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Drug-eluting embolic microspheres: State-of-the-art and emerging clinical applications

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Abstract

Introduction: Drug-eluting embolic (DEE) microspheres, or drug-eluting beads (DEB), delivered by transarterial chemoembolization (TACE) serve as a therapeutic embolic to stop blood flow to tumors and a drug delivery vehicle. New combinations of drugs and DEE microspheres may exploit potential synergy between mechanisms of drug activity and local tissue responses generated by TACE to enhance the efficacy of this mainstay therapy.

Areas covered: This review provides an overview of key drug delivery concepts related to DEE microspheres with a focus on recent technological developments and promising emerging clinical applications as well as speculation into the future.

Expert opinion: TACE has been performed for nearly four decades by injecting chemotherapy drugs into the arterial supply of tumors while simultaneously cutting off their blood supply, trying to starve and kill cancer cells, with varying degrees of success. The practice has evolved over the decades but has yet to fulfill the promise of truly personalized therapies envisioned through rational selection of drugs and real-time multi-parametric image guidance to target tumor clonality or heterogeneity. Recent technologic and pharmacologic developments have opened the door for potentially groundbreaking advances in how TACE with DEE microspheres is performed with the goal of achieving advancements that benefit patients.

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Declaration of interest

BJ Wood and NIH have intellectual property in the space. Patents on imageable beads and imageable drug-eluting bead technologies include patent #US10307493 Imageable Embolic Microsphere, PCT WO 2014/152488 Imageable Embolic Microsphere, Japan JP6420817 Imageable embolic microspheres, and Bismuth image-able drug eluting microsphere for chemoembolization. The NIH has a Cooperative Research and Development Agreement with each of the following: Philips Research, Biocompatibles UK Ltd / BTG now Boston Scientific Corporation, Celsion Corp, and Siemens Healthineers. BJ Wood is the Principle Investigator for these research agreements. WF Pritchard, AS Mikhail, M Mauda-Havakuk, AH Negussie, and JW Owen participate in the related research. AL Lewis was previously VP of R&D for BTG (now Boston Scientific) and is currently Director for R&D at OwenMumford Ltd. The content of this manuscript does not necessarily reflect the views or policies of the U.S. Department of Health and Human Services. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as an actual or implied endorsement of such products by the United States government.

Keywords

microsphere; bead; embolization; TACE; DEB; chemoembolization; drug delivery; doxorubicin; hepatocellular carcinoma; embolic

1. Introduction

Liver cancer is a leading cause of cancer-related death worldwide and has continued to increase in incidence and mortality for several decades [1]. Hepatocellular carcinoma (HCC), the predominant form of liver cancer, is usually diagnosed at advanced stages of tumor development when transplantation, surgical resection, or local thermal ablation may not be feasible. For many of these patients, minimally invasive regional therapy with transarterial chemoembolization (TACE) may be a palliative option.

TACE is the recommended first-line therapy for patients with intermediate-stage HCC [2] and it has also been used to treat patients with hepatic metastasis from primary gastrointestinal, breast, melanoma, and neuroendocrine tumors [3–6]. The procedure is performed by delivering chemotherapy and embolic materials directly into tumor-supplying arteries under imaging guidance. The rationale for TACE is that selective disruption of blood flow to hepatic arteries parasitized by tumors and concomitant delivery of chemotherapy results in tumor-localized ischemic and cytotoxic effects. For conventional TACE (cTACE), a chemotherapeutic drug is emulsified in ethiodized oil (Lipiodol®) and administered transarterially followed by an embolic. More recently, TACE has also been performed with drug-eluting embolic (DEE) microspheres, also referred to as drug-eluting beads (DEB), that serve both as an embolic and locoregional drug delivery vehicle. TACE with DEE microspheres results in greater drug concentrations in tumors and reduced systemic drug exposure compared to intra-arterial infusions of free drug [7, 8] or cTACE [9, 10].

Despite its widespread adoption, there remains little agreement on treatment parameters including the type and size of embolic, drug selection, catheter selectivity for tumor arteries, or definition of treatment endpoints. This has contributed to substantial variability in local operator practices and procedural techniques at the expense of reproducibility and comparability. In addition, there is a lack of understanding regarding the relative contribution of the drug *versus* the embolic towards treatment efficacy. Indeed, results comparing the outcomes of embolization using bland microspheres (no drug) to embolization using DEE microspheres loaded with doxorubicin, a chemotherapeutic drug, have been mixed and there remains no consensus regarding which approach is superior. This has spurred investigations into drugs with various different modes of action with the goal of better exploiting potential synergy between mechanisms of drug activity and local tissue responses generated by TACE.

The ideal drug candidate for delivery with DEE microspheres should load into the microspheres in high concentrations, have a sustained or tunable drug release profile, and penetrate readily into surrounding tissue. Moreover, its activity should be maintained or enhanced within the post-TACE tumor microenvironment and counteract or promote biologic processes stimulated by embolization. For example, antiangiogenic drugs eluted

from DEE microspheres may help to counteract neoangiogenic pathways stimulated by TACE-induced tissue hypoxia that can promote tumor recurrence. With the development of immunotherapy, combinations of DEE microspheres and immune-modulating agents could provide potent local, and potentially systemic, therapeutic effects by promoting antitumor immune responses within the inflammatory microenvironment created by TACE, or by helping to overcome pathways of immune resistance or tolerance.

Novel DEE platforms are also under development which may complement or provide new capabilities for TACE and enhance drug delivery and efficacy. Recently, DEE microspheres that are visible on intra-procedural fluoroscopy and cone beam CT (CBCT) imaging have been developed [11–14] raising the intriguing possibility that microsphere radiopacity could serve as a surrogate for spatial drug levels, enabling spatial drug dosimetry [15]. DEE microspheres containing a variety of radiopacifiers have been developed that could potentially enable differentiation on CT imaging between microspheres loaded with different drugs enabling rational delivery of specific drugs to discrete microenvironments within the tumor [16, 17].

The goal of this review is to highlight recent advances in DEE microsphere-based drug delivery, and to explore emerging and yet-to-be-explored strategies to improve the efficacy of TACE with DEE microspheres.

2. Tumor-localized drug delivery using DEE microspheres

The concept of using microspheres to enhance drug delivery to the liver dates back several decades [18, 19] and is based on the original idea that temporary or permanent occlusion of hepatic blood flow following intra-arterial drug infusion can increase drug retention in tumors, reduce washout, and limit systemic exposure, potentially reducing side effects and enhancing efficacy. Drug-eluting microspheres combine both embolic and drug delivery mechanisms into a single vector, with the delivered drug initially co-localized with the embolic microspheres.

The safety, efficacy, and pharmacokinetics of DEE microspheres loaded with doxorubicin were demonstrated by landmark clinical trials reported in 2007 and 2010 which showed significant reductions in peak plasma drug concentrations and area under the curve, as well as increased objective response in patients with advanced HCC, compared to cTACE [9, 10, 20]. In this section, we provide an overview of DEE microspheres developed using permanent or biodegradable materials with the capacity to entrap and release drug in a controlled manner.

2.1 Commercially available DEE microspheres

Currently there are six commercial DEE microspheres with CE marking: five non-degradable and one biodegradable (Table 1). Initial clinical experience with an additional DEE microsphere, Callispheres, has been reported [21–23]. No microspheres have been approved by the U.S. Food and Drug Administration for marketing as DEE devices therefore their use is investigational or off-label when drug-loaded. The microspheres' chemical compositions and physical characteristics are summarized in Table 1.

