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Traditional and emerging applications of microspheres: A Review

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Abstract: The uses of particulate drug delivery systems covers all areas of medicine such as cardiology, endocrinology, gynecology, immunology, pain management, oncology and molecular biology. Microsphere is already having an impact on products as diverse as novel foods, medical devices, chemical coatings, personal health testing kits, sensors for security systems, water purification units for manned space craft, and high throughput screening techniques. Most of the advanced drug delivery systems utilize microspheres or microcapsules for the encapsulation of drugs and proteins. The purpose of writing this review was to compile the various traditional and recent applications of microspheres.

Key words: Traditional and emerging applications of microspheres

Introduction

Microspheres have many applications in medicine, with the main uses being for the encapsulation of drugs and proteins. Traditionally microspheres are used as drug carriers by delivering drug to a localized diseased state. The drug loaded microspheres are delivered to the target area by passive means (trapping by size) or active means (magnetic targeting) and slowly release the encapsulated drug over a desired time period, the length of which is determined by the drug's biological half-life and release kinetics of the microsphere matrix. The bio-distribution and final fate of the microsphere is highly dependent on their size and surface charge. Even very unstable substances such as hormones, interferon[1] or neuro active peptides[2] can be given in once daily dose instead of several daily injections. Oral applications of insulin are also possible.[3]These systems have significant importance in biomedical applications. Microspheres also has novel application in the foods, medical devices, chemical coatings, personal health testing kits, sensors for security systems, biochemical sensors water purification units for manned space craft, and high throughput screening techniques. The purpose of writing this review was to compile the recent literature with special focus on the emerging applications of microspheres.

Applications of microspheres:

Microspheres sized 10 to 30 µm are larger than capillaries and will be trapped in the first capillary bed that they encounter. This effect is used for radioembolization therapy in which microspheres are injected into the artery that leads to the tumor of interest. Positively charged microspheres sized in the micrometer range are quickly taken out of the blood pool by the reticuloendothelial cells of the liver and spleen[4]. Particles smaller than 0.1 µm are able to pass the fenestration in the liver and may be able to target the hepatocytes, although most are still taken up by the liver's Kupffer cells. Negatively charged or neutral nanospheres such as small PEG-coated nanospheres or liposomes can evade this fast uptake and circulate in the blood system for up to several days[5]. A more active way of increasing the concentration of nano- or microspheres in the target tissue is to bind antibodies against the target cells on the nanospheres' surface[6].Alternatively, nanospheres, microspheres and colloids with a high affinity for white blood cells can

be prepared. Such particles are rapidly taken up by the white blood cells and then concentrate in inflammatory regions because of chemotaxis and phagocytosis[7]. The brief outline of various applications of microspheres are explained as follows.

(A) Magnetic microspheres:

Magnetic drug delivery by particulate carriers is a very efficient method of delivering a drug to a localized disease site. Fig: 1 highlights the concept of magnetic targeting. In magnetic targeting, a drug or therapeutic radioisotope is bound to a magnetic compound, injected into a patient's blood stream, and then stopped with a powerful magnetic field in the target area[8].

Depending on the type of drug, it is then slowly released from the magnetic microspheres. It is thus possible to replace large amounts of freely circulating drug with much lower amounts of targeted magnetically to locally diseased sites, reaching effective upto several fold increased localized drug levels[9,10,11].

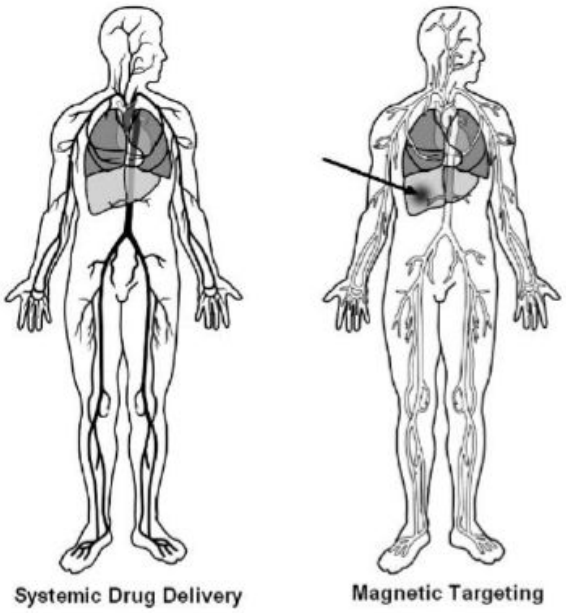

Fig 1: Concept of magnetic targeting

Magnetic carriers receive their magnetic responsiveness to a magnetic field from incorporated materials such as magnetite, iron, nickel, cobalt, iron-boron or samarium cobalt. Matrix materials that have been tested for the magnetic microspheres includes Chitosan, dextran, poly (lactic acid), starch, poly(vinyl alcohol), polyalkylcyanoacrylate, polyethylene imine, carbon, polysaccharides, gelatin and proteins.

