ZINC NANOPARTICLES FOR THE TREATMENT OF INFECTIONS AND CANCER

Abstract: The present invention provides for production and use of pharmaceutically-acceptable zinc nanoparticles having cores comprising elemental zinc without significant amounts of other metals or metal oxides. The use of these nanoparticles in cancer therapy and treatment of infection disease, along with medical imaging, is provided.
DESCRIPTION

ZINC NANOPARTICLES FOR THE TREATMENT OF INFECTIONS AND CANCER

BACKGROUND OF THE INVENTION

This application claims benefit of priority to U.S. Provisional Application Serial No. 61/234,374, filed August 17, 2009, the entire contents of which are hereby incorporated by reference.

I. Field of the Invention

The present invention relates generally to the fields of oncology, infectious disease and medicine. More particularly, it provides novel pharmaceutical formulations of zinc nanoparticles for the treatment of cancer and infections.

II. Description of Related Art

A. Zinc-Induced Cell Killing

Zn is an essential nutrient and is present throughout the body. It is required as either a catalytic, co-catalytic or structural component for a large number of enzymes and, as such, contributes to a wide variety of important biological processes including gene expression, replication, hormonal storage and release, neurotransmission, and memory. There is compelling evidence from a number of reviews with regards to the role of zinc in the development and progression of malignancy. Zinc has also been shown to impact the invasive capabilities of cancer cells. Zinc ions had the ability to suppress invasion of cancer cells in Matrigel. In studies, evaluating intracellular zinc levels within SCCHN, it was shown that tumor levels of zinc were associated with outcome. Higher zinc levels resulted in greater survival presumably by inhibition of tumor metastasis.

Zinc treatment has long been recognized to inhibit tumor development and growth and the reverse, zinc deficiency has been associated with the development of many tumor types. An early 1918 version of the Merck Manual lists various zinc salts as treatment methods for different cancers. Zinc metal readily dissolves into zinc salts in acid conditions. Zinc salts have long been recognized as an astringent and are used
as a fixative for skin lesions in Moh's surgery. This astringency effect is modulated by extracellular zinc. Intracellular zinc accumulation has been reported by numerous groups to be cytotoxic in a wide number of cell types. This effect is further corroborated in the inventors' studies using pyrithione, a zinc ionophore. At non-toxic levels of zinc and pyrithione, in combination, toxicity is seen at zinc concentrations as low as 1 µM. Zinc-oxide nanoparticles have been shown to accumulate in both endosomes and lysozomes depending on the cell type and extracellular conditions.

It has been shown that zinc can both induce and prevent apoptosis (see Fraker and Telford, 1997; Fraker and Telford, 1996). These reports art showed that 80-200 µM zinc induced apoptosis in 40% of CD4+ CD8+ αβTCR10CD3(10) thymocytes. The killing was viewed as being caused by apoptosis.

B. Cancer

Current treatment methods for cancer, including radiation therapy, surgery, and chemotherapy, have vastly improved the chances for short and long term survival of those patients afflicted with cancer. However, malignancies continue to be the a major causes of mortality around the world, and the cost of treatment and care for cancer patients continues to be a major drain on society. The development of new and innovative therapies for cancer is, therefore, critical to the improvement of cancer healthcare.

U.S. Patent Publication 2007/0212331 showed that both cellular apoptosis and necrosis can be induced in cells - including cancer cells - by increasing the intracellular concentration of zinc above a certain threshold. Moreover, the data further demonstrated the killing of p53-negative cells, which are not able to undergo canonical apoptosis. Liposomal delivery of zinc was provided as a particular embodiment.

C. Infection

Most nosocomial infections are caused by the contamination of medical devices resulting in serious hospital-acquired infections. Nosocomial pneumonias are the second most common nosocomial infections, and are associated with the highest attributable mortality and morbidity. Recent data have shown that at least 300,000
episodes of nosocomial pneumonia occur annually in the United States (Official Statement, American Thoracic Society). The attributable mortality of this infection is 33-50%, hence, around 100,000 patients die annually because of nosocomial pneumonia (CDC, 1993; Leu et al., 1989). The risk of nosocomial pneumonia increases 6- to 20-fold from the use of mechanical ventilation (Official Statement, American Thoracic Society).

Antibiotics and antiseptics have been used to impregnate vascular catheters. The concern with the use of antibiotics has been that resistance might develop to antibiotics, preventing their use therapeutically and systemically in hospitalized patients. Furthermore, the durability of the existing antiseptics has been limited. What is needed is an effective antiseptic having broad spectrum activity against resistant staphylococci, vancomycin-resistant enterococci, resistant Pseudomonas aeruginosa and Candida species, to be used in conjunction with indwelling devices that will inhibit or prevent the nosocomial infections typically associated with the use of these indwelling devices.