Differences in chemical structures among these microspheres impart different drug loading capacities and rates of drug release [44], parameters that directly impact *in vivo* pharmacokinetics. Differences in size, size range and dispersity, and mechanical properties, e.g., compressibility and deformability, may influence the *in vivo* performance of microspheres including their distribution and packing density in arteries, ischemic effects, and treatment-specific toxicity. These properties can also affect microsphere handling characteristics and compatibility with micro-catheters. Clinical investigations of DEE microspheres should be performed in compliance with applicable regulations after consultation with appropriate regulatory bodies.

2.2 Drug loading and release

All of the currently marketed DEE microspheres are loaded at the point of use (normally in the hospital pharmacy) by mixing with a drug solution followed by occasional shaking. The time needed for drug loading is dependent upon drug concentration and type, as well as the size, quantity, and type of microspheres [48–50]. Drug loading occurs via an active uptake mechanism driven by the ionic interaction between cationic drugs, usually containing one or more protonated amine groups, and negatively charged sulfonate (SO_3^-) or carboxylate (COO^-) moieties within the microsphere polymer structure [44, 48, 51]. Formation of drug salts in acidic solution can improve drug solubility and promote loading into the microspheres by providing one or more positive charges for interaction with the anionic moieties of the microspheres.

Drug release occurs by ion-exchange whereby the drug is displaced by positively charged ions in the elution media, blood or tissues. The rate of drug release is a function of one or more factors including the strength of the ionic interactions between drug and microspheres, drug-drug interactions within the microspheres (as in the case of doxorubicin) and the ionic strength of the elution medium [52–54]. As a result, under the same conditions, different drugs have different relative rates of release from microspheres ranging from water soluble drugs with little or no interaction with the microspheres that release quickly, to those with moderate (e.g. irinotecan), and relatively slow (e.g. doxorubicin) release rates [55]. Drugs with chemical structures that promote molecular aggregation within the microspheres may prolong drug elution [53, 56]. In addition, smaller microspheres tend to have faster elution rates due to their higher surface area to volume ratio relative to larger microspheres [53]. Drug solubility and concentration in the elution medium can also influence the rate of drug release and diffusion from the microspheres [53].

In vitro drug elution studies are normally conducted using techniques for dissolution testing described in the US pharmacopeia (Chapter <711>) [57]. However, no single technique can accurately predict drug release kinetics *in vivo*. Instead, *in vitro* elution studies provide a means for comparing relative release rates among drugs under controlled conditions and may provide an indication of the relative extent of systemic exposure *in vivo*.

One limitation of current commercially available DEE microspheres is that, in their native form, they can only load and release positively charged drugs due to the anionic nature of their polymer matrices. Other DEE microspheres with different physicochemical properties and drug delivery mechanisms may load and release other varieties of drugs [58]. Some

drugs that cannot be actively loaded into DEE microspheres may be incorporated into the microspheres using solvents or by precipitating poorly water-soluble drugs into the microsphere matrix [59, 60].

Although there is no currently approved DEE microsphere available in which the drug is pre-loaded into the microsphere matrix, a number of clinical studies have used this product format [10, 61–63]. In these studies, doxorubicin, irinotecan, or vandetanib was loaded into the microspheres by the manufacturer and lyophilized in order to produce a sterile vial of dry microspheres that were hydrated with water at the point of use. This approach has the advantage of eliminating preparation time normally needed for drug loading, providing rapid intraprocedural access to drug-loaded microspheres.

2.3 Biocompatibility and safety

Commercial DEE microspheres are required to undergo biocompatibility evaluations in order to demonstrate that the unloaded microsphere matrix has no long-term adverse effects. This may include various tests for cytotoxicity of leachable chemicals as well as short-, medium- and long-term studies of local effects including tissue reactions following DEE microsphere delivery in animals [34, 64–66].

TACE with DEE microspheres for treatment of HCC tumors is a safe procedure with reported rates of serious adverse events ranging from 6.7 to 20.4% [10, 20, 67, 68]. Post embolization syndrome following TACE with DEE microspheres, as also seen with cTACE or embolization with bland microspheres, consisting of abdominal pain, fever, nausea and vomiting has been reported in 24.7–84% of patients but is self-limiting [10, 20, 68, 69]. Transient elevations in liver enzymes including aspartate aminotransferase, alanine aminotransferase, and bilirubin are known to occur in humans [70] and animal models [71–73]. Lower rates of chemotherapy-related adverse events have been reported for TACE with doxorubicin-loaded DEE microspheres compared to cTACE and is likely attributable to lower systemic levels of doxorubicin [20].

2.4 Biodegradable DEE microspheres

The rationale for biodegradable (resorbable) DEE microspheres is that they may be used for temporary occlusion of blood vessels to permit repeat procedures or in an effort to limit the duration of tissue hypoxia which has been implicated in the stimulation of neoangiogenesis. Biodegradable DEE microspheres have been developed using natural [74–77] or synthetic [78–81] materials that degrade primarily by hydrolysis. However, depending on local hemodynamic and coagulative conditions, it is possible for biodegradable embolics (or any embolic) to cause permanent vessel occlusion if a thrombus or vessel damage persists despite microsphere degradation. Overall, the potential benefits of biodegradable DEE microspheres remain speculative.

There are a number of potentially important characteristics that should be considered when designing biodegradable DEE microspheres. For example, the microspheres should degrade into small soluble components, avoiding potential dislodgement of large fragments that could lead to non-target embolization or inflammation. In addition, drug release should occur in a controlled manner governed by or within the timeframe of microsphere

degradation. Similarly, microspheres should degrade at a desirable rate so as to balance the duration of tissue ischemia with desired vessel recanalization.

One example of a synthetic resorbable DEE microsphere was developed from poly(ethylene glycol) methacrylate (PEGMA) crosslinked with a hydrolyzable copolymer consisting of poly(lactide-co-glycolide) (PLGA) and poly(ethylene glycol) (PEG) (PLGA-PEG-PLGA) [82, 83]. To shorten the size of the polymer degradation products, additional hydrolyzable ester linkages were incorporated into the hydrogel matrix using 2-methylene-1,3-dioxepane [82, 83]. The proposed benefit of this approach is that small, water soluble degradation products can be cleared by renal elimination [84].

2.5 Antiangiogenic agents loaded into DEE microspheres

Loading and elution of various antiangiogenic agents have been evaluated with DEE microspheres [30, 32, 85, 86] and performance of the loaded microspheres tested in preclinical models [27, 73, 79, 87, 88]. TACE-induced hypoxia can stimulate neoangiogenesis through increased expression of vascular endothelial growth factor (VEGF) which can lead to local tumor recurrence [89–91]. Antiangiogenic agents, such as the multikinase inhibitor sorafenib, have shown efficacy in HCC but are associated with systemic toxicities. Local delivery of these agents with DEE microspheres may be an effective strategy to mitigate systemic toxicity and inhibit neoangiogenic pathways induced by TACE.