(A). (1) Therapeutic magnetic microspheres:

Magnetic targeting can be used to deliver chemotherapeutic drugs to liver tumors and also therapeutic radio isotopes. The advantage of this method over external beam therapy is that the dose can be increased, resulting in improved tumor cell eradication,without harm to nearby normal tissue[12]. Magnetic targetted carriers which are more magnetically responsive iron carbon particles, have been radiolabelled with isotopes such as ^{188}Re, ^{90}Y, ^{111}In and ^{125}I[13]. Similar to chemotherapeutic drugs, many other drugs including peptides and proteins can be absorbed or encapsulated into magnetic microspheres. A very recent development in the field of magnetic targetting is the use of magnetically enhanced gene therapy. Advantages of such an approach are targetted gene transfection at rapid speed and high efficiencies[14].

The magnetic component in microspheres can also be used for purposes other than targetting. This is possible by filling an additional magnetic component into capsules or tablets. The speed of travel through the stomach and intestines can then be slowed down at specific positions by an external magnet, thus changing the timing and/or extent of drug absorbtion in stomach or intestines[15].

(A). (2) Diagnostic magnetic microspheres:

It acts as contrast agents for magnetic resonance imaging. Smaller supramagnetic iron oxides have been developed into unimodular nanometer sizes and have since 1994 been approved and used for the imaging of liver metastases or to distinguish loops of the bowel from other abdominal structures[16].

(B) Radioactive microspheres:

(B).(1)Therapeutic radioactive microspheres:

Many radio labeled microspheres are appropriate for therapy once the encapsulated diagnostic radioisotope has been exchanged for a therapeutic one from the α- or β-emitter group. Typical uses in the last 20 to 40 years include local application sfor the treatmant of rheumatid arthritis, liver tumors and cystic brain tumors. However, their use remains experimental because of smaller than expected target uptake, unwanted toxicity and insufficient treatment effects that have resulted from radio chemical instability and suboptimal biodistribution of the radiopharmaceutical. In addition there exists a general negative attitude towards the use of radioactive substances inspite of proven superior results of many radiation therapies[17-19]. Few therpeutic applications of radioactive microspheres are tabulated in Table:1

Table:1 Therapeutic applications of microspheres

Type of radioactive microspheres	Applications
^{90}Y-glass microspheres, ^{186}Re/^{188}Re-glass microspheres	Radioembolisation of liver and spleen tumors
^{35}S-colloid, ^{90}Y-resin microspheres, ^{169}Er.citrate	Radiosynovectomy of arthritis joints
^{90}Y-labeled poly(lactic acid) microspheres, ^{211}At-microspheres, ^{212}Pb-sulfur colloid	Local radiotherapy
Chromium ^{32}P-phosphate, ^{90}Y-silicate	Intracavity treatment

(B).(2) Diagnostic radioactive microspheres:

Diagnostic studies with radiopharmaceuticals include dynamic and static imaging and invivo function tests. Dynamic imaging provides information about the biodistridution and pharmacokinetics of drugs in organs. Performed with a γ-camera, dynamic studies are generally carried over apreset length of time and provide clues to the functioning of the organ being examined.

The first such microsphere in clinical use were red and white blood cells, which were taken from a patient, labelled with ^{111}In or ^{51}Cr, and then re-injected. Red blood cells labelled with ^{51}Cr commonly used for the measurement of red blood cell mass and imaging of the spleen. Another common application of radiolabelled red blood cells is the accurate determination of total systemic arterial flow or venous return,as well as for blood flow determination within specific organs.[20] Various diagnostic applications of radioactive microspheres are as follows:

Table:2 Diagnostic applications of radioactive microspheres

Type of radioactive microspheres	Applications
^{111}In or ^{51}Cr-labelled red blood cells	Gated blood pool study
^{111}In-labeled platelets ^{99m}Tc-sulfur colloid	Thrombus imaging in deep vein thrombosis
Polystyrene microspheres labeled with γ-emitters ^{141}Ce, ^{57}Co, ^{114m}In, ^{85}Sr, ^{51}Cr	Blood flow measurements
^{3}H, ^{14}C-labelled microspheres ^{141}Ce-polystyrene microspheres	Investigation of biodistribution and fate of drug loaded microspheres
^{99m}Tc-impregnated carbon particles ^{99m}Tc-macro aggregated human serum albumin	Lung scintigraphy
^{99m}Tc-macro aggregated human serum albumin	Radioembolisation
^{99m}Tc-macro aggregated human serum albumin	Liver and spleen imaging
^{99m}Tc-sulfur colloid ^{99m}Tc-antimony sulfide colloid	Bone marrow imaging

