of the total diagnosed cases of cancer

[74]. Other than colon cancer, inflammatory bowel disorder, which includes Crohn's disease and ulcerative colitis is also one of the major prevalent colon ailments which affected almost 6.8 million people across the globe in 2017 [73]. Treatment of these diseases demands a successful delivery of the therapeutic to the colonic sites. However, the harsh environment of the GI tract and the inter and intrapersonal variations makes the task difficult. Therefore, several approaches are adopted to target the drugs to the desired site in the colon. Some of these approaches include the prodrug approach [75], colon specific biodegradable systems [76], pH-sensitive matrix-based systems [77], time-controlled systems [78] and multiparticulate systems [79]. Microspheres are one of the multiparticulate systems, which can pass through the GI tract more easily due to their small size. The microsphere approach for colon targeting can provide sustained release of the drugs, while at the same time, it protects the drugs from degradation which are otherwise degraded in the highly acidic gastric environment. Also, they provide advantages of improved bioavailability and reduces the local irritation and risk of systemic toxicity [80].

Microspheres coated with enteric coating polymers is one of the widely used formulation strategies for colon targeting. For example, a study with the use of enteric coating polymers was carried out by Jain, Ashish et al. where they prepared pH-dependent sustained-release clarithromycin loaded Tulsion® microspheres with thermocoat L 30 D 55 polymer as a release retardant by using quasi-emulsion solvent diffusion method. The formulation masked the metallic taste of the drug

as well as increased the half-life of the drug by 1.2 folds [26]. The use of magnetite in microsphere formulation can further increase the drug concentration in the targeted area by means of an external magnet [81, 82]. Based upon this hypothesis, a study reported the formulation of magnetic chitosan microspheres with coatings of Eudragit L 100 for local delivery of sulfasalazine for inflammatory bowel disease (IBD) treatment. The cell line study showed that the increased in down regulation of Cyclooxygenases 2 (COX2) by formulation with compared to control and marketed formulation [83]. Similar to clarithromycin, some anticancer drugs like 5-FU have also been formulated as eudragit coated microspheres for colon targeting. This approach has also proven effective in preventing the degradation of some small protein molecules like insulin, as reported in a recently published study [84].

In addition to the synthetic polymers like Eudragit, many polymers from natural sources are also being used these days for the preparation of colon targeted microspheres. H.H. Gadalla et al. incorporated progesterone (PG) in pectin/NaCMC (Sodium Carboxy Methyl Cellulose) microspheres cross-linked with  $Zn^{2+}$  and  $Al^{3+}$ . The oral microspheres were formulated with an objective to find an alternative to currently available formulations of PG like IM injection and vaginal inserts. The formulation showed pH-dependent swelling in dissolution medium, insignificant release of PG in simulated gastric fluid and prolonged release in simulated intestinal fluid; thereby overcoming the problems of shorter half-life and extensive intestinal and first pass metabolism [85]. Similarly, in order to evade the pitfalls in the treatment of colorectal