2.6 Radiopaque DEE microspheres

Conventional DEE microspheres are radiolucent and thus cannot be directly visualized on intra-procedural fluoroscopy or other X-ray imaging modalities. Early imageable DEE microspheres were formulated by impregnating LC Bead with Lipiodol [92] enabling intra- and post- procedural visualization of the microspheres as demonstrated in preclinical models [93–95]. To address leaching of the entrapped Lipiodol from the microspheres over time, intrinsically radiopaque embolic microspheres were developed by conjugating a portion of the hydroxyl functional groups available on DC Bead with 2,3,5-triiodobenzoic acid via a linker [12]. Later, direct conjugation was achieved using 2,3,5-triiodobenzaldehyde [11, 13, 64]. These microspheres (DC Bead LUMI™) were shown to possess acceptable catheter deliverability, conspicuity on intra- procedural fluoroscopy, and persistent and stable image-ability on follow-up CT [11, 14, 64, 96]. It should be noted that the radiopaque microspheres do not shrink upon drug loading, in contrast to non-radiopaque DC Bead [97]. Recently, small diameter (40–90 μm) radiopaque microspheres were introduced to promote more distal arterial distribution [34].

Use of radiopaque DEE microspheres in humans has demonstrated real-time feedback regarding anatomic localization of microspheres during TACE [14, 98, 99]. Although the clinical utility of radiopaque DEE microspheres continues to be evaluated, it is postulated that they may facilitate refinement of treatment endpoint, visualization of non-target embolization, and early detection of undertreated regions of a tumor thus enabling same-procedure completion of treatment [14]. It has also been proposed that

microsphere radiopacity and distribution on imaging may serve as a surrogate for local drug concentrations enabling spatial drug dosimetry [15].

3. Drug effects and distribution following TACE with DEE microspheres

3.1 Drug vs ischemia

Results comparing the outcomes of embolization with bland microspheres and doxorubicin-loaded DEE microspheres have been mixed, fueling an ongoing debate as to the relative contributions of ischemia and drug to the efficacy of TACE [100]. There is evidence suggesting that both ischemia and drug may contribute to therapeutic effects. Some preclinical and clinical studies have demonstrated greater local cytotoxic effects surrounding DEE microspheres compared to bland microspheres [87, 101–104]. A retrospective study found that TACE with DEE microspheres loaded with epirubicin resulted in greater tumor necrosis compared to embolization with bland microspheres in embolized livers explanted for liver transplantation [105]. In a single center prospective randomized trial, TACE with DEE microspheres demonstrated superior time to progression than bland microspheres [69]. However, a single center randomized study by Brown *et al.* found no difference in response using RECIST 1.0 criteria as primary outcome between DEE microspheres and bland microsphere embolizations, although different bland and DEE microspheres were used [68].

3.2 Factors that influence drug distribution

To be most effective, chemotherapeutic agents must penetrate tissue effectively, maximizing the number of cells exposed to therapeutic drug concentrations. The extent to which this requirement applies to immunotherapeutic agents is not known, since exposure of discrete immunologic targets may be sufficient to stimulate systemic antitumor immune responses. There are a number of factors that contribute to the intratumoral distribution and effects of drugs delivered using DEE microspheres for TACE.

3.2.1 Microsphere distribution—Due to the relative colocalization of drug and microspheres, the extent of tumor drug coverage is largely dependent on the distribution of microspheres within the tumor vasculature. Indeed, the volume and attenuation of radiopaque DEE microspheres measured on CT in spatially discrete tumor samples was found to positively correlate with the amount of doxorubicin measured in the samples [15]. Upon injection, DEE microspheres are carried by blood flow and thus preferentially accumulate in regions of high arterial blood supply commonly associated with certain tumors. Smaller DEE microspheres become lodged more distally from the catheter within arteries and are therefore capable of providing superior drug coverage compared to larger microspheres prone to more proximal vessel occlusion [95, 106].

3.2.2 Drug release—To exert any effect, the drug must first be released from the microspheres. The amount of bioavailable drug, i.e. the fraction of drug eluted, in the tumor over time depends on the rate of drug release from the microspheres and rate of clearance from the tumor by washout or metabolism. *In vivo*, the drug release profile from DEE

microspheres may be defined by several phases with differing rate-determining mechanisms (Figure 1).

Drug release is most rapid immediately following DEE microsphere infusion, due in part to the high surface area of microspheres exposed to ions in blood and the “burst” release that is characteristic of initial drug release from DEE microspheres. As microspheres accumulate in arteries, blood flow is gradually reduced until flow stasis is reached and a microsphere-thrombus mass is formed. During this phase of the release profile, diffusion of ions into and drug out of the microspheres slows. Elevated drug levels in tissue immediately surrounding the microspheres may further reduce the rate of drug diffusion from the microspheres as the concentration gradient between the microspheres and surrounding tissue diminishes and equilibrates. This phenomenon may be particularly relevant for drugs that penetrate poorly into the tumor extravascular space, resulting in elevated concentrations in the immediate vicinity of the microspheres and prolonging drug release. In the case of doxorubicin, *in vivo* drug release can occur over a period of at least one month [108].

3.2.3 Tissue penetration—Intratumoral drug distribution is subject to a number of biologic barriers that can inhibit tumor drug penetration and coverage including large intervascular distances, dense cellular and extracellular compartments, and elevated interstitial fluid pressure (Figure 2). Drug transport in tumors is also influenced by the physicochemical characteristics of the drug including molecular size, charge, and solubility as well as the extent of cell uptake and binding [109–112]. Once released from DEE microspheres in tissues, drug transport is likely to occur predominantly by diffusion since convection is reduced or eliminated following embolization of blood vessels. Some studies have evaluated the distribution of drugs in tumors following embolization with DEE microspheres and found that doxorubicin remains within ~ 600 μm radially of the microspheres [15, 95, 103, 108], in contrast to sunitinib which penetrates further into surrounding tissues [113].

It is possible that tumor drug penetration following drug release from DEE microspheres may be improved by co-delivery of microenvironment modulators, such as drugs that normalize or remodel blood vessels or the extracellular matrix. Similarly, locoregional therapies (LRT) or other technologies, such as high intensity focused ultrasound or irreversible electroporation, may potentiate drug distribution by heating, convection, or mechanical disruption or permeabilization of tissue.

3.3 The tumor microenvironment

The tumor microenvironment plays an important role in determining local drug effects. Even if a drug is homogeneously distributed throughout a tumor, its effects may differ from one region to another due to spatial heterogeneity in cell phenotypes, proliferation rates, metabolism, and tissue oxygenation [114]. This may be particularly relevant for conventional cytostatic drugs that exert their effects in a cell cycle dependent manner. Tissue hypoxia induced by embolization may affect the susceptibility of cells to certain drugs such as doxorubicin, potentially increasing the dose required to achieve cytotoxic effects or selecting for a more aggressive tumor phenotype [115, 116]. However, the exact mechanisms

by which potential changes in the tumor microenvironment post-embolization, including possible alterations in interstitial fluid pressure, microvascular perfusion, and tumor stromal content, influence drug distribution and therapeutic effects require further exploration [117–120]. DEE microspheres loaded with hypoxia-activated or antiangiogenic drugs may be a promising strategy to exploit changes within the tumor microenvironment resulting from TACE.

3.4 Measuring drug distribution in tumors following TACE with DEE microspheres

A commonly employed method to assess drug accumulation in preclinical tumor models consists of tumor tissue homogenization, drug extraction, and quantification. Although this approach provides information regarding the total amount and concentration of drug in the entire tissue sample, information regarding the spatial distribution of drug in the sample is lost. For investigations of TACE with DEE microspheres, this approach additionally fails to differentiate between the eluted drug fraction, which is bioavailable, and the amount of drug sequestered in the microspheres, which does not contribute to the therapeutic effect. A number of techniques that rely on tissue homogenization have been employed to evaluate drug concentrations in tumors following TACE with DEE microspheres, but relatively few techniques have been used that preserve drug spatial distribution relative to the microspheres (Table 2).