(C) Perfect count microspheres:
These are meant for invitro diagnostic use.These are designed for determining absolute counts of cells in peripheral blood, bone marrow, leukapheresis and culture medium samples using flow cytometry. These are micro-bead-based single platform system for absolute counts, which can be used in combination with monoclonal antibodies conjugated with different flurochromes, which makes it possible to identify the cell subpopulations for which the absolute count is intended[21,22].

(D) Microspheres for high throughput screening assays:
The role of microspheres in these screens is similar to their traditional role in immunoassays, namely as asolid phase to either enhance detection, separation or both. The predominance of radioactive assays in high throughput screening,along with the desire to find alternative means of detection, have led to research on substituting alternative fluorescent technologies. An example is a format using the same approach as the scintillation proximity assay, but substituting fluorescence for radioactivity. These are commonly referred to as *fluorescence energy transfer latex.*
Several companies have researched assays using this microsphere-based technology[23]. An example for high throughput screening assays involves using large, non-magnetic, Streptavidin- coated microspheres which are trapped by filter-bottom plates. Using a protein kinase assay, this approach can be diagrammed as shown in the fig:2.
In addition to these, microspheres are now being used as the basis for entire high throughput screening platform technologies, such as the electro chemiluminescent technology. This is a versatile technology using Sterptavadine-coated magnetic microspheres as the solid phase, and has already been implemented for use in drug discovery by several leading pharmaceutical companies.[23]

(E)Microsphere sensors:
Optical microspheres are proving to be excellent candidates for label-free biochemical sensors. Light of resonant frequencies circulates on the surface of the microsphere in the form of whispering-gallery modes (WGMs). Because of the high-*Q* factor of microspheres the evanescent interaction between the WGM and the surrounding medium is significantly enhanced. As a result, the WGM's resonant wavelength is extremely sensitive to changes in refractive index near the sphere's surface when molecules bind to or are removed from the surface. The applications of microsphere sensors include detection of protein and DNA molecules heavy-metal detection and refractometric sensing. Another important application of microsphere sensors is small-molecule detection. This is of significance for molecular pharmacology as related to drug design as well as for biochemical sensing applications such as peptide cleavage, as the molecular mass of those target molecules ranges from a few tens to a few hundreds of daltons. Detecting small molecules is challenging because the transduction signal in labelfree sensors is generally proportional to the mass of the target molecule[24].