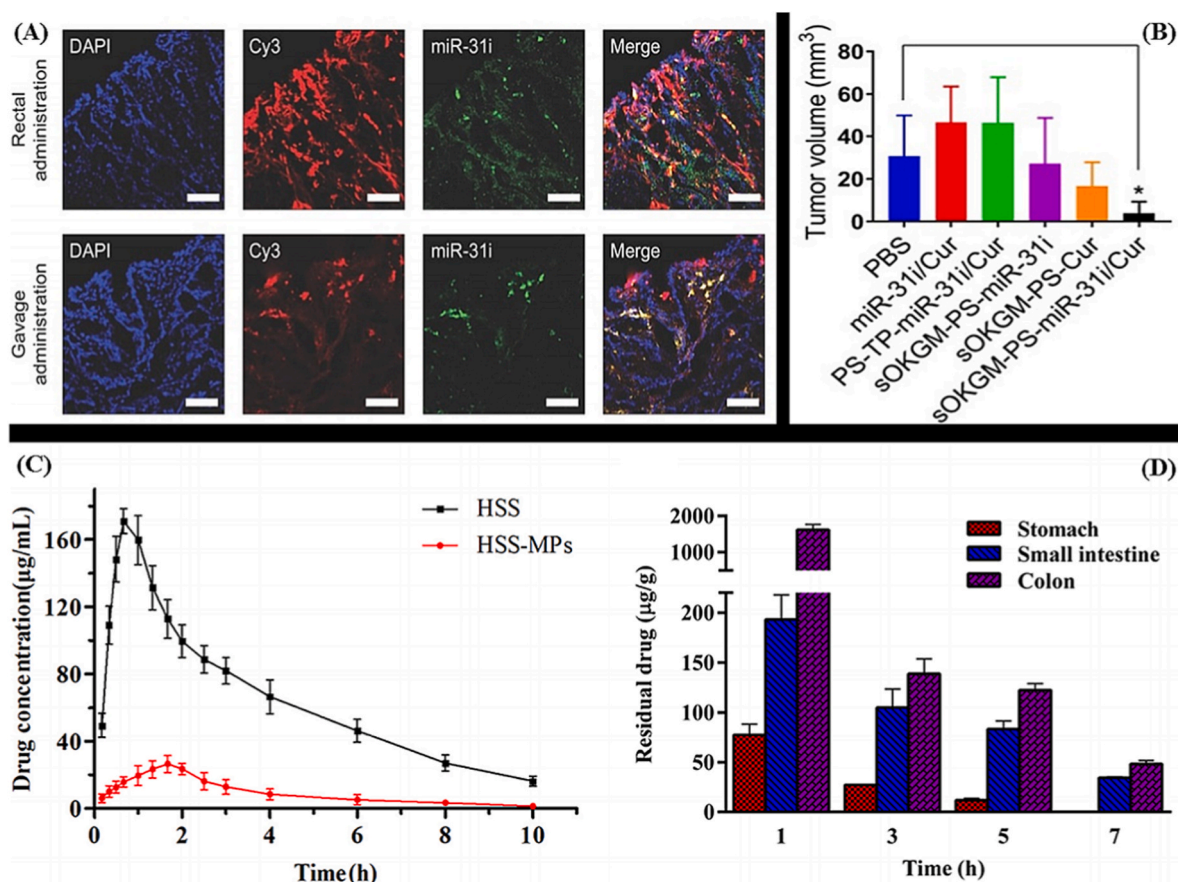


**Fig. 4.** Colon targeting. (A) Images of fluorescence microscopy of AOM-DSS induced tumor cells following 12 h of sOKGM-PS-miR-31i/Cy3 gavage and rectal administration, depicting the localization of PS (labeled by Cy3, red) and miR-31i (labeled by Cy3, red). Scale bar, 100 µm, Reprinted with permission from Ref. [86]; (B) Diagram showing decrease in tumour volume after rectal delivery of sOKGM-PS-miR-31i/Cur microspheres compared to blank PBS and other formulations *in-vivo*., Reprinted with permission from Ref. [86]; (C) Diagram showing pharmacokinetic studies for the investigation of HSS absorption after oral administration in rats, with a dose of 20 mg/kg, Reprinted with permission from Ref. [111], (D) The distribution of HSS in GIT of mice after administration of 1.2 mg HSS-HMS suspended in 0.5% NaCMC solution., Reprinted with permission from Ref. [111]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cancer, Zhao, Ran et al. formulated novel mucoadhesive-to-penetrating peptosomes in microspheres coloaded with *anti*-miR31 oligonucleotide and curcumin for colon targeting by rectal and oral route. NPs of  $\alpha$ -lactalbumin peptosomes ( $\alpha$ -LA-PS) targeting colon cancer stem cell antigen CD133, co-loaded with miR-31 and Curcumin (Cur) were incorporated into oxidized Konjac glucomannan (o-OKGM) microspheres forming sOKGM-PS-miR-31i/Cur composite microspheres. The novel formulation showed that the microspheres penetrated the colonic mucosa and delivered the therapeutics i.e., curcumin and mi-R31 to the colon tumour cells [Fig. 4(A)], suppressing the growth efficiently [Fig. 4(B)]. Also, the microspheres were shown to have increased retention time, stability in the harsh gastrointestinal environment, pH responsiveness and decreased toxicity [86]. Another strategy that was tested using natural polymers encapsulated 5-FU in epichlorhydrin crosslinked Portulaca-alginate microspheres. The natural mucilage proved to be a useful excipient to combat the issues of biocompatibility and biodegradability. The pharmacokinetic studies showed enhanced bioavailability of the active, and GI distribution studies indicated drug localization in the colon [87].