For drugs that are naturally fluorescent, such as doxorubicin and sunitinib, spatial drug concentrations can be evaluated in tumor sections using fluorescence microscopy (Figure 3) [15, 95, 113]. However, quenching of fluorescence signal can restrict accurate quantification of the fraction of drug retained in the microspheres. To avoid this problem, infrared microspectroscopy has been used to quantify drugs inside microspheres and microspectrofluorimetry to detect drug in surrounding tissue [103].

For drugs that are not fluorescent, their distribution may be measured by radiolabeling, or, in some cases, inferred by assessing specific drug bioeffects as a surrogate [128]. For example, qPCR and microdissection were used to measure interleukin-6 in tissue as a marker of ibuprofen released by DEE microspheres [129].

4. DEE microspheres: emerging paradigms and innovations

4.1 Drug dosimetry

The advent of intrinsically radiopaque DEE microspheres has opened the door to the possibility of estimating spatial drug concentrations on intraprocedural CBCT or postprocedural CBCT or multidetector CT using microsphere radiopacity as a surrogate [130]. Recently, the volume and attenuation of radiopaque DEE microspheres measured on CT was shown to be linearly proportional to the amount of doxorubicin measured in liver samples in a rabbit model [15]. This correlation was then used to predict the amount of doxorubicin in the liver based on CT. One can imagine that similar models could enable real-time intraprocedural estimation of spatial drug dosimetry using CBCT to identify regions of tumor at risk of undertreatment (Figure 4). A limitation of this approach is that the ability to image radiopaque microsphere distribution clinically is limited by the

spatial resolution of imaging systems [131]. Further study is required to better understand the relationship between the imaged three-dimensional distribution of radiopaque DEE microspheres, drug distribution, and therapeutic effects of TACE. The ability to predict local drug levels could help to clarify the relative contributions of drug and ischemia to treatment efficacy.

4.2 Imaging of radiopaque DEE microspheres for rational drug targeting

The ability to image radiopaque microspheres with standard CBCT or multidetector CT is limited by the spatial resolution of both technologies [131]. The image is based on X-ray absorption alone and cannot be used to discriminate between different absorbers, such as calcium, iodine or bismuth. Spectral imaging with dual energy CT (DECT) generates two scans with different X-ray energy spectra that can be used to selectively identify a single k-edge absorber, e.g., iodine, in images and enables limited differentiation between two materials with different k-edge energies [132, 133]. Photon-counting CT systems under development use X-ray detectors that can quantify the number and energy of incident photons, enabling material decomposition for more than one radioabsorber, based on k-edges and measured absorption spectra [16, 134, 135]. These techniques could be used to differentiate DEE microspheres containing different radiopacifiers, such as iodine, bismuth or tantalum, from soluble contrast agents used during microsphere delivery or from each other. Furthermore, photon-counting CT systems offer higher spatial resolution than current CT scanners and may allow more complete imaging of radiopaque bead distribution [136].

Preliminary work on the synthesis of bismuth-containing microspheres and their differentiation on imaging from soluble iodinated contrast using DECT has been reported [17, 137]. Bismuth microspheres may be distinguished from iodine-based liquid contrast on photon counting CT and, by extension, from iodinated microspheres as its k-edge energy (90.52 KeV) is higher than that of iodine (33.2 KeV) [16, 135]. Although speculative, radiopaque DEE microspheres containing different radiopacifiers and loaded with different drugs could provide a means for rational targeting of different drugs to different regions of tumor based on their mechanisms of action, with the option to uniquely image their distribution with spectral or photon-counting CT (Figure 5).

4.3 DEE microspheres and immunomodulation

It has been hypothesized that the number of patients that respond to immunotherapy may be improved by combination with LRT such as transarterial chemoembolization, radioembolization, and thermal ablation (Figure 6) [138]. The rationale is that destruction of tumor cells induced by LRT may enhance tumor immunogenicity by promoting the expression of tumor-associated antigens and accumulation of tumor-infiltrating lymphocytes (TILs). Recently, augmentation of LRT-induced immune activation by concomitant systemic administration of check point inhibitors (CPI) was tested in a phase II trial and found to be safe and effective in a subset of patients with HCC refractory disease [139]. However, low response rates and systemic toxicities remain limiting characteristics of immunotherapy.

Strategies to increase tumor immunogenicity are being actively persued in several clinical trials delivering CPI with antiangiogenic agents with or without LRT [140]. Among the

immune effects caused by antiangiogenic agents are restoration of dendritic cell maturation, and reduction of T-regulatory cells [141, 142]. Enhancement in recruitment and activation of CD8+ T cell response has also been reported [143–146]. A recent clinial study demonstrated better overall and progression-free survival for the systemic combination therapy of atezilumab, an anti-PD-L1 monoclonal antibody, and bevacizumab, an anti-VEGF monoclonal antibody, compared to sorafenib. However, severe adverse events were reported in 56% of the patients in the combination arm [147].

Another promising approach to augment antitumor immune responses is by pharmacologic targeting of immune stimulatory pathways including but not limited to STING, toll-like receptors (TLR) [148, 149], VISTA, TIM-3, Btk/Itk, and SHIP1 [150–152]. However, these agents have potentially serious non-target side effects when delivered systemically. Intratumoral bolus injection may require multiple or repeated administrations due to rapid clearance from the tumor and poor intratumoral distribution. DEE microspheres may enable better control of the pharmacokinetics and distribution of immune modulating agents, with the potential to approximate metronomic or continuous delivery schedules. To date, there is limited clinical experience with transarterial delivery of immune modulators [153–155].

There are currently a number of clinical trials underway exploring the combination of LRT with immunotherapy [156]. The potential systemic toxicity of many immune-modulating agents suggests that they may be ideal candidates for tumor-localized delivery with DEE microspheres. However, loading of monoclonal antibodies in DEE microspheres is poor. Recently, small molecule CPI targeting the PD-1/PD-L1 pathway have been developed that may provide greater flexibility of route of administration, improved toxicity profiles, and reduced costs compared to antibody-based CPIs [157, 158]. However, more research is needed to effectively combine immune modulating agents with DEE microspheres and to optimize drug selection, loading, and elution profiles.

5. Expert opinion

TACE is a minimally-invasive LRT that employs catheters and imaging-based vascular “roadmaps” to deliver therapy directly to tumors, precisely where it is needed. This exquisite access to tumors presents vast opportunities for locoregional drug delivery using controlled release drug delivery systems, such as DEE microspheres, to reduce systemic drug exposure, increase intratumoral drug concentrations, and provide greater control over spatial and temporal drug distribution.

Doxorubicin remains the most commonly used drug for TACE despite its abandonment as a systemic treatment for HCC and evidence suggesting other drugs may have greater activity *in vitro* [159]. Outcomes of trials comparing drug-loaded microspheres to bland microspheres have been mixed, further fueling the debate as to the contribution of doxorubicin to treatment efficacy and whether it is the optimal drug for delivery with DEE microspheres. Nevertheless, pre-treatment biopsies may soon dictate rational drug selection, based on single cell transcriptomics with clusters of common mutations or RNA expression patterns that define susceptibilities to TACE [160]. Yet, mutational heterogeneity within a

single tumor and between synchronous multifocal tumors may limit the ability to capture the full mutational landscape with a single biopsy specimen.

