CHAPTER 1

MICRO AND NANO SYSTEMS IN BIOMEDICINE AND DRUG DELIVERY

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Abstract: Micro and nano sytems sysnthesized from organic and inorganic materials are gaining great attention in biomedical applications such as design of biosensors, construction of imaging systems, synthesis of drug carrying and drug targeting devices, etc. Emulsions, suspensions, micelles, liposomes, dendrimers, polymeric and responsive systems are some examples for drug carrier devices. They have lots of advantages over conventional systems since they enhance the delivery, extend the bioactivity of the drug by protecting them from environmental effects in biological media, show minimal side effects, demonstrate high performance characteristics, and are more economical since minimum amount of expensive drugs are used. This chapter provides brief information about micro and nano systems used in biomedicine, nanobiotechnology and drug delivery

Keywords: micelles, liposomes, dendrimers, drug carriers, responsive polymers

1. INTRODUCTION

Development of metal, ceramic, polymer or materials of biological origin for use in medicine is a very important research area of the last decades. Scientists made great innovations in the production of artificial organs and tissues such as dental and orthopedic prostheses, artificial veins and heart valves, contact lenses, tissue engineering scaffolds, diagnostic systems, etc. As the knowledge on materials and biological systems improved, new areas such as interaction between the material and cells, effect of therapeutic agents at molecular level, the relation between the molecular structure and macroscopic properties became important research lines. Scientists are increasingly interested in mimicking the biological systems, understanding cell-cell communications and modeling the structures that already exist

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in nature. This curiosity makes them search individual molecules, study interactions between the functional groups, signaling between the cells at micro and nano levels to be able to control the properties of the artificial and biological systems. Technologies based on micro and nano levels involve synthesis and utilization of materials, devices and systems in which at least one dimension is less than 1 mm or in the submicron range, respectively.

2. MICRO AND NANO TECHNOLOGY IN MEDICINE

Micro and nanotechnology have significant applications in the biomedical area, such as drug delivery, gene therapy, novel drug synthesis, imaging, etc. In diagnostics and treatment of many disorders, micro-electro-mechanical systems (MEMS) and biocompatible electronic devices have great potentials. MEMS are formed by integration of mechanical elements, sensors, actuators and electronics on a common silicon wafer with microelectronics and micromachining technologies. Sensors collect information from the environment by measuring mechanical, thermal, biological, chemical, optical or magnetic parameters; electronics process these information and actuators respond by moving, positioning, regulating, pumping or filtering. Therefore a desired response occurs against the stresses and environment is controlled by the system.

Use of nano devices in imaging is another important area especially in the detection of tumor cells. In principle, nanoparticles injected into the body detect cancer cells and bind to them. They behave as contrast agents making the malignant area visible so that the anatomical contours of the cancer lesion can be defined. For this purpose iron-oxide nanoparticles whose surfaces were modified by amines were prepared by Shieh et al (2005) and a fast and prolonged inverse contrast effect was shown in the liver in vivo that lasted for more than 1 week. Medical applications of metallic nanoparticles were studied by different groups. For example Dua et al (2005) constructed a non-toxic, biomimetic interface for immobilization of living cells by mixing colloidal gold nanoparticles in carbon paste and studied its electrochemical exogenous effect on cell viability. Pal et al (2005) prepared gold nanoparticles in the presence of a biopolymer, sodium alginate by UV photoactivation. Carrara et al (2005) prepared nanocomposite materials of poly(*o*-anisidine) containing titanium dioxide nanoparticles, carbon black and multi-walled carbon nanotubes for biosensor applications. The synthesized materials were deposited in thin films in order to investigate their impedance characteristics. Lee et al (2005) prepared ultrafine poly(acrylonitrile) (PAN) fibers containing silver nanoparticles. Silver ions in a PAN solution were reduced to produce Ag nanoparticles and the resulting solution was electrospun into ultrafine PAN fibers.

Morishita et al (2005) associated HVJ-E (hemagglutinating virus of Japan-envelope) with magnetic nanoparticles so that they can potentially enhance its transfection efficiency in the presence of a magnetic force. It was reported that, heparin coated maghemite nano particles enhanced the transfection efficiency in the analysis of direct injection into the mouse liver. They proposed that the system could potentially help overcome fundamental limitations to gene therapy in vivo.

3. MICRO AND NANO DRUG DELIVERY SYSTEMS

One of the most attractive areas of micro and nano research is drug delivery. This includes the design of micro and nano carriers, synthesis of nanomedicines and production of nanosystems that are able to deliver therapeutic drugs to the specific organs or tissues in the body for appropriate periods. For drug delivery vehicles it is very important that these systems have good blood and biocompatibility properties. They themselves or the degradation products should not have any toxic, allergic or inflammatory effects. The systems should also protect the activity of the drugs and improve their transport through the biological barriers. If some specific functionality is added on the system, it would also be possible to deliver the drug to the target site where the system is stimulated by an appropriate signal.

In the design and formulation of delivery systems, the key parameters are the size of the device, entrapment method, stability of drug, degradation parameters of the matrix and release kinetics of drugs. Nanosystems have many advantages over the micro systems such as circulation in blood stream for longer periods without being recognized by macrophages, ease of penetration into tissues through capillaries and biological membranes, ability to be taken up by cells easily, demonstrating high therapeutic activity at the target site, and sustaining the effect at the desired area over a period of days or even weeks. In the last decades, numerous publications came up to describe the design of delivery systems with novel preparation methods, physicochemical properties, and bioactivities.

Drug delivery is an interdisciplinary area of research that aims to make the administration of complex drugs feasible. Over the recent years there has been an increasing interest in developing new delivery systems by collaborative research of basic scientists, engineers, pharmacologists, physicians and other health related scientists. The main purpose is to deliver the drug to the desired tissue in the biological system so that it would achieve higher activity for prolonged period at the site without risk of side effects. Micro and nano drug delivery systems are developed for these purposes especially to target the drugs to a specific area or organ in a more stable and reproducible controlled way.

Entrapment or conjugation of a drug to a polymeric system may protect the drug from inactivation and help to store its activity for prolonged durations, decrease its toxicity, as well as may achieve administration flexibility. Various delivery systems, such as emulsions, liposomes, micro and nanoparticles, are of major interest in the field of biomedicine and pharmaceutics. Generally biodegradable and bioabsorbable matrices are preferred so that they would degrade inside the body by hydrolysis or by enzymatic reactions and does not require a surgical operation for removal.