Apart from enteric coating, pH sensitive hydrogels are also used for colon targeting. However, the lump forming property of hydrogels makes it unsuitable by oral route. For this reason, small particulate drug delivery systems with pH sensitivity and controlled size are highly preferred [88]. As evidenced by previous research, microspheres in the size range between 10 and 300  $\mu$ m are suitable for targeting to inflammatory site of GIT after oral administration [89]. Shi, Xianpeng et al. developed the pH sensitive P(LE-IA-MEG) microspheres for colon targeting in the treatment of ulcerative colitis and performed there *in-vitro* and *in-vivo* study. The smart pH-responsive microsphere system was able to overcome the limitations associated with the systemic absorption of an anti-inflammatory drug, hydrocortisone sodium succinate (HSS). The pharmacokinetic studies carried out in rats revealed that HSS-MPs (Hydrocortisone Sodium Succinate- Microparticles) reduced the systemic absorption of the drug and therefore it could overcome the side effects associated with the drug [Fig. 4(C)]. The formulation was unaffected at acidic pH, while released the drug at the basic pH of the intestine, which was evident from the GIT distribution studies, where it was shown that the drug was predominantly released in colon [Fig. 4(D)]. Further, it reduced the systemic absorption of HSS and showed ameliorative effects on ulcerative colitis [88].

Another interesting approach, called Nanoparticle in microsphere oral delivery system (NiMOS) has been studied by a few researchers [90, 91]. Such a type of system is especially studied for encapsulating siRNA and protein therapeutics. The unique formulation combines two particulate systems to achieve better therapeutic outcomes. It exploits the passive targeting, mucous penetrating and membrane permeating ability of nanoparticles, and the microspheres provide a larger depot and infer better stability to the protein therapeutics. One such reported study encapsulated Salmonella effector enzyme, AvrA in chitosan/alginate microspheres. The enzyme has proven anti-inflammatory and anti-apoptotic properties. The system was able to effectively deliver the enzyme to small intestine and colon. The results indicated that microspheres protected the enzyme in simulated gastric fluid *in-vitro* and also decreases the clinical symptoms in a murine DSS induced pre/co-treatment model. Thus, it was concluded that such a system could be further exploited to delivery of other bacterial protein therapeutics antibodies, and vaccines to the colon via oral route [92].

## 2.5. Bone targeting

In the body the skeletal system, consist of bones, ligaments and connective tissues has a complex anatomical structure, involving a complex meshwork of different types of cells, particularly in the avascular cartilage region. An increase in the number of bone disorders like osteoporosis and bone metastasis with increasing age makes it very important to emphasize targeted therapies to the bone. The bone

disorders share a common characteristic of local inflammation and/or results in the exposure of hydroxyapatite (HAP) to the blood. These two features of bone disorders can be exploited to deliver more amount of drugs load to specific bone tissues. Dilation of the vasculature due to cytokines causes the inflamed tissue to fill with exudates, and therefore the macromolecular therapeutics can retain at the site due to EPR (Enhanced Permeation and Retention) effect. Exposure to HAP also presents unique possibilities of targeting to bone tissues. Substances such as bisphosphonates have an ability to bind with inorganic HAP part of the bones. This binding affinity is retained even after its conjugation with other drug molecules or carriers, presenting them as ideal candidates for developing TDDS to bones [93,94]. The treatment of bone disorders with conventional oral therapies and bolus injections is associated with secondary adverse effects to other tissues and organs and lesser concentration of drugs being available to the bone tissues. Therefore, it becomes important to emphasize on local targeting approaches to improve the treatment outcomes in various bone disorders. An extensive literature is available on various types of delivery approaches to deal with bone disorders like beads, ceramics, cements, conjugates, hydrogels, implants, liposomes, nanoparticles, nanohybrids and microspheres. Microspheres have been explored for various bone disorders like osteoporosis, osteomyelitis, osteosarcoma and also for bone regeneration [95]. Unlike the targeting to other organ systems of the body, where mostly polymeric microspheres are used, bone targeted microspheres are of inorganic type, in the majority of the cases.