A similar scenario may be envisioned in which drug selection could be informed non-invasively using imaging features to identify potential therapeutic targets. For instance, multi-parametric AI and deep learning models trained with digital pathology, genomic data, and imaging could potentially identify the most effective combination of DEE microspheres and drugs, and where to deliver them, based on pre-treatment imaging. Indeed, different drugs could be specifically delivered to discrete regions of tumors based on surrogate imaging markers that correlate with specific tumor phenotypes, molecular signatures, or immunologic status. Imageable DEE microspheres containing different radiopacifiers distinguishable using photon counting CT could facilitate image-guided spatial drug delivery of multiple agents.

As our understanding of post-embolization changes in the tumor microenvironment grows, so do the opportunities to use drugs that specifically target local conditions created by TACE. Tumor hypoxia may be a double edge sword: a natural consequence of ischemia induced by embolization that causes necrosis, but also a promoter of neoangiogenesis leading to local recurrence. The hypoxic microenvironment may present a rationale for use of hypoxia-activated drugs or other targeted agents as a means to enhance drug efficacy. Vascular agents that target VEGF or hypoxia-inducible factor 1-alpha (HIF1alpha) may serve to limit angiogenesis induced by tumor hypoxia, regarded as a mechanism of local tumor recurrence after TACE, or even promote beneficial antitumor immune effects. A number of multikinase inhibitors of various other pathways remain to be explored in combination with DEE microspheres and present numerous intriguing possibilities for local delivery. Counteracting mediators of pro-oncogenic effects following LRT such as TACE, for example by suppressing tumor stress responses by local delivery of inhibitors using DEE microspheres, may also be possible [161].

The recent success of immunotherapy in the form of checkpoint inhibitors has ushered in a new era of cancer therapy. However, many of these treatments are limited by poor response rates and undesirable systemic and non-target effects. There is growing evidence that response rates to immunotherapy may be augmented by LRT, based on the observation that LRT may elicit immunologic cell death and enhance tumor-antigen presentation. In this way, LRT and local administration of immune modulating agents, for example by delivery using DEE microspheres (“immunobeads”), may help to convert tumors from immunologically “cold’ desert or immune-excluded tumors to “hot” and immune-susceptible, in order to expand the number of patients that may benefit from immunotherapy. Heterogeneous regions of tumors with more aggressive and active clonality or neoantigens could be identified with imaging, then targeted for destruction or enhancement of antigen release and presentation via TACE, ablation, or DEE microspheres loaded with immune modulators.

The diverse types of immune cells, receptors, and molecular pathways that could potentially be targeted by drugs presents a wealth of opportunities for local drug delivery with DEE microspheres. Many of these agents may be used to target specific inherent tumor characteristics, potentially identified by next-generation sequencing technologies, or

biologic processes initiated by TACE itself. As we move beyond the use of conventional chemotherapeutic agents for TACE, an emphasis on systematic comparative preclinical and clinical studies, supported by collaborative, inter-disciplinary research efforts, will be crucial to expedite the translation of next-generation DEE microsphere–drug combinations for clinical use.

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Abbreviations

cTACE	Conventional Transarterial Chemoembolization
CPI	checkpoint inhibitor
DEB	Drug-eluting bead
DECT	dual energy CT
DEE	drug-eluting embolic
HCC	hepatocellular carcinoma
LRT	locoregional therapy
PCCT	photon counting CT
TACE	transarterial chemoembolization
TLR	toll-like receptor
VEGF	vascular endothelial growth factor

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Article highlights

- Drug-eluting embolic microspheres / beads serve as an embolic agent to stop blood flow to tumors and as a drug delivery vehicle for transarterial chemoembolization (TACE) of hepatic malignancies.
- DEE microspheres increase drug concentrations in tumors and reduce systemic drug exposure compared to conventional drug delivery methods
- Rational selection of drugs for combination with DEE microspheres could exploit potential synergy between mechanisms of drug activity and biologic responses to TACE to enhance efficacy.
- Image-able DEE microspheres may enable “image-guided drug dosimetry” for rational drug targeting of specific drugs to discrete microenvironments within heterogeneous tumors.
- Combinations of DEE microspheres and immune-modulating agents could provide potent local and systemic therapeutic effects by promoting anti-tumor immune responses generated by TACE

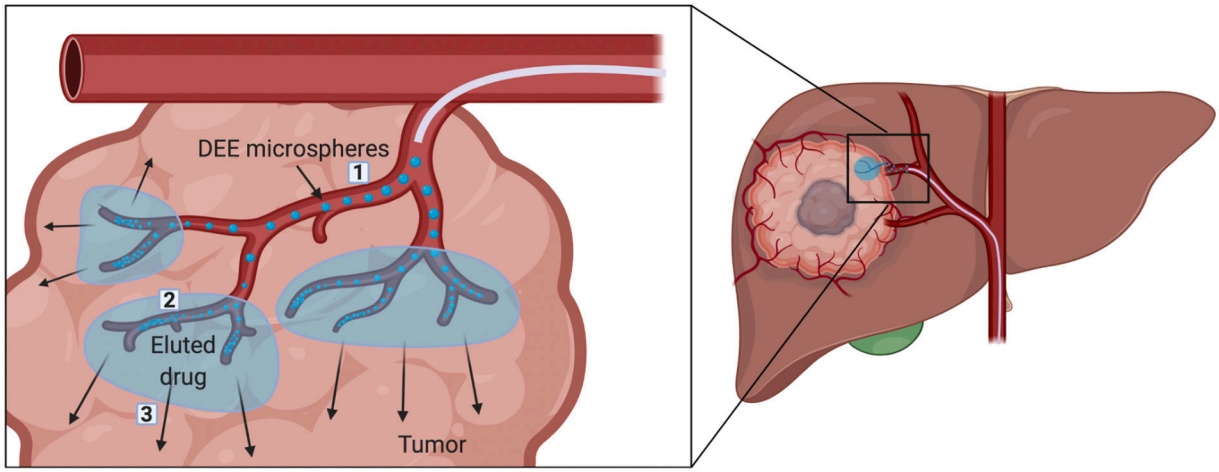

Figure 1.

Schematic representation of theoretical phases of DEE microsphere-based drug delivery, *in vivo*. 1) DEE microspheres are injected via intra-arterial catheter into hepatic arteries supplying the tumor. Drug release is rapid as DEE microspheres are transported with high surface area exposed to flowing blood rich in ions. 2) DEE microspheres become packed in arteries, reducing the overall surface area exposed to blood. Blood flow begins to slow and the rate of drug release diminishes. A blood clot is formed, reducing or eliminating blood flow, and further reducing the rate of drug release. 3) The eluted drug traverses the blood vessel wall and penetrates into the interstitial space primarily by diffusion. Adapted from [107] with permission from Elsevier.