Targeted delivery can be achieved by either active or passive targeting. Active targeting of a therapeutic agent is achieved by conjugating the therapeutic agent or the carrier system to a tissue or cell-specific ligand. Passive targeting is achieved by coupling the therapeutic agent to a macromolecule that passively reaches the target organ. Muvaffak et al (2002, 2004a, 2004b, 2005) prepared anticancer drug-containing gelatin microspheres and conjugated antibodies on the surfaces of these biodegradable microspheres. It was reported that the systems prepared in this

way demonstrated specific activity towards its antigen. Monsigny et al (1994) reviewed the main properties of neoglycoproteins and glycosylated polymers which have been developed to study the properties of endogenous lectins and to carry drugs which can form specific ligands with cell surface receptors. The glycoconjugates have been successfully used to carry biological response modifiers such as *N*-acetylmuramyldipeptide which is hundreds of times more efficient in rendering macrophages tumoricidal when it is bound to this type of carriers. Complexes of polycationic glycosylated polymers with plasmid DNA molecules are also very efficient in transfecting cells in a sugar-dependent manner.

Bioactive agents can be incorporated in micro and nano systems or in systems which have microporous structures. Local delivery of drugs or growth factors which are embedded in microporous gelatin structures was reported by Ulubayram and coworkers (2001, 2002). They examined release kinetics of bovine serum albumin proteins from gelatin matrices (Ulubayram et al 2002) and also reported fast and proper healing of full skin defects on rabbits with application of gelatin sponges loaded with epidermal growth factor (EGF) (Ulubayram et al 2001). EGF was added in gelatin microspheres which were crosslinked with various amounts of crosslinkers (Ulubayram et al 2001, 2002). Similar systems were studied by Sakallioglu and colleagues (2002, 2004) and positive effects of low-dose EGF loaded gelatin microspheres in colonic anastomosis were reported. Uguralp et al (2004) also reported positive effects of sustained and local administration of EGF incorporated to biodegradable membranes on the healing of bilateral testicular tissue after torsion. Guler et al (2004) examined the effects of locally applied fibroblast containing microporous gelatin sponges on the testicular morphology and blood flow in rats.

There are a large number of studies investigating the drug releasing responses to various stimuli such as pH, temperature, electric field, ultrasound, light, or other stresses. Kim et al (2000) prepared nanospheres with core-shell structure from amphiphilic block copolymers by using PEO-PPO-PEO block copolymer (Pluronic) and poly(ε-caprolactone). Release behaviors of indomethacin from Pluronic/PCL block copolymeric nanospheres showed temperature dependence and a sustained release pattern. Chilkoti et al (2002) described recursive directional ligation approach to synthesis of recombinant polypeptide carriers for the targeted delivery of radionuclides, chemotherapeutics and biomolecular therapeutics to tumors by using a thermally responsive, elastin-like polypeptide as the drug carrier. Determan et al (2005) synthesized a family of amphiphilic ABCBA pentablock copolymers based on the commercially available Pluronic® F127 block copolymers and various amine containing methacrylate monomers. The systems exhibited both temperature and pH responsiveness. They suggested that the copolymers have high potential for applications in controlled drug delivery and non-viral gene therapy due to their tunable phase behavior and biocompatibility. Micro and nano systems for drug delivery applications can be studied in the classes of micelles, liposomes, dendrimers, and particles of polymeric and ceramic materials as explained in the following sections.

3.1. Micelles

Micelles are ideal bioactive nanocarriers, especially for water insoluble agents. Many amphiphilic block copolymers can be used for this purpose. Polymers can self-associate to form spherical micelles in aqueous solution by keeping hydrophilic ends as the outer shell and the hydrophobic ends as the core. Hydrophobic drugs can be entrapped in the core during micelle formation process. Polymeric micelles have good thermodynamic stability in physiological solutions, as indicated by their low critical micellar concentration, which makes them stable and prevents their rapid dissociation in vivo. The sizes of micelles are generally less than 100 nm in diameter. This provides them with long-term circulation in blood stream and enhanced endothelial cell permeability in the vicinity of solid tumors by passive diffusion. If site-specific ligands or antibodies are conjugated to the surface of the micelles, the drug targeted delivery potential of polymeric micelles can be enhanced.

Kataoka et al (2000) studied the effective targeting of cytotoxic agents to solid tumors by polymeric micelles. They conjugated doxorubicin to poly(ethylene glycol)-poly(β,α-aspartic acid) block copolymers and showed that these micelles achieved prolonged circulation in the blood compartment and accumulated more in the solid tumor, leading to complete tumor regression against mouse C26 tumor. Rapoport (1999) studied stabilization and activation of Pluronic micelles for tumor-targeted drug delivery. Aliabadi et al (2005a) examined the potential of polymeric micelles to modify the pharmacokinetics and tissue distribution of cyclosporine A (CsA). Their results demonstrated that PEO-b-PCL micelles can effectively solubilize CsA confining CsA to the blood circulation and restricting its access to tissues such as kidney, perhaps limiting the onset of toxicity. They also investigated micelles of methoxy poly (ethylene oxide)–b–poly (ε–caprolactone) (PEO–b–PCL) as alternative vehicles for the solubilization and delivery of Cyclosporine A (Aliabadi et al 2005b). They concluded that these nanoscopic PEO–b–PCL micelles have high potential as drug carriers for efficient solubilization and controlled delivery of CsA. Prompruk et al (2005) synthesized a functionalized copolymer with three polymeric components, poly (ethylene glycol)–block–poly (aspartic acid–stat-phenylalanine) and investigated its potential to form micelles via ionic interactions with diminazene aceturate as a model water-soluble drug.

Wasylewska et al (2004) entrapped human prostatic acid phosphatase (PAP) entrapped in AOT–isooctane–water reverse micelles and studied the kinetics of 1–naphthyl phosphate and phenyl phosphate hydrolysis, catalyzed by PAP. Wang et al (2004) prepared polymeric micelles from poly (ethylene glycol)–distearoyl phosphoethanolamine conjugates (PEG–DSPE) loaded with Vitamin K3 (VK3) and with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU). These micelles were stable for 6 months during storage at 4°C and no change in their size or release of the incorporated drugs were observed. They showed that these loaded micelles resulted in synergistic anticancer effects against both murine and human cancer cells in vitro. Kang et al (2004) prepared A-B-A triblock and star-block amphiphilic copolymers such as poly (*N*–(2–hydroxypropyl) methacrylamide)–*block*–poly

(D,L–lactide)–*block*–poly (*N*–(2–hydroxy propyl) methacrylamide), poly (*N*–vinyl-2–pyrrolidone)–*block*–poly (D,L–lactide)–*block*–poly (*N*–vinyl–2–pyrrolidone), star–poly (D,L–lactide)–*block*–poly (*N*–(2–hydroxypropyl) methacryl amide) and star–poly (D,L–lactide)–*block*–poly (*N*–vinylpyrrolidone). They reported that all copolymers self-assembled in aqueous solution to form supramolecular aggregates of 20–180 nm in size. The prepared triblock copolymer micelles were examined as carriers for two drugs, indomethacin and paclitaxel, which are poorly water-soluble. Carrillo and Kane (2004) studied the formation and characterization of self–assembled nanoparticles of controlled sizes based on amphiphilic block copolymers synthesized by ring-opening metathesis polymerization. They showed that the monomer undergoes living polymerization and forms assembled nanoparticles of controlled size. The obtained micelles were fairly monodisperse with dimensions of 30–80 nm depending on the composition of the block polymer.