A very interesting study encapsulating Doxorubicin was reported, where the drug was encapsulated in hollow porous calcium phosphate-phosphorylated adenosine (CPPA) microspheres for the treatment of osteosarcoma. CPPA is a pH-responsive material, which shows the release of the encapsulated drug in the acidic tumour environment while remains stable at normal physiological pH [Fig. 5(A)]. The biocompatible and biodegradable system had a dual function, drug delivery system for DOX from CPPA/DOX microspheres for the treatment of osteosarcoma and CPPA microspheres itself plays important role in osteogenic induction system for subsequent bone regeneration. The anticancer properties of the system, tested *in-vitro* and *in-vivo* demonstrated that the targeted intratumoural injection of CPPA can significantly reduce the tumour weight and volume [Fig. 5(B)]. Also, no distinguished organic damage was observed to heart, liver, spleen and kidney tissues in histopathological examination. Osteogenic differentiation studies of CPPA on human bone mesenchymal stem cells (hBMSC) depicted elevated expression of the genes responsible for osteogenesis, suggesting that the organic-inorganic hybrid system can have promising application in osteosarcoma treatment [29].

The inorganic microspheres have also been used for the treatment of osteoporosis, an issue which is faced by people, especially by females with increasing age. The deleterious effects are accountable for the multiple fractures that can occur due to the decrease in bone density. Although there are few therapeutics prescribed to osteoporotic patients, efforts are focused on the development of an efficient preventive medicine. Indeed, with an objective of strengthening the bones and preventing fractures, MIDO's project (Minimally Invasive Delivery of Osteospheres), received a grant from NIH (National Institute of Health Research). The project is aimed at developing porous microspheres of calcium phosphate which can act as vessels for stem cells. After recognizing low bone mineral density areas, the scientists expect the possibilities of injecting the patients with their own stem cells, stored in these developed microspheres, in order to strengthen and repair the bones, in the treatment of fractures [96,97]. This provides an insight into the potential of inorganic porous microspheres in preventive medicine for osteoporosis in the coming years.

In addition to inorganic microspheres, bone seeker type with special functional groups attached on the surface have also been investigated. Rotman, Stijn G., et al. developed the formulation for bone targeting, in which bone-seeker functionalized microspheres contains antibiotics for bone infections (osteomyelitis) treatment [98]. Treatment of bone