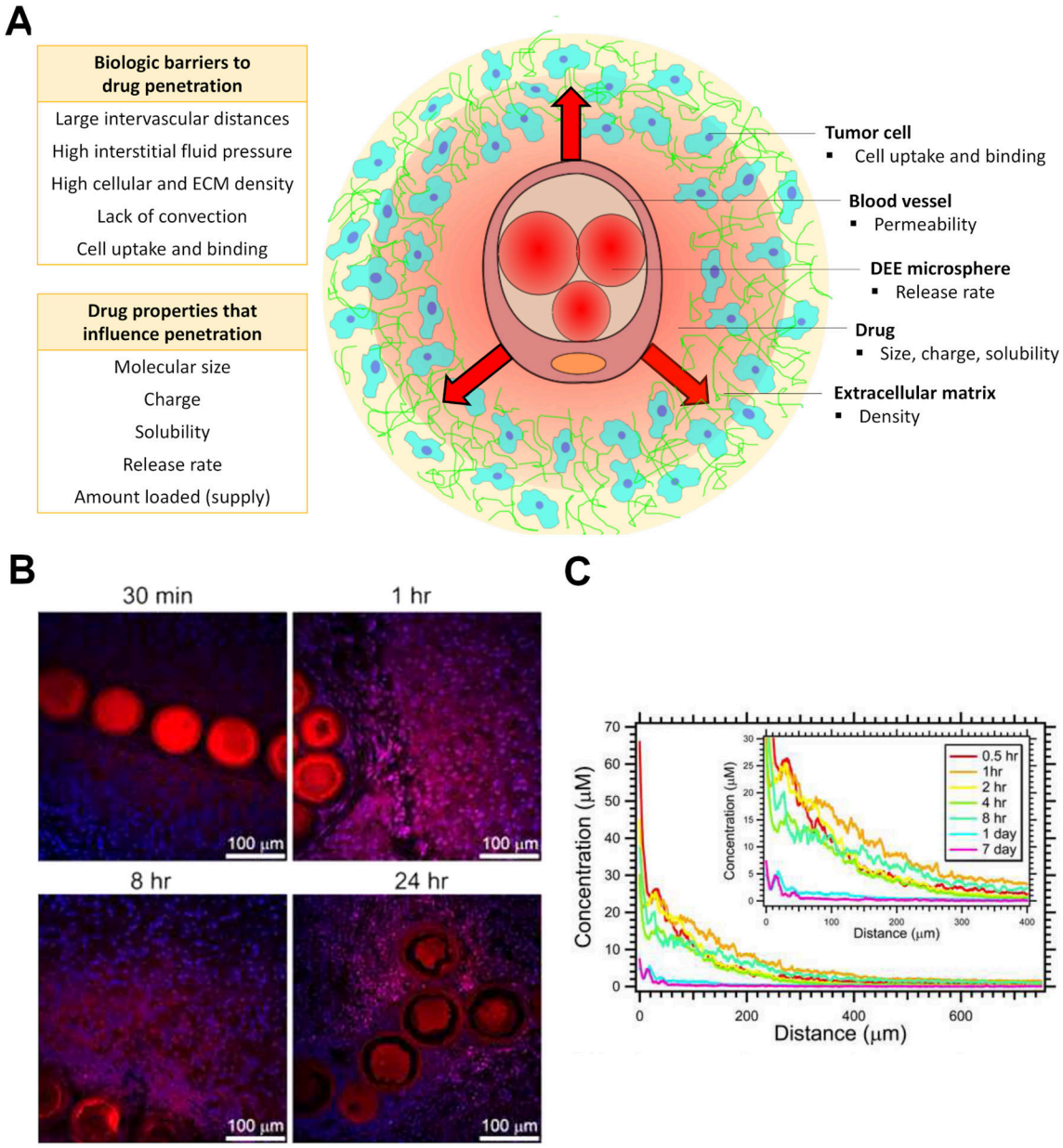

Figure 2.

(A) Factors that may affect drug penetration in tumors after release from DEE microspheres. (B) Distribution of doxorubicin (red) relative to DEE microspheres and cell nuclei (blue) over time following TACE in swine liver. C) Doxorubicin concentration versus distance from microspheres in swine liver embolized with 70–150 µm DEE microspheres. B and C reproduced from [95] with permission from Elsevier.

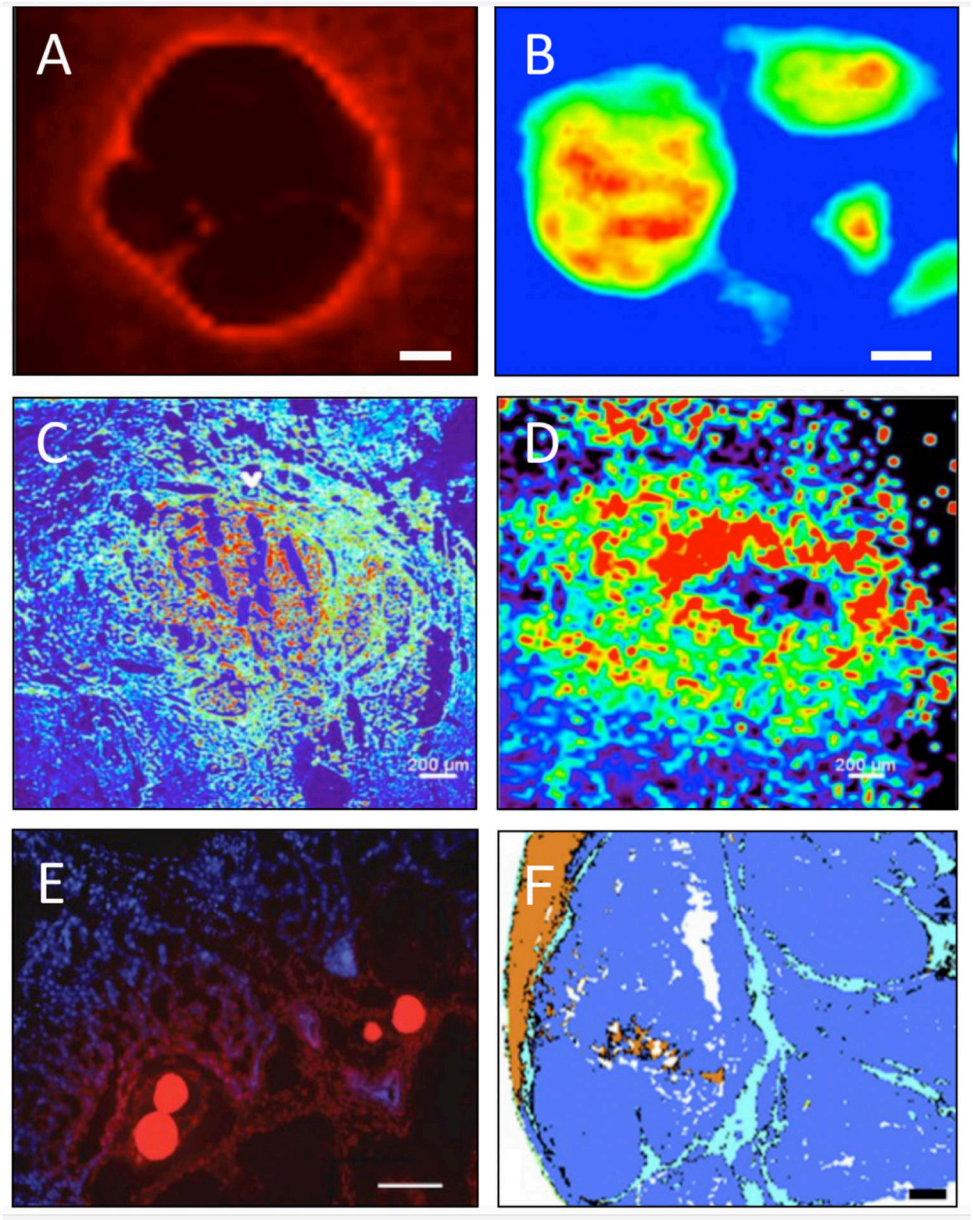

Figure 3.