Synthetic copolymers containing phosphorylcholine structure can also be used in the formation of micelles. Phosphorylcholine-based polymers mimic the surface of natural phospholipid membrane bilayers and therefore demonstrate good biocompatability. Salvage et al (2005) copolymerised 2-methacryloyloxyethyl phosphorylcholine (MPC) with two pH responsive comonomers, 2–(diethylamino) ethyl methacrylate (DEA) and 2–(diisopropyl amino) ethyl methacrylate (DPA), in order to develop pH responsive biocompatible drug delivery vehicles. Koo et al (2005) studied sterically stabilized micelles (SSM) and evaluated camptothecin-containing SSM (CPT–SSM) as a new nanomedicine for parenteral administration where camptothecin is a well-established topoisomerase I inhibitor against a broad spectrum of cancers. Konno et al (2001) have shown that 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer immobilized on poly (l–lactic acid) nanoparticles effectively suppressed any unfavourable interactions with biocomponents and improved the blood compatibility of the nanoparticles. It has been suggested that the nanoparticles immobilized with the MPC polymer have the potential use as long–circulating micelles and are good candidates for carrying drugs and diagnostic reagents which can come in contact with blood components. Nishiyama et al (2005) published a review article about construction and characteristic behaviors of intracellular environment-sensitive micelles that selectively exert drug activity and gene expression in live cells. Xiong et al (2005) grafted poly (lactic acid) to both ends of Pluronic F87 block copolymer (PEO–PPO–PEO) to obtain amphiphilic P(LA-*b*-EO-*b*-PO-*b*-EO-*b*-LA) block copolymers. Various types of particles consisting of small micelles were obtained due to the complex structure of the copolymers and a constant initial release rates were observed for procain hydrochloride. Sot and coworkers (2005) investigated the behaviour of *N*–hexadecanoyl sphingosine (Cer16), *N*–hexanoylsphingosine (Cer6) and *N*–acetyl sphingosine (Cer2) ceramides in aqueous media and in lipid-water systems. Cer16 behaved as an insoluble non-swelling amphiphile while both Cer6 and Cer2 behaved as soluble amphiphiles in aqueous solutions. They observed micelle formations for Cer6 and Cer2 at high concentrations as well as phospholipid monolayer formation when the air-water interface is occupied by a phospholipid.

Responsivity can be added to micelles by combining pH or temperature sensitive functional groups into the structures. Cammas et al (1997) prepared thermo-responsive polymeric micelles from amphiphilic block copolymers composed of N–isopropylacrylamide as a thermo-responsive outer shell and styrene as hydrophobic inner core. Leroux et al (2001) studied N–isopropylacrylamide bearing pH-responsive polymeric micelles and liposomes as a delivery system for the photosensitizer aluminum chloride phthalocyanine (AlClPc), which was evaluated in photodynamic therapy. pH-responsive polymeric micelles loaded with AlClPc were found to exhibit increased cytotoxicity against EMT-6 mouse mammary cells in vitro. Liu et al (2003) synthesized cholesteryl end-capped thermally responsive amphiphilic polymers with two different hydrophobic/hydrophilic chain-length ratios from the hydroxyl-terminated random poly (N–isopropylacrylamide–co–N, N–dimethylacrylamide) and cholesteryl chloroformate. The micellar nanoparticles prepared from the amphiphilic polymers demonstrated temperature sensitivity. It was suggested that these nanoparticles would make an interesting drug delivery system. Nostrum (2004) reviewed the results of photosensitizers for photodynamic therapy including drug loading, biodistribution studies, and therapeutic efficiency and concluded that pH-sensitive micelles appeared to be promising candidates for photosensitizer delivery.

3.2. Liposomes

Liposomes are small spherical vesicles in which one or more aqeous compartments are completely enclosed by molecules that have hydrophilic and hydrophobic functionality such as phospholipids and cholesterol. Properties of liposomes vary substantially with composition, size, surface charge and method of preparation. They can be formed as single lipid bilayer or in multiple bilayers. Liposomes containing one bilayer membrane are termed small unilamellar vesicles (SUV) or large unilamellar vesicles (LUV) based on their size ranges (Mozafari and Sahin 2005). If more than one bilayer is present then they are called multilamellar vesicles (MLV). Liposomes are commonly used as model cells or carriers for various bioactive agents including drugs, vaccines, cosmetics and nutraceuticals.

The introduction of positively or negatively charged lipids provides the liposomes a surface charge. Drugs associated with liposomes have markedly altered pharmacokinetic properties compared to free drugs in solution. Liposomes are also effective in reducing systemic toxicity and preventing early degradation of the encapsulated drug after introduction to the body. They can be covered with polymers such as polyethylene glycol (PEG) – in which case they are called pegylated or stealth liposomes – and exhibit prolonged half-life in blood circulation (Mozafari et al 2005). Furthermore, liposomes can be conjugated to antibodies or ligands to enhance target-specific drug therapy. Visser et al (2005) studied targeting of pegylated liposomes loaded with horse radish peroxidase (HRP) and tagged with transferrin to the blood-brain barrier in vitro. They have shown effective targetting of liposomes loaded with protein or peptide drugs to the brain capillary endothelial

cells and suggested that the system is an attractive approach for drug delivery to brain. Lopez-Pinto and coworkers (2005) examined the dermal delivery of a lipophilic drug, minoxidil, from ethosomes versus classic liposomes by appliying the vesicles non-occlusively on rat skin. They studied the permeation pattern, depth into the skin and the main permeation pathway of different liposomal systems. Ozden and Hasirci (1991) prepared small unilamellar vesicles composed of phosphatidylcholine, dicetyl phosphate and cholesterol and entrapped glucose oxidase in them. They obtained loading efficiency as one protein per liposomal vesicle.

Liposomes containing the expression vector pRSVneo coding for neomycin phosphotransferase–II were studied by Leibiger et al (1991) for a gene transfer into rat liver cells in vivo. After intravenous application of liposomes to male Wistar-rats, nonintegrated vector DNA was detected by blot-hybridisation in isolated nuclei of hepatocytes. Cirli and Hasirci (2004) prepared calcein encapsulated reverse phase evaporation vesicles carrying photoactive destabilization agent suprofen in the lipid bilayer. They investigated the effect of UV photoactivation of liposomal membrane-incorporated suprofen on the destabilization of the liposome bilayer and the release of encapsulated calcein as a model active agent.