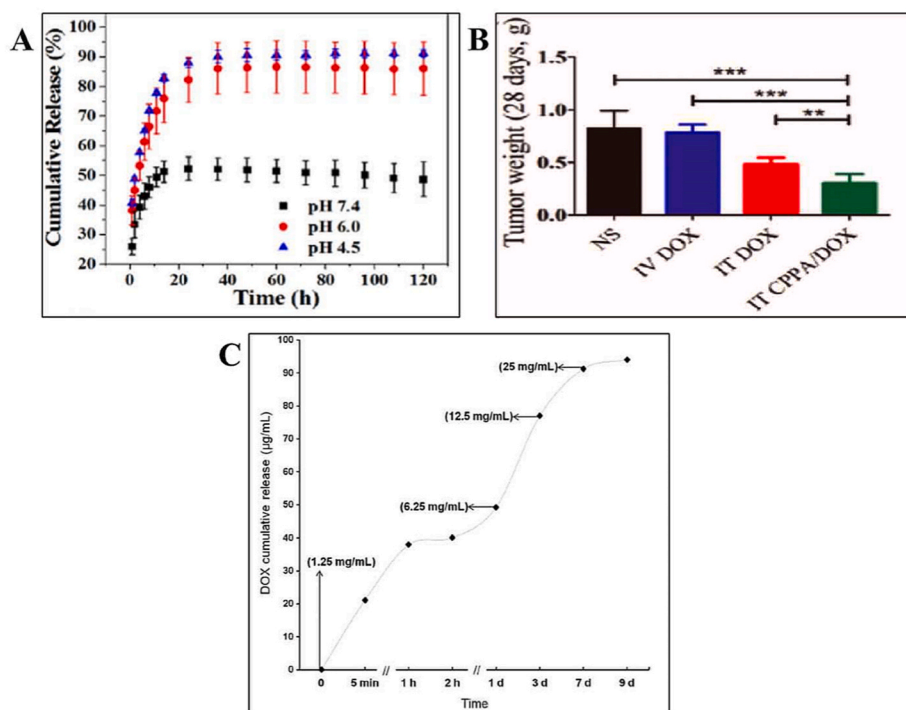


**Fig. 5.** Bone targeting. (A) The effect of various pH conditions on the release profiles of DOX from CPPA/DOX drug delivery system, Reprinted with permission from Ref. [112]; (B) The beneficial therapeutic effects of DOX-loaded CPPA; illustrated by decrease in tumor weight after 28 days in 143B osteosarcoma tumour mouse model (each group,  $n=6$ ): the tumour weight in NS (normal saline), IV DOX, IT DOX, and IT CPPA/DOX groups. Data are the mean  $\pm$  SD. \* denotes  $p < 0.05$ , \* denotes  $p < 0.01$ , \*\*\* denotes  $p < 0.001$ ; Reprinted with permission from Ref. [112] (C) Diagram showing the burst release (20%) in the initial 24 h, followed by continuous and increased drug release up to 9 days, Reprinted with permission from Ref. [99].

infections by intravenous administration of antibiotics is challenging because of the impaired vascularity of bones, and the inadequate capacity of the bone tissue to hold the drug for a longer period of time. Therefore, a synergistic combination consisting of a bone-seeking or bone-binding agent combined with a carrier which gives sustained release was investigated. A hydrophobic analogue of gentamycin, di-octyl sulfosuccinate was encapsulated in PCL (poly-caprolactone), and PLLA (poly D, L-lactic acid) microspheres which were then functionalized with alendronate (ALN) which has a high affinity for inorganic HAP of the bone. The findings depicted 15 times increment in the binding of microsphere to bone mimicking materials and also proved its efficient anti-microbial activity against the major bone infection due to *S. aureus*. A very similar study was carried out by Soriano-Souza et al., where doxycycline containing HAP ceramic microspheres were prepared for drug delivery to bone targeting. The high internal porosity of HAP structure contributed to the increased doxycycline adsorption in HAP structure. The microspheres showed 20% release in the initial 24 h, followed by continuous release up to 9 days [Fig. 5(C)]. The formulation showed anti-microbial activity up to 7 days and histological observation clearly demonstrated new bone formation around the microspheres [99].

## 2.6. Microspheres for targeting other organs

In addition to the aforementioned applications in targeting various organs like lungs, brain, liver, colon and bones, microspheres have got minor applications in targeting other organs and tissues of the body.

For example, PDGF $\beta$ R (Platelet Derived Growth Factor Receptor beta) targeted polymeric microspheres were prepared by N. Teekamp et al. for the treatment of fibrotic kidney. They developed a sustained release injectable microsphere formulation for delivering PDGF $\beta$ R-targeted drug carrier protein pPB-HSA, comprised of multiple PDGF $\beta$ R-recognizing moieties (pPB) attached to human serum albumin (HSA), by subcutaneous route [100].

Further, the recent developments in the use of natural carriers have opened new arena in the field of targeting by microspheres. One such novel natural carrier is  $\beta$ -glucan, obtained from baker's yeast (*Saccharomyces cerevisiae*). The spherical particles have hollow and porous

structures which can accommodate guest drug molecules. The particles with  $\beta$ -glucan group are easily taken up by phagocytic cells by receptor mediated cell uptake through dectin receptors [101–103] and have been studied for encapsulating various payloads including DNA, RNA [104], proteins and small molecules [105]. For example, Mesoporous Silica Nanoparticles (MSN) containing DOX were encapsulated in glucan particles (GP) to target macrophages based upon the hypothesis that, in solid tumours macrophages can serve as Trojan Horses which carries the drugs and release it [105]. Peptide and amine modified  $\beta$ -glucan particles encapsulating siRNA have been shown to target the macrophages and silence an inflammatory cytokine, osteopontin (OPN) in the adipose tissue of obese mice. This represents a promising system with desirable efficacy to deliver therapeutic siRNA to phagocytic cells *in-vivo* for modulating inflammatory responses [106]. Moreover, targeted drug delivery to macrophages using  $\beta$ -glucan is indeed one of the good strategies to deal with inflammatory disorders too. Considering this, one recently reported study depicted the preparation of  $\beta$ -glucan microspheres doped with ortho-vanillin to exacerbate the anti-arthritis effects of methotrexate by targeted delivery to the macrophages. Instead of directly adsorbing the drug molecules, nanoparticles with BSA were prepared, to decrease the amount of burst release from microspheres. Ortho-vanillin is an inhibitor of toll-like receptor, while methotrexate is a dihydrofolate reductase inhibitor. The combination of the two therapeutics, coupled with the targeting ability of  $\beta$ -glucan showed significant improvement in inflammation in adjuvant induced arthritis in rats. The findings of *in-vivo* studies suggested a significant decrease in the levels of TNF- $\alpha$  and IL-6 in the animals treated group with the novel formulation in comparison to the mixture of two drugs in free form or each drug alone [107].