Techniques used to assess drug distribution or treatment effects in tumors after embolization with DEE microspheres. A) Fluorescence microspectroscopy image of eluted doxorubicin surrounding microspheres in a blood vessel (scale bar 50 μm) and B) infrared microspectroscopy demonstrating quantification of doxorubicin inside microspheres, 28 days post embolization in swine liver (scale bar 70 μm) [103]. C) Fluorescence imaging (pseudo-colored intensity heatmap) and D) mass spectrometry imaging of sunitinib in a tissue section from a rabbit VX2 tumor embolized with sunitinib-eluting microspheres [113]. E) Fluorescence microscopy image of rabbit VX2 tumor section containing doxorubicin-loaded radiopaque microspheres showing doxorubicin (red) *in vivo* elution into tissue (blue cell nuclei) 1 hour after embolization [15]. F) Automated identification of tumor

necrosis (blue), fibrosis (light blue), and liver parenchyma necrosis (brown) using infrared microspectroscopy-based prediction model in a rabbit VX2 tumor section (scale bar 1 mm) [127]. A, B, C, D, and F reproduced with permission from Elsevier. E reproduced with permission from The Radiological Society of North America.

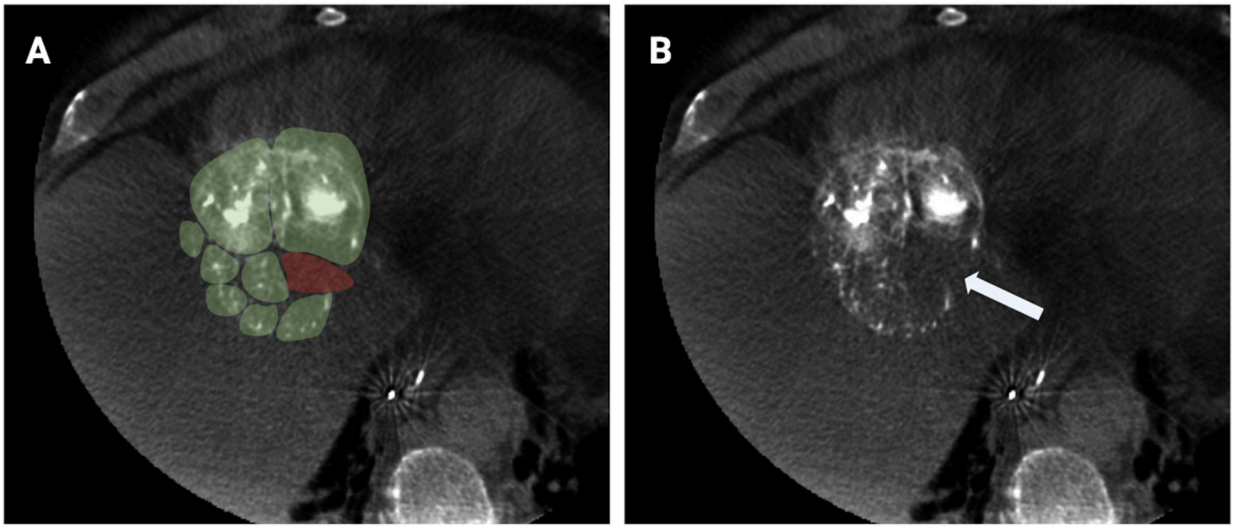

Figure 4.

Hypothetical representation of predictive models of spatial drug dosimetry based on radiopaque DEE microsphere attenuation and distribution in an HCC tumor. A) Axial CBCT slice of HCC tumor with green overlay representing hypothetical regions of tumor adequately treated with drug, and red overlay representing potentially undertreated regions. B) CBCT of same tumor showing a paucity of radiopaque DEE microspheres in the region that was illustrated as undertreated. More research is needed to transform this illustration into a method for quantitative prediction of drug distribution and therapeutic effects based on imaged distribution of radiopaque DEE microspheres.

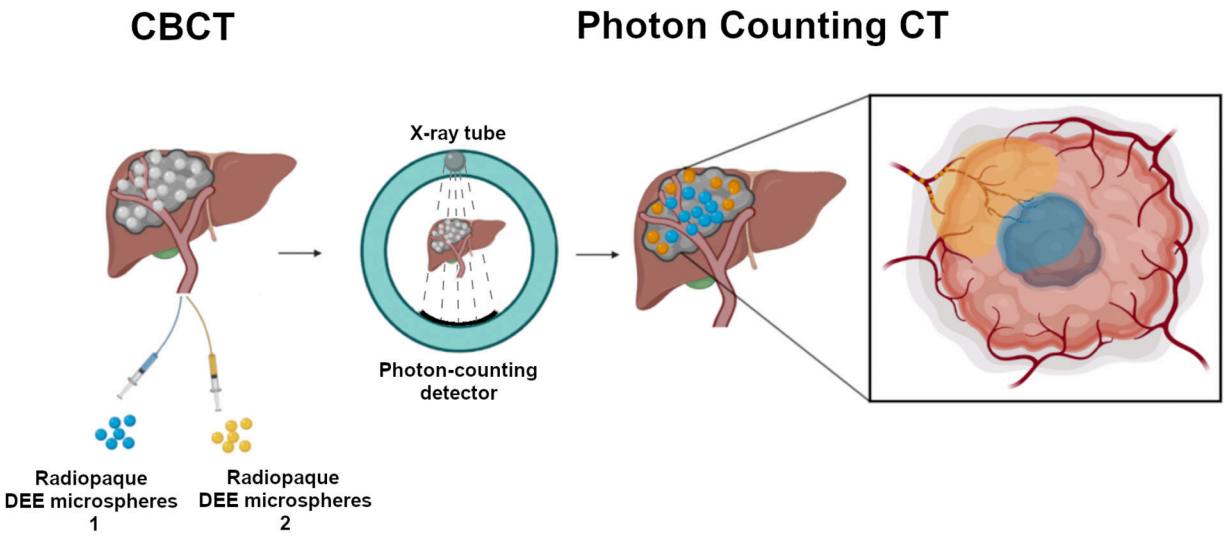

Figure 5.

Concept of "Dual Drug – Dual Microsphere" TACE: delivery of DEE microspheres containing different radiopacifiers and different drugs to specific regions within a tumor, imaged with photon counting CT. Microspheres containing different radiopacifiers and loaded with different drugs are delivered sequentially to different parts of the tumor for rational targeting of discrete intratumoral regions based on drug mechanism of action and expected tumor microenvironment. For example, microspheres with a hypoxia activated drug (blue) may be delivered to hypoxic tumor regions while microspheres with an immune-modulating agent may be directed to immunologically active regions at the tumor margin (yellow). The microspheres cannot be distinguished based on standard unfiltered CBCT (left). Subsequent photon counting CT shows spatial distribution of the two DEE microspheres with different radiopacifiers (center) allowing inferences as to the distribution of the two associated drugs, as shown on the inlay.