Liposomes are also studied as carriers for cells, genes or DNA fragments. Ito et al (2004) studied the effect of magnetite cationic liposomes which have positive surface charge to enrich and proliferate Mesenchymal stem cells (MSCs) in vitro. Kunisawa et al (2005) established a protocol for the encapsulation of nanoparticles in liposomes, which were further fused with ultra violet-inactivated Sendai virus to compose fusogenic liposomes and observed that fusogenic liposome demonstrated a high ability to deliver nanoparticles containing DNA into cytoplasm. Ito et al (2005) investigated whether coating the culture surface with RGD (Arg–Gly–Asp) conjugated magnetite cationic liposomes (RGD-MCLs) was able to facilitate cell growth, cell sheet construction and cell sheet harvest using magnetic force without enzymatic treatment. They reported that cells adhered to the RGD-MCLs coated bottom of the culture surface, spreaded and proliferated to confluency. Detachment and harvesting of the cells did not need enzymatic process. Fuentes et al (2003) studied the adjuvanticity of two gamma inulin/liposomes/Vitamin E combinations in the mouse, in contraceptive vaccines by using sperm protein extracts or a synthetic HE2 peptide (Human Epididymis gene product; residues 15–28) as antigen. They showed that the gamma inulin/liposomes/Vitamin E combination, with sperm protein extracts, was better than Freund's adjuvant. When the synthetic HE2 peptide was used as antigen, the gamma inulin/liposomes/Vitamin E combination was less effective than Freund's adjuvant.

Vierling et al (2001) published a review on fluorinated liposomes made from highly fluorinated double-chain phospho- or glyco-lipids as well as fluorinated lipoplexes, e.g. complexes made from highly fluorinated polycationic lipospermines and a gene. The properties of the fluorinated lipoplexes including stability and in vitro cell transfection in the presence of serum or bile were reported. El Maghraby et al (2004) showed that incorporation of activators (surfactants) into liposomes improved estradiol vesicular skin delivery. They examined the

interactions of additives with dipalmitoylphosphatidylcholine (DPPC) membranes by using high sensitivity differential scanning calorimetry. Lopes and colleagues (2004) investigated the encapsulation of acid (AD) and sodium diclofenac (SD) in small unilamellar liposomes (SUV) prepared by sonication from multilamellar liposomes containing soya phosphatidylcholine and diclofenac at various proportions. The interactions of the drug with the bilayers were examined. They proposed a schematic model for interaction of SD with phosphatidylcholine of the liposomes in which the diclofenac anion interacts with the ammonium group of the phospholipid and the dichlorophenyl ring occupies a more internal site of bilayer near phosphate group. Simard et al (2005) prepared multilamellar vesicles by shearing a lamellar phase of lipids and surfactants. They reported formation of vesicles with mean diameter of less than 300 nm in which hydrophilic drugs can be loaded with high yield. They coated the vesicles with PEG and loaded them with 1-β-D-arabinofuranosylcytosine. Following injection of the vesicles intravenously to rats they observed that the surface-modified liposomes exhibited longer circulation times compared to uncoated liposomes.

Koynova and MacDonald (2005) examined the lipid exchange between model lipid systems, including vesicles of the cationic lipoids ethyl dimyristoyl phosphatidylcholine, ethyl dipalmitoyl phosphatidylcholine or their complexes with DNA, and the zwitterionic lipids by using differential scanning calorimetry. They observed that, exchange via lipid monomers was considerably more facile for the cationic ethylphosphatidylcholines than for zwitterionic phosphatidylcholines and for the cationic liposomes. The presence of serum in the dispersing medium strongly promoted lipid transfer between cationic vesicles while almost no effect was reported for zwitterionic liposomes. This phenomenon was proposed as an important point for the application of cationic liposomes as nonviral gene delivery. Foco et al (2005) studied the delivery of sodium ascorbyl phosphate (SAP), an effective oxygen species scavenger to prevent the degenerative effects of UV radiation on skin. SAP was encapsulated into liposomes to improve its penetration through the stratum corneum into the deeper layers of the skin. They prepared two types of multilamellar vesicles, one from non-hydrogenated and the other from hydrogenated soybean lecithin, together with cholesterol. Sinico et al (2005) studied transdermal delivery of tretinoin and examined the influence of liposome composition, size, lamellarity and charge on transdermal delivery. They studied positively or negatively charged liposomes of different types, i.e. multilamellar vesicles (MLV) or unilamellar vesicles (ULV), prepared from hydrogenated soy phosphatidylcholine (Phospholipon® 90H) or non-hydrogenated soy phosphatidylcholine (Phospholipon® 90) and cholesterol, in combination with stearylamine or dicetylphosphate. It was reported that negatively charged liposomes strongly improved newborn pig skin hydration and tretinoin retention.

Arcon et al (2004) encapsulated an anticancer agent, cisplatin, in sterically stabilized liposomes and studied the systems with extended X-ray absorption fine structure (EXAFS) method, and concluded that the liposome-encapsulated drug is chemically stable and does not hydrolyze. Sapra and Allen (2003) published

a review article about the ligand-targeted liposomes (LTLs) for the delivery of anticancer drugs. In this article, new approaches used in the design and optimization of LTLs was discussed and the advantages and potential problems associated with their therapeutic applications were described.

3.3. Ceramic Nanoparticles

Use of ceramics in medicine is especially significant in dental and orthopedic applications as strengthening materials for the hard tissue implants. Hydroxyapatite (HA) is a ceramic naturally existing in the bone structure and therefore its use in the hip or knee prosthesis can reduce the risk of rejection and stimulate the production of osteoblasts which are the cells responsible for the growth of the bone matrix.

Ceramic particles effectively protect the doped molecules (enzymes, drugs, etc) against denaturation induced by external pH and temperature. In addition, their surfaces can be easily modified with different functional groups. They can be conjugated to a variety of monoclonal antibodies or ligands for targeting purposes in vivo. Ceramic particles with entrapped biomolecules have a great potential in delivery of drugs. Such particles, including silica, alumina, titania, etc, are known for their compatibility with biological systems. They have several advantages such as the ease of preparation with the desired size, shape and porosity under ambient conditions, high stability such as no swelling or change in shape in environmental conditions.

McQuire et al (2005) synthesized hydroxyapatite sponges by using aminoacid coated HA nanoparticles dispersed within a viscous polysaccharide (dextran sulfate) matrix and examined the use of these materials for the viability and proliferation of human bone marrow stromal cells in order to search possibility for cartilage or soft tissue engineering. Rusu et al (2005) studied size-controlled hydroxyapatite nanoparticles prepared in aqueous media in a chitosan matrix from soluble precursors salts bone for the purpose of tissue engineering applications. Serbetci et al (2000, 2002, 2004) prepared acrylic bone cements with addition of HA microparticles. They examined the effect of HA addition on the properties of the cement. They reported enhancement of mechanical, thermal and biological properties depending on the added amount of HA.