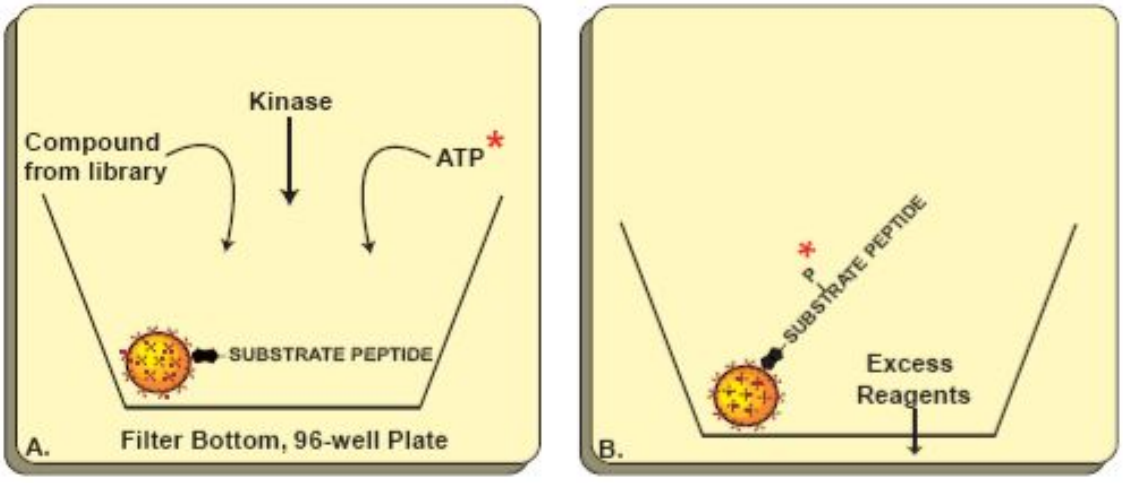

Fig:2 Radioactive protein kinase assay

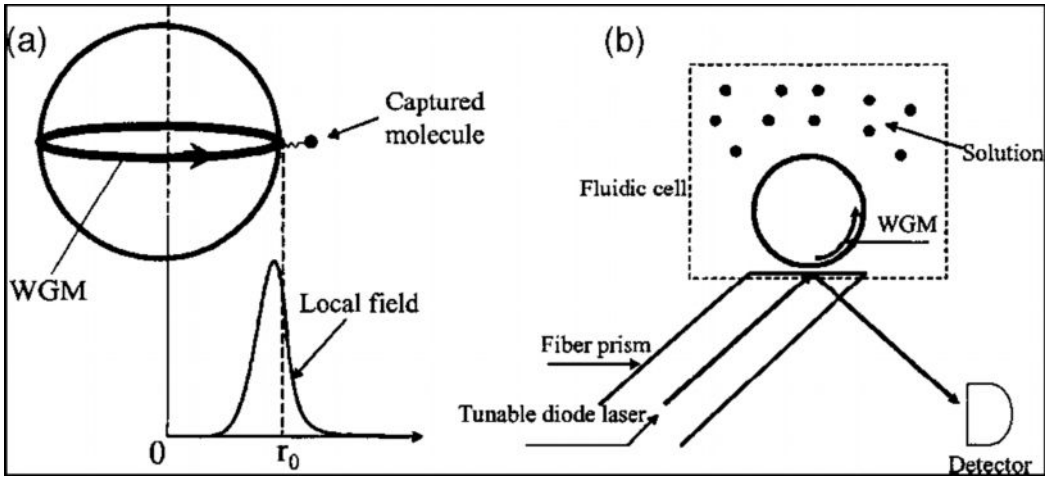

Fig:3- A microsphere is sensitive to changes in molecules near the sphere's surface. (b) Experimental setup.

(G) Fluorescent microspheres:

These are made of polystyrene or poly vinyl toluene, mono disperse system ranging in size from 20nm to 4µm. Preparation of our fluorescent microspheres comprising: Swelling the polymeric microsphere so that fluorescent dyes may enter the microspheres pores. Unswelling the poIymeric microspheres so that the fluorescent dyes become physically entrapped in the pores. The main applications for Estapor® (commercial fluorescent microspheres) Fluorescent Microspheres are the following: Membrane-based technologies Flow cytometry, Confocal Microscopy FLISA: Fluorescent Linked Immuno-Sorbent Assay, Toxicology, Cell Biology, Microbiology, Embolization, BioSensors, BioChips and Microfluidics[25].

(H) Microspheres in molecular biology:

Advances in our understanding of the underlying genetic causes of disease have highlighted a need for multiplexed analytical genotyping methods. Although microarray platforms have attempted to address this need, their acceptance in the clinical diagnostic setting has been limited. This describes a novel, microsphere-based universal array genotyping platform, the Tag-ItTM platform. They used universal, minimally cross-hybridizing tags combined with solution-phase kinetics characteristic of microsphere-based hybridization reactions to improve signal-to-noise ratios. They used microsphere-based arrays to avoid the quality-control/quality-assurance obstacles encountered by first-generation arrays in which "each" chip represents a new entity. Unlike first-generation arrays, the described assay may easily be modified to reflect the ever-changing panel of relevant mutations associated with a given condition. This report demonstrates the use of this microsphere-based universal array genotyping platform for the detection of six single-nucleotide polymorphisms (SNPs)3 believed to be associated with venous thromboembolism, a classic example of a complex, multifactorial disorder involving multiple genetic abnormalities [(26-30)].

Pyrosequencing is real-time DNA sequencing-by-synthesis[(31,32)] Pyrosequencing currently has many applications, including determination of singlenucleotide polymorphisms, resequencing of PCR products, microbial typing, and analysis of secondary DNA structures such as hairpins[31]. Current pyrosequencing technique utilizes DNA templates attached to magnetic microspheres, which can easily be put on an electrowetting chip in solution. On a digital microfluidic platform, pyrosequencing could be accomplished by merging reagent or wash droplets with the droplet containing the magnetic microspheres and then resolving the double-volumed droplet through droplet splitting[32].

Conclusion

Microspheres offers several improvements over existing technologies. These have emerged as an exciting new platform for biologists to adopt into their armory of techniques in the investigation of biomolecule interactions and cellular processes. In recent years there have been increasing numbers of studies in which microspheres have been used in more diverse applications and it is evident that the range of potential applications is enormous. The future certainly looks bright for these microspheres, particularly in the areas of genomics, proteomics and drug discovery.

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