A unique strategy was adopted by Chiang, B., et al. in the form of sustained release PLA microspheres for targeting supraciliary space of eye to decrease the intra ocular tension in the treatment of glaucoma. The drug brimonidine was encapsulated in PLA microspheres and delivered to the targeted site by microneedle injections having  $750 \pm 50$   $\mu$ m length. The formulation showed to have better bioavailability compared to eyedrops and improved patient adherence owing to its sustained release for one month. Also, the dose was reduced by 7.5 folds, thereby taking care of the side effects of dry mouth associated with

brimonidine. However, some foreign body response was observed at the site of puncture across the sclera [108]. L. Mayol et al. tested the possibility of delivering therapeutic proteins to the posterior segment of the eye by intravitreal injection. Bovine Serum Albumin (BSA) was selected as the model drug and was encapsulated in PLGA microspheres, which were further coated with a mucoadhesive polymer, hyaluronic acid (HA). The mucoadhesive property of HA rendered the microspheres motionless in the vitreal space and showed prolonged release up to 30 days [27].

In addition to the applications of the microspheres for treating malignancies associated with many organs, as cited in this article in the earlier sections, folate conjugated microspheres have been prepared by a group of scientists, finding applications in triple negative breast cancer therapy. Curcumin loaded biopolymeric gum acacia microspheres conjugated with folic acid as the ligand selectively targeted Triple Negative Breast Cancer cells (TNBC) due to the overexpression of folate receptors on malignant cells. The system effectively delivered the drug i.e. curcumin in the acidic tumour microenvironment and also showcased the implications of a cost-effective biopolymer to be used as a carrier for encapsulating therapeutics [28]. A very similar study was performed by P. Zhang et al. where polyamine/salt assembled microspheres were coated with hyaluronic acid, which can precisely recognize and bind with CD44 receptors overexpressed on various tumour cells. Unfortunately, *in-vivo* studies were not carried out with the prepared formulation, but the cell lines studies carried out using HeLa cell lines and NIH 3T3 cell lines revealed that the rate of receptor mediated endocytosis was quite high for HeLa cell lines compared to NIH 3T3 cell lines, which suggests that the cellular uptake depends upon the relative number of receptors present on the cell [109].

### 3. Conclusion

It can thus be concluded that microspheres are being investigated for targeting almost all the major organ systems of the body. They have been proven efficacious compared to conventional drug delivery systems, as they are able to overcome a number of formidable physiological barriers. Also, their versatility and the ability of modifications in their structure as per the requirement give them an added advantage. The availability of intense literature in context of various polymers and other fabrication materials as well as the preparation methods, aids the formulators to select appropriate materials and methods, depending upon the ultimate objective of their studies. However, there are challenges still existing, in terms of poor reproducibility, lower encapsulation efficiency and lack of suitable technologies for mass production of such dosage forms. Future research will focus on technological aspects to solve these problems and to come up with newer and innovative approaches to achieve more intensive targeting, so as to achieve the desired outcomes, especially in case of malignancies. Considering the potential of microspheres, they can serve as an excellent drug delivery carrier for sustained as well as targeted delivery of 'difficult to deliver' therapeutics like proteins/peptides, biologicals etc.

### Credit authors statement

Ankita N. Yawalkar: Writing- Original Draft, Writing-Review and Editing, Manoj A. Pawar: Writing -Original Draft, Writing-Review and Editing, Pradeep R. Vavia: Writing-Review and Editing.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

This is a review article. The references have been cited for the represented literature.

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