Christel and co-workers (1984) implanted calcium phosphate bioglass ceramics in the tibiae of rabbits to study the interface of bioceramics. It was reported that hydroxyapatite surface give rise to a closer contact with new bone than calcium phosphate glass ceramics. Lin and colleagues (1996) implanted bioglass discs into the condyle area of rabbits. The failure load, when an implant detached from the bone or when the bone itself broke, was measured by a push-out test and compared with sintered hydroxyapatite bioceramic. Vogel and coworkers (2001) implanted bioglass particles in the distal femoral epiphysis of rabbits and examined bone formation at the implant site. They discussed the parameters (implantation model, particle size and surface-area-to-volume ratio) as possible parameters determining bone regeneration. Recently Amaral and colleagues (2002) studied wettability and

surface charge properties of Si_3N_4–bioglass biocomposites. They determined that the examined bioglass had comparatively higher hydrophilic character and surface tension value than the most common bioceramics. The presence of very high negative zeta potential at neutral pH influenced albumin adsorption. They also studied mechanisms in terms of entropy and enthalpy gains from conformational unfolding and cation coadsorption (Amaral et al 2002).

Zeng and co-workers (2002) prepared Al_2O_3–A/W bioglass coating through tape casting process by selecting low melting point A/W bioglass to decrease the Al_2O_3 sintering temperature and modify the bioactivity of implant. On the other hand, Xin and colleagues (2005) investigated the formation of calcium phosphate (Ca-P) on various bioceramic surfaces in simulated body fluid (SBF) and in rabbit muscle. The bioceramics were sintered porous solids, including bioglass, glass-ceramics, hydroxyapatite, α-tricalcium phosphate and β-tricalcium phosphate. They compared the ability of inducing Ca-P formation and obtained similar results in SBF but observed considerable variations in vivo.

3.4. Dendrimers

Dendrimers are small molecules which have a core and a series of branches symmetrically formed around the core resulting in a monodisperse, symmetrical macromolecule. They can be synthesized either starting from the core molecules and going out to the periphery by connecting the branch groups or by forming the branches first and then collecting all around the core. Functionality of the branching units is generally 2 or 3, which makes the layer of branching units doubles or triples. The interior cavity is very suitable for the entrapment of the drugs and their unique properties such as high degree of branching, multivalency, globular architecture and well-defined molecular weight, make dendrimers promising new carriers for drug delivery. Their nanometer size, ease of preparation and functionalization, and their ability to display multiple copies of surface groups for biological reorganization processes increase their attraction in biomedical applications.

Interaction of dendrimer macromolecules with the molecular environment is predominantly controlled by their terminal groups. By modifying their termini, the interior of a dendrimer may be made hydrophilic while its exterior surface is hydrophobic, or vice versa. Drug molecules can be loaded both in the interior of the dendrimers as well as attached to the surface groups. Water-soluble dendrimers are capable of binding and solubilizing small molecules and can be used as coating agents to protect or deliver drugs to specific sites in the body or as time-release vehicles for transporting biologically active agents. In the last decades, research has increased on the design and synthesis of biocompatible dendrimers and their application to many areas of bioscience including drug delivery, immunology and the development of vaccines, antimicrobials and antivirals gained great attantion.

A series of lipidic peptide dendrimers based on lysine with 16 surface alkyl (C_{12}) chains has been synthesised by Florence et al (2000). A fourth generation dendrimer with a diameter of 2.5 nm was studied for its absorption at different organs after

oral administration to female Sprague–Dawley rats. The results showed that the total percentage of the dose absorbed through Peyer's patches depend on the loaded dose as well as the size of the nanoparticules. Wang et al (2000) investigated the fifth generation of ethylenediamine core dendrimer for its ability to enhance gene transfer and expression in a clinically relevant murine vascularized heart transplantation model. They formed complexes of the plasmids with dendrimers which were perfused via the coronary arteries during donor graft harvesting, and reporter gene expression was determined by quantitative evaluation. Yoo and Juliano (2000) studied the behavior of dendrimer-nucleic acid complexes at the cell interior. They prepared dendrimers conjugated with the fluorescent dye Oregon green 488 and used these in conjunction with oligonucleotides labeled with a red (TAMRA) fluorophore in order to visualize the sub-cellular distribution of the dendrimer-oligonucleotide complex and of its components by two-color digital fluorescence microscopy. They observed that oregon green 488-conjugated dendrimer was a better delivery agent for antisense compounds than unmodified dendrimers.

Sashiwa and Aiba (2004) investigated the role of individual functional groups in applications of chitosan. They modified chitosan by attaching sugars, dendrimers, cyclodextrins, crown ethers, and glass beads to chitosan and concluded that among these derivatives, sugar-modified chitosans were excellent candidates as drug delivery systems or for cell culture while chitosan–dendrimer hybrids were interesting multifunctional macromolecules in biomedicinal applications.

The most commonly synthesized and studied dendrimers are the ones prepared from polyamidoamine (PAMAM). Wiwattanapatapee et al (2000) investigated the effects of size, charge, and concentration of PAMAM dendrimers on uptake and transport across the adult rat intestine in vitro using the everted rat intestinal sac system. They used cationic PAMAM dendrimers (generations 3 and 4) and anionic PAMAM dendrimers (generations 2.5, 3.5, and 5.5) and labelled the dendrimers with I-125. They concluded that, the anionic PAMAM dendrimers displayed serosal transfer rates faster than that of other synthetic and natural macromolecules (including tomato lectin). PAMAM dendrimers were also prepared by Tripathi et al (2002) by linking methyl methacrylate and ethylenediamine successively on an amine core and the surfaces were modified with fatty acids. They studied the release rates of chemotherapeutic drug, 5-fluorouracil (5-FU), which was entrapped in dendrimer grafts. In vitro studies, release rate was examined across cellulose tubing in PBS, and in vivo studies release rates were performed in albino rats by determining the amount of 5-FU in plasma. Jevprasesphant et al (2004) investigated the mechanism of transport of G3 PAMAM dendrimer nanocarriers and surface-modified (with lauroyl chains) dendrimers across Caco-2 cell monolayers. Optical sectioning of cells incubated with fluorescein isothiocyanate (FITC)-conjugated dendrimer and lauroyl–dendrimer using confocal laser scanning microscopy revealed colocalisation of a marker for cell nuclei (4',6-diamidino-2-phenylindole) and FITC fluorescence, also suggesting cellular internalisation of dendrimers. Effect of various concentrations PAMAM dendrimers (generations 2, 3, and 4) on human red blood cell morphology, and membrane integrity was studied by

Domanski et al (2004). They observed a change in erythrocyte shape from biconcave to echinocytic in dendrimers as well as cell aggregation and haemolysis depending on concentration and generation of dendrimers. Sagidullin et al (2004) studied the self-diffusion coefficients and nuclear magnetic relaxation of poly (amidoamine) dendrimers with hydroxyl surface groups (PAMAM-OH) by dissolving dendrimers in methanol over a wide range of concentrations. The generalized concentration dependence of PAMAM-OH self-diffusion coefficients were found to be coincide with analogous curve obtained for poly (allylcarbosilane) dendrimers of high generations.