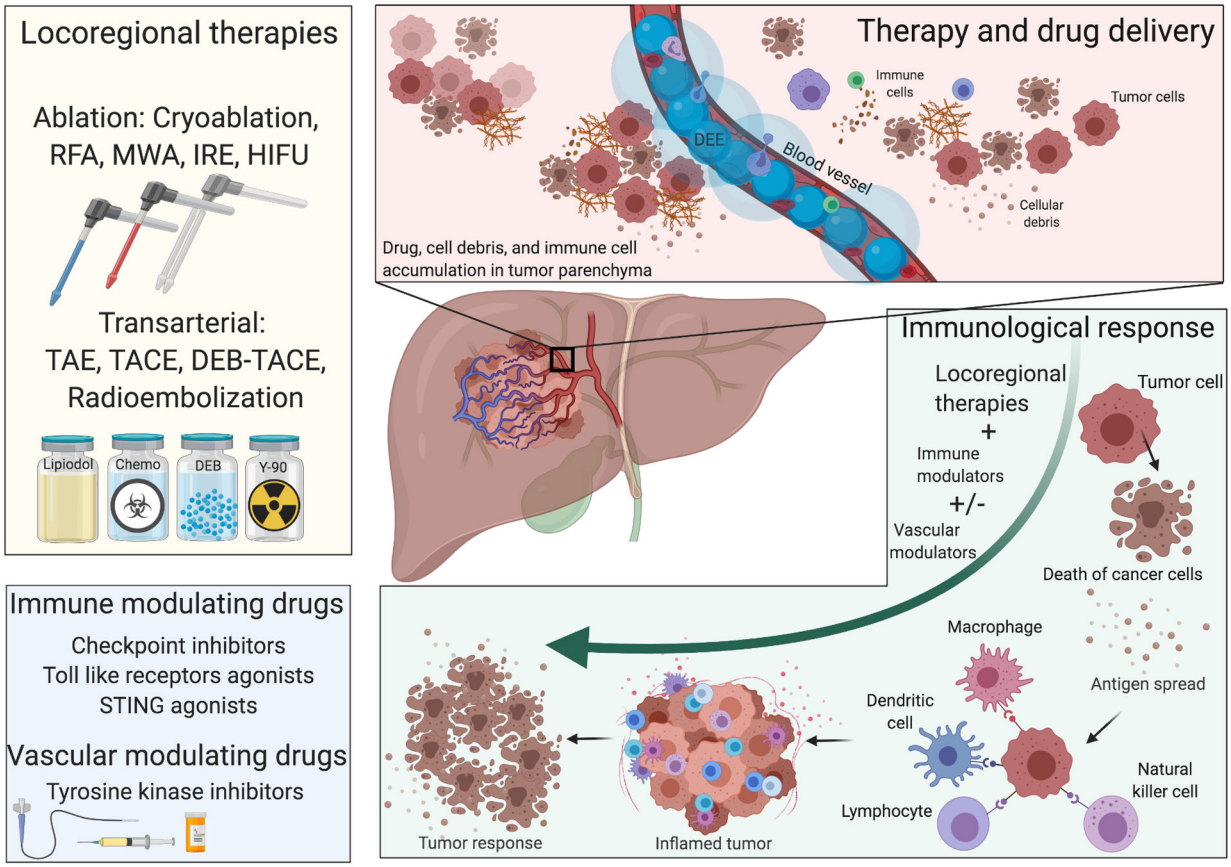

Figure 6.

Immune effects and potential therapeutic strategies using DEE microspheres loaded with immune-modulating agents with or without vascular modulators. Clinically approved drugs as well as novel immune modulators (bottom left) can be delivered in combination with locoregional therapies (top left). This potentially includes TACE in which the immune modulators may be loaded into DEE microspheres as a novel treatment paradigm in HCC. The hypothesis is that intra-arterial delivery of these drugs using DEE microspheres may reduce systemic toxicities, provide greater control over drug spatio-temporal distribution, and promote local immunologic effects initiated by TACE. For example, antigens and cell debris generated by TACE or other locoregional therapies can accumulate in the tumor microenvironment (top right) and trigger a cascade of immunologic events, promoted by immune-modulating agents delivered locally at the site, that include recruitment of immune cells and cytokine release ultimately leading to promotion of antitumor immune responses (bottom right). (RFA: radiofrequency ablation; MWA: microwave ablation; IRE: irreversible electroporation; HIFU: high intensity focused ultrasound; TAE: transarterial embolization)

Table 1.

Characteristics of DEE microspheres and drugs with CE marking, and other drugs that have been loaded.

Commercial Name	Material	Size (μm)¶	Non-degradable or biodegradable	Examples of drugs loaded	References
DC Bead™#(Boston Scientific, USA)	Polyvinyl alcohol (PVA) cross-linked with 2-acrylamido-2-methyl propane sulfonate	70–150, 100–300, 300–500, 500–700	Non-degradable	doxorubicin*, irinotecan*, epirubicin, sorafenib, sunitinib, idarubicin, topotecan, mitoxantrone, bevacizumab§, rapamycin‡	[24–31]
DC Bead LUMI™#(Boston Scientific, USA)	PVA cross-linked with 2-acrylamido-2-methyl propane sulfonate followed by conjugation of iodinated moiety	40–90 (“M0”), 70–150 (“M1”)	Non-degradable	doxorubicin*, irinotecan*, topotecan, vandetanib	[13, 32–34]
Embozene TANDEM™//(Varian Medical Systems Inc., USA)	Poly(methylmethacrylate) coated with poly(bis[trifluoro-ethoxy]phosphazene)	40±10, 75±15, 100±25	Non-degradable	doxorubicin*, irinotecan*, idarubicin	[35–37]
HepaSphere™//(Merit Medical Systems, USA)	Sodium acrylic acid-vinyl alcohol copolymer	30–60, 50–100, 100–150, 150–200	Non-degradable	doxorubicin*, irinotecan, epirubicin, idarubicin, oxaliplatin, cisplatin	[37–43]
LifePearl®(Terumo European Interventional Systems, Belgium)	Polyethylene glycol (PEG) and 3-sulfopropyl acrylate	100±25, 200±50, 400±50	Non-degradable	doxorubicin*, irinotecan*, idarubicin*, epirubicin*	[37, 44–46]
BioPearl®†(Terumo European Interventional Systems, Belgium)	–	–	Biodegradable	doxorubicin*, idarubicin*, epirubicin*	[47]

*Drug CE marked for loading in the corresponding listed DEE microspheres. The remaining drugs are not approved for use with the listed microspheres but their loading has been reported in pre-clinical or clinical investigations. No microspheres are approved for use as drug-eluting devices in the USA.

#DC Bead and DC Bead LUMI are cleared for marketing within the US as bland embolic devices.

//Embozene TANDEM and HepaSphere are marketed as Embozene™ and QuadraSphere®, respectively, in the US as bland embolic devices.

¶Size ranges reported by vendor

§Loaded using layer-by-layer microsphere coating

‡Drug loaded by precipitation within microspheres

†Information based on vendor press release

Table 2.

Examples of techniques used to measure drug in tumors following TACE with DEE microspheres.

Technique	Examples of drugs measured	Sample preparation	Drug concentration measured in tissue	Drug spatial distribution measured in tissue	Drug measured in microspheres	
LC-MS/MS	Doxorubicin / doxorubinicol, sunitinib, vandetanib,irinotecan (CPT-11, SN38, SN38-G)	Homogenized tissue or biopsy	✓	X	X	[7, 62, 65, 73, 121–123]
LC-FD	Doxorubicin	Homogenized tissue	✓	X	X	[15]
Fluorescence microscopy	Doxorubicin, sunitinib	Tumor section	✓	✓	X	[7, 15, 95, 113]
Autoradiography	^{14}C doxorubicin	Whole-body section (rat)	✓	✓	X	[124]
Atomic absorption spectroscopy	Doxorubicin / doxorubinicol	Homogenized tissue	✓	X	X	[102]
Microspectrofluorimetry	Doxorubicin	Tissue section	✓	✓	X	[103, 108, 122]
Infra-red microspectroscopy	Doxorubicin, ibuprofen	Tissue section	X	X	✓	[103, 125]
Mass spectroscopy imaging	Sunitinib and metabolites	Tissue section	✓	✓	X	[113]
Atomic fluorescence	Arsenic trioxide	Homogenized tissue	✓	X	X	[126]