To establish an effective nonviral gene transfer vector to hepatocytes, various oligo-carrier complexes were developed by Mamede et al (2004) by employing dendrimer (G4) and avidin–biotin systems (Av–bt). It was reported that for In-111-labeled-oligo, without any carriers, low uptake in normal organs other than the kidney were observed. In contrast, In-111-labeled-oligo coupled with avidin through biotin had very high accumulation in the liver. If G4 complexed forms are used, high uptake in the kidney and spleen were observed with relatively low hepatic uptake. They concluded that avidin–biotin systems have high potential as a carrier of oligo-DNA to the liver. ^{111}In-oligo-bt-Av, which exhibited the highest hepatic uptake in vivo, showed high and rapid internalization into hepatocytes. Okuda et al (2004) also studied non-viral gene delivery systems and showed that dendritic poly (L-lysine) of the 6th generation (KG6) had high transfection efficiency into several cultivated cells with low cytotoxicity. They synthesized KGR6 and KGH6, in which terminal amino acids were replaced by arginines and histidines, respectively. DNA-binding analysis showed that KGR6 could bind to the plasmid DNA as strongly as KG6, whereas KGH6 showed decreased binding ability. Wada et al (2005) studied in vitro and in vivo gene delivery efficiency of polyamidoamine starburst dendrimer (generation 2) conjugate with α-cyclodextrin bearing mannose with various degrees of substitution of the mannose moiety as a novel non-viral vector in a variety of cells. Sampathkumar et al (2005) described bifunctional PAMAM-based dendrimers that selectively target cancer cells. The targeting moiety for the folate receptor was complexed to an imaging or therapeutic agent by a DNA zipper. Choi et al (2005) produced amine-terminated, generation 5 polyamidoamine dendrimers conjugated to different biofunctional moieties (fluorescein and folic acid), and then linked them together using complementary DNA oligonucleotides to produce clustered molecules that target cancer cells that over express the high-affinity folate receptor. Kolhe et al (2003) studied the interaction between the drug and polyamidoamine dendrimers (generations 3 and 4 with $-NH_2$ functionality) and Perstrop Polyol (generation 5, hyperbranched polyester with –OH functionality) by using ibuprofen as a model drug. They found that hyperbranched Polyol (with 128 –OH end groups) appears to encapsulate approximately 24 drug molecules.

Singh and Florence (2005) synthesized lipidic polylysine dendrimers. They examined the effect of concentration on the diameter and stability of nanoparticles formed from two short homologous series of dendrimers. Raju et al (2005) described the synthesis of a new scaffold derived from iminodipropionic acid for

the preparation of peptide dimers and tetramers. Pan et al (2005) synthesized polyamidoamine (PAMAM) dendrimer on the surface of magnetite nanoparticles to allow enhanced immobilization of bovine serum albumin (BSA). They concluded that there were two major factors that improved the BSA binding capacity of dendrimer-modified magnetite nanoparticles: either the increased surface amine can be conjugated to BSA by a chemical bond; or the available area has increased due to the repulsion of surface positive charge.

Schatzlein and colleagues (2005) studied the transfection activity of polypropylenimine dendrimers and the effect of the strength of the electrostatic interaction between carrier and DNA on gene transfer. They evaluated the in vivo gene transfer activity of low molecular weight, non-amphiphilic plain and quaternary ammonium gene carriers and concluded that the polypropylenimine dendrimers were promising systems, which may be used in gene targeting. Recently Namazi and Adeli (2005) applied citric acid–polyethylene glycol–citric acid triblock dendrimers as biocompatible compounds for drug-delivery. They investigated the controlled release of molecules and drugs in vitro conditions and reported that the drug/dendrimer complexes were stable while the drugs were not released after storage at room temperature for about 10 months. Marano and co-workers (2004) described the synthesis of lipid–lysine dendrimers and their ability to deliver sense oligonucleotide ODN-1 to its target. It is important to mediate the reduction in VEGF concentration both in vitro and in vivo during ocular neovascularisation. They demonstrated that lipophilic, charged dendrimer mediated delivery of ODN-1 resulted in the down-regulation of in vitro VEGF expression. Time course studies showed that the dendrimer/ODN-1 complexes remained active for up to two months indicating the dendrimer compounds provided protection against the nucleases. Ooya and colleagues (2003) developed systems to increase the aqueous solubility of paclitaxel (PTX), a poorly water-soluble drug. They reported that graft and star-shaped graft polymers consisting of poly (ethylene glycol) (PEG 400) graft chains increased the PTX solubility in water by three orders of magnitude. Polyglycerol dendrimers dissolved in water at high concentrations without significantly increasing the viscosity and by increasing the solubility of PTX while the release rate was found as a function of the star shape and the dendrimer generation. Rittner and co-workers (2002) studied the design of basic amphiphilic peptides, ppTG1 and ppTG20 (20 amino acids), and evaluated their efficiencies *in vitro* and *in vivo* as single-component gene transfer vectors. Based on the structure–function studies, and sequence variants, they suggested that the high gene transfer activity of these peptides was correlated with their propensity to exist in α-helical conformation, which seems to be strongly influenced by the nature of the hydrophobic amino acids.

Dendrimers were also studied in the production of biosensors. For example, Alonso et al (2004) used ferrocene–cobaltocenium dendrimers in the preparation of glucose electrodes. For this purpose, enzyme glucose oxidase (GOx) was immobilized electrostatically onto carbon and platinum electrodes which were modified with dendrimers and the effects of the substrate concentration, the dendrimer

generation, and the thickness of the dendrimer layer, interferences, and storage on the response of the sensors were investigated. Devarakonda et al (2004) investigated the effect of low generation (G0–G3) ethylenediamine (EDA) core poly (amidoamine) dendrimers on the aqueous solubility of nifedipine in different pH values. It was reported that generation size, surface functional group and the pH of the aqueous media determined the aqueous solubility and solubility profiles of nifedipine. For amine and ester terminated dendrimers the highest nifedipine solubility was observed at pH 7.0.

Smith et al (2005) published a review about the properties of dendritic molecules and focused on examples in which individual dendritic molecules are assembled into more complex arrays via non-covalent interactions. This review emphasises how the structural information programmed into the dendritic architecture controls the assembly process, and as a consequence, the properties of the supramolecular structures which are generated, and how the use of non-covalent (supramolecular) interactions provide the assembly process with reversibility, with a high degree of control. The review also illustrates how self-assembly offers an ideal approach for amplifying the branching of small, synthetically accessible, relatively inexpensive dendritic systems (e.g. dendrons), into highly branched complex nanoscale assemblies and how assembled structures encapsulate a templating unit.

3.5. Polymeric Micro and Nano Particles

In the delivery of bioactive agents, generally the agent is dissolved, entrapped, adsorbed, attached or encapsulated in a polymeric matrix that has a micro or nano dimension. Depending on the method of preparation, micro or nano particles, spheres or capsules can be obtained with different properties and different release characteristics. Capsules are vesicular systems in which the drug is trapped in the central cavity which is surrounded by a polymeric membrane, whereas spheres are systems in which the drug is physically and uniformly dispersed in the matrix. Scientists have carried out numerous studies describing the effect of preparation parameters on the properties of micro and nano particles. Boguslavsky et al (2005) prepared polyacrylonitrile nanoparticles in sizes ranging from approximately 35 to 270 nm by dispersion/emulsion polymerization of acrylonitrile. They investigated the influence of various polymerization parameters (e.g. concentration of monomer and initiator, type and concentration of surfactant, temperature and time of polymerization, ionic strength, pH and co-solvent concentration) on the properties (e.g. size and size distribution, yield, stability, etc.) of the particles. Recently He and colleagues (2005) prepared polyaniline nanofibers and polyaniline/CeO_2 composite microspheres by stabilizing the emulsion by CeO_2 nanoparticles. They also synthesized sub-micrometer fibers of polyaniline/nano-ZnO composites in a toluene/water emulsion stabilized by ZnO nanoparticles and examined effects of volume ratio of toluene to water on properties of the composites. Akin and co-workers (1990) designed and synthesized polymeric hydrophobic membranes which have micro hydrogel channels and examined permeabilities towards various chemicals. They

found that, permeability depends on the crosslinking of hydrogel part, as well as the chemical structure and the charge of the permeant.

Nanoparticles of poly (DL-lactic acid) (PDLLA), poly (DL-lactic-co-glycolic acid) (PLGA) and poly (ethylene oxide)–PLGA diblock copolymer (PEO–PLGA) were prepared by the salting-out method by Zweers et al (2004). They examined the in vitro degradation of the prepared nanoparticles in PBS (pH 7.4) at 37°C. The effects of particle size, molecular weight of the polymers and the amount of lactic and glycolic acids on the degradation were examined. It was reported that, PDLLA nanoparticles gradually degraded over a period of 2 years while faster degradation was observed for PLGA nanoparticles such as complete degradation in 10 weeks.

Natural polymers such as gelatin, chitosan, proteins and starch are all interesting materials for medical applications since they are biodegradable and bioabsorbable where the degradation products do not have any toxic effect. Akin and Hasirci examined the properties of gelatin microspheres prepared under different conditions (1995) and also examined release of 2,4-D from these systems (1994). Burke et al (2000, 2002) examined iron ion adsorption capacity of chitosan microspheres to remove iron from the blood for the treatment purpose of thalasemmia. Yilmaz et al (2002) also examined chelating capacity of chitosan flakes and microspheres for complexed iron (III) for the removal of iron ions. Ulubayram et al (2001, 2002) examined cytotoxicity of microporous gelatin sponges prepared with different crosslinkers. In a series of studies Muvaffak et al (2002, 2004a, 2004b, 2005) prepared gelatin microspheres and conjugated antibodies to their surfaces. They studied targeting and release of chemotherapeutic drugs such as 5-fluoroucil and colchicines and showed that the system had a high affinity towards its antigens and the release rate of drugs depended on the preparation parameters of microspheres. They suggested the systems are promising and have high potential as anticancer drug targeting systems to specific tumor locations.

One advantage of delivery systems is that they allow the delivery of drugs that are highly water-insoluble or unstable in the biological environment. Zhang and Zhuo (2005) prepared a BAB type amphiphilic triblock copolymers consisting of poly (ethylene glycol) (PEG) (B) as hydrophilic segment and poly (ε-caprolactone) (PCL) (A) as hydrophobic block. A poorly water-soluble anticancer drug 4'-dimethyl-epipodophyllotoxin (DMEP) was encapsulated into the polymeric nanoparticles for controlled drug release. In vitro results showed that the drug release rate can be modulated by the variation of the copolymer composition. Long-term sustained delivery is a desired property and is affected by the diffusion kinetics of the drug and degradation of the matrix which controls the rate of drug release. It is possible to extend this period from hours to months. A review was published by Sinha et al (2004) about long-term delivery from poly-ε-caprolactone (PCL) microspheres and nanospheres. They reported that biodegradation of PCL is very slow in comparison to other polymers, which makes it suitable for long-term delivery, extending the release duration to more than one year.

Alonso and colleagues (2004) studied nanosystem drug carriers for mucosal administration. In vitro cell culture studies and in vivo experiments have proved the

potential of nanocarriers in overcoming mucosal barriers such as intestinal nasal and ocular barriers. Recently Dinauer et al (2005) prepared gelatin nanoparticles and antibodies specific for the CD3 antigen of lymphocytic cells were conjugated to the nanoparticle surface. Cellular uptake and effective internalization of antibody-conjugated nanoparticles into CD3 expressing cells were examined. Dinauer et al (2004) also developed a carrier system for antisense oligonucleotides (AS-ODN) and antisense phosphorothioate analogs (AS-PTO). They prepared nanoparticles by using protamine to complex AS-ODN and AS-PTO and concluded that cellular uptake of these nanoparticles significantly enhanced the uptake in comparison to naked oligonucleotides. Dong and Feng (2005) prepared poly (d,l-lactide-co-glycolide)/montmorillonite (PLGA/MMT) nanoparticles by emulsion/solvent evaporation method as bioadhesive drug delivery system for oral delivery of paclitaxel. It was reported that the system extended residence time in the gastrointestinal (GI) tract and promoted the effect of the drug.

Ciardelli et al (2004) studied formation of poly (methyl methacrylate-*co*-methacrylic acid) nanospheres which were imprinted with theophylline through template radical polymerization. Effect of the nature of the functional monomer in the recognition and in the release of template was studied. These systems can be considered as promising systems for the recognition and isolation of the biologically important template molecules. Chen and Subirade (2005) prepared chitosan/β-lactoglobulin core–shell nanoparticles with the aim of developing a biocompatible carrier for the oral administration of nutraceuticals. Uniform size nanoparticles were prepared by ionic gelation with sodium tripolyphosphate and were highly sensitive to medium pH. When transferred to simulated intestinal conditions, the β-lactoglobulin shells of the nanoparticles were degraded by pancreatin.

Responsive hydrogels gained great importance in 1990‘s and lots of research is going on since then. Yoshida et al (1989) synthesized some thermo-responsive hydrogels containing α-amino acid groups as side chains from copolymerizing 2-hydroxypropyl methacrylate and polyethylene glycol dimethacrylate, using gamma irradiation. They investigated swelling-deswelling as well as thermo-responsive kinetics of drug release. Dong and Hoffman (1990) investigated progesterone release from thermally reversible hydrogels of N-isopropylacrylamide (NIPAAm) and bis-vinyl-terminated polydimethylsiloxane (VTPDMS) synthesized by gamma irradiation. They proposed existance of microdomain structure in the gels based on differential scanning calorimetry results and observed zero-order release of progesterone. Kabra et al (1992) synthesized poly (vinyl methyl ether) thermally responsive gels by gamma irradiation and examined the shrinking rates of the gels. They observed that enhancement in rate was related to the development of a microporous structure which allows the convective expulsion of solvent from the network which occurs more quickly than the diffusive motion of the network. Low et al (2000) designed microactuator valves made of metal or polymeric substances for responsive delivery of drugs. The reversible polymeric valve systems acted as artificial muscle and were prepared from a blend of redox polymer and hydrogel (polyaniline and poly (2–hydroxyethylmethacrylate)–poly (N–vinylpyrrolidinone).

They concluded that responsive controlled drug delivery by these microactuator valves is possible. Shantha and Harding (2000) examined biocompatible and biodegradable pH-responsive hydrogels based on N-vinyl pyrrolidone (NVP), polyethylene glycol diacrylate (PAC) and chitosan. In-vitro release profiles of theophylline and 5-fluorouracil were examined in enzyme-free simulated gastric and intestinal fluids, observing that more than 50% of the entrapped drugs were released in the first 2 h in gastric pH. Goldraich and Kost (1993) prepared hydrogel matrices for immobilization of glucose oxidase and release of insulin responsive to glucose concentration. They did the synthesis by chemical polymerization of 2-hydroxyethyl methacrylate, N,N-dimethyl-aminoethyl methacrylate, tetraethylene glycol dimethacrylate, ethylene glycol in the presence of water solutions of glucose oxidase, bacitracin or insulin. They observed faster and higher swelling and release rates at lower pH or at higher glucose concentrations. Chen et al (2000) prepared colloidal platinum nanoparticles in the size range of 10–30 Å in the presence of poly (*N*-vinylisobutyramide) (PNVIBA). The formed colloidal PNVIBA–Pt nanoparticles exhibited inverse temperature solubility and a cloud-point temperature of 38.9°C in water.

Gomez-Lopera et al (2001) prepared colloidal particles responsive to magnetic field. They did the synthesis of biodegradable poly (dl-lactide) polymer around a magnetite nucleus by using biodegradable poly (dl-lactide) with a double-emulsion technique. The main purpose was to develop responsive drug delivery systems. Vihola et al (2002) investigated behaviours and release kinetics of model drugs (β-blocking agents nadolol and propranolol and a choline-esterase inhibitor tacrine) from thermally responsive polymeric nanoparticles composed of poly (N-vinylcaprolactam) (PVCL). They observed that the more hydrophobic drug substances, propranolol and tacrine, considerably swell the PVCL-microgels. The β-blocking agents were tightly bound to the microgels especially at higher temperatures and on the contrary, the release of tacrine across the cellulose membrane was increased when PVCL particles were present. Taniguchi et al (2003) investigated temperature, pH, and salinity effects for adsorption and desorption of anti-α-feto protein (anti–AFP) onto polystyrene-core-poly (*N*-isopropylacrylamide)-shell particles. They observed that adsorption was mainly governed by electrostatic interactions. Twaites et al (2004) prepared poly (*N*-isopropyl acrylamide) (PNIPAm) co-polymers responsive to temperature and pH. They examined the binding of plasmid DNA to these materials and to control polymers of poly (ethyleneimine) (PEI) and poly (ethyleneimine)-octanamide. They observed the complexes of plasmid DNA with thermoresponsive cationic polymers displayed variations in gel retardation behaviour above and below polymer phase transition temperatures such as, lesser affinity for high molecular weight linear cationic PNIPAm co-polymer complexes, and higher affinity for branched PEI-PNIPAm co-polymers above LCST. Zhang et al (2004) prepared composite membranes from nanoparticles of poly (N-isopropylacrylamide-co-methacrylic acid) of various NIPAAm:MAA ratios dispersed in a matrix of a hydrophobic polymer. Permeation of N-Benzoyl-L-tyrosine ethyl ester HCl, momany peptide, Leuprolide, vitamin B12, insulin,

and lysozyme were examined as a function of temperature. Kovacs et al (2005) demonstrated that anionic microspheres coated with an ornithine/histadine-based cationic peptide (O10H6) were effective carriers of short oligonucleotides. They reported that microspheres stabilize the DNA and O10H6 through complexation. They proposed that, this self-assembly system can be an effective delivery vehicle for DNA-based formulations. Venkatesan et al (2005) studied the feasibility of nanoparticulate adsorbents in the presence of an absorption enhancer for the administration of erythropoietin (EPO) to the small intestine. Liquid filled nano and micro particles were prepared using solid adsorbents such as porous silicon dioxide, carbon nanotubes, carbon nanohorns, fullerene, charcoal and bamboo charcoal. The serum EPO levels were compared for the prepared systems. Among the adsorbents studied, carbon nanotubes showed the highest capacity. Recently Jo and coworkers (2004) carried out mathematical modeling of release of encapsulated indomethacin from poly (lactic acid-co-ethylene oxide) nanospheres and investigated in vitro release behavior based on the proposed mathematical models. Effects of several key parameters were examined according to two different types of mathematical models.

4. CONCLUSION

Use of micro and nano particles in biomedicine and especially in drug delivery has a great deal of advantages over conventional systems such as: the enhanced delivery, high performance characteristics of the product, use of lesser amounts of expensive drugs in the delivery systems, extension of the bioactivity of the drug by protecting it from environmental effects in biological media, more effective treatment with minimal side effects. In addition, research for the design of more effective delivery systems is more economical for the discovery of a new bioactive molecule. Micro and nano colloidal drug delivery systems such as emulsions, suspensions and liposomes have been used for decades for this purpose and recently, nanosized systems with dimension of less than 100 nm gained significant attention. Nanotechnology promises to generate a library of sophisticated drug delivery systems that integrate molecular recognition, diagnostic and feedback. Nanotechnology is expected to create lots of innovations and play a critical role in various biomedical applications including the design of drug and gene delivery systems, molecular imaging, biomarkers and biosensors. By understanding the signalling and interaction between the molecules at nano levels, it would be possible to mimic biological systems.

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