Though zinc is needed for normal cellular function, numerous studies exist reporting zinc-containing compositions have antibiotic activity, particularly at higher concentrations (Sødeberg et al., 1990; Atmaca et al., 1998). More recently, Chang and Leung (2008) report that ceramic powders of zinc oxide (ZnO) showed marked anti-bacterial activity. Nanocomposites containing nano zinc oxide (average size 20 nm) in titanium dioxide (titania) sol-gel matrix were synthesized with various loading weight percentage of nano zinc oxide powder with respect to titania sol weight. Nanocomposites with 20% and 30% by weight of ZnO nanoparticles in TiO_2 solgel matrix (TiO_2-ZnO20 and TiO_2-ZnO30, respectively) inhibited 40 to 95% of both antibacterial proliferation from different batch of nanocomposite products. Both nanocomposites selectively inhibited E. coli compared with S. aureus. A clear dose-dependent response between TiO_2-ZnO20 and TiO_2-ZnO30 was recorded in S. aureus assay.
SUMMARY OF THE INVENTION

Thus, in accordance with the present invention, there is provided a zinc nanoparticle composition comprising (a) a zinc core substantially free of other metals or metal oxides; and (b) a non-zinc shell or coating. The zinc nanoparticle may be dispersed in a pharmaceutically acceptable buffer, carrier or diluent. The non-zinc shell may comprise a material permitting conjugation of an agent to said zinc nanoparticle, such as a thiolic acid. The zinc nanoparticle may further comprise an agent conjugated to said zinc nanoparticle. The agent may be a cell or tissue targeting agent, a diagnostic agent, or a therapeutic agent. The non-zinc shell or coating comprises a biologically inert gel, polymer or matrix. The zinc nanoparticle may be between about 5 nm and 100 nm, or between 10 nm and 50 nm.

In still another embodiment, there is provided a method of treating a subject with a solid tumor comprising administering to said subject a zinc nanoparticle composition comprising (a) a zinc core substantially free of other metals or metal oxides; and (b) a non-zinc shell or coating. The solid tumor may be prostate tumor, basal cell carcinoma tumor, squamous cell carcinoma tumor, or melanoma tumor. The subject may be a human, a non-human primate, a dog, a cat, a horse, a cow, a goat, a rat, a pig, a rabbit, or a mouse. The administering may comprise systemic administration, intravenous injection, or loco-regional administration, such as intratumoral injection, injection into tumor vasculature, or injection regional to said tumor. The method may further comprise administering to said subject a second anti-cancer therapy, such as radiotherapy, chemotherapy, immunotherapy, gene therapy, hormone therapy, and antibiotic therapy. The cancer may be recurrent, metastatic or multi-drug resistant.

In a further embodiment, there is provided a method of treating a subject with an infection comprising administering to said subject a zinc nanoparticle composition comprising (a) a zinc core substantially free of other metals or metal oxides; and (b) a non-zinc shell or coating. The infection may be bacterial, viral, fungal or parasitic. The subject may be a human, a non-human primate, a dog, a cat, a horse, a cow, a goat, a rat, a pig, a rabbit, or a mouse. The administering may comprise systemic administration, intravenous injection, or loco-regional administration, such as topical administration or injection regional to said infection. The method may further
comprise administering to said subject a second anti-biotic therapy. The infection may be caused by a drug resistant organism.

In still yet an additional embodiment, there is provided a method of imaging a site in a subject comprising (i) administering to said subject a zinc nanoparticle composition comprising (a) a zinc core substantially free of other metals or metal oxides; and (b) an agent that targets said zinc nanoparticle to said site, and (ii) imaging said zinc nanoparticle in said subject. The zinc nanoparticle may be further comprise a detectable label, such as a fluorescent, chemilluminescent or radiolabel, or may further comprise a therapeutic agent, such as an antibiotic, an anti-fungal, and anti-parasitic, an anti-viral, a chemotherapeutic, or a radiotherapeutic. The subject may have a tumor or an infection.

In some aspects, the shell or coating is composed of a polymer. Examples of such polymeric surfaces include polyvinyl chloride, polyurethane, polyethylene, silastic elastomers, polytetrafluoroethylene, dacron, collodion, carboethane or nylon, esters of polylactide, polyglycolide, polycaprolactone, polyethylene glycol, polyethylene oxide and all copolymers of the above derivatives. Alternatively, the surface may be composed of silicone or silk.

In another embodiment, there is provided a medical device coated with zinc nanoparticle composition comprising zinc nanoparticles comprising a zinc core substantially free of other metals or metal oxides. The device may be an implanted or indwelling device, such as a catheter, stent, pump, or suture. The zinc nanoparticle may further comprise a non-zinc shell or coating, such as a biologically inert gel, polymer or matrix. The nanoparticle may be between about 5 nm and 100 nm, or between about 10 nm and 50 nm. The non-zinc shell or coating may comprise a material permitting conjugation of a therapeutic agent to said zinc nanoparticle, such as a thiolic acid.

In yet other embodiments, the zinc nanoparticles can be use to impregnate an inorganic surface. Examples of such inorganic surfaces include floors, table-tops, counter-tops, surfaces of a hospital equipment, wheelchair surfaces, etc. Virtually any surface comprising a material that is capable of being coated by, impregnated with, absorbing or otherwise retaining the antiseptic compounds of the invention may be disinfected and/or sterilized using the present antiseptic compounds and their compositions. Thus, the antiseptic compound of the invention can be used to disinfect, sanitize and sterilize a wide variety of surfaces.
The invention also provides medical devices coated with zinc nanoparticles. Examples of medical devices include endotracheal tubes, a vascular catheter, an urinary catheter, a nephrostomy tube, a biliary stent, a peritoneal catheter, an epidural catheter, a central nervous system catheter, an orthopedic device, a prosthetic valve, and a medical implant. The vascular catheter may be a central venous catheter, an arterial line, an pulmonary artery catheter, and a peripheral venous catheter. The central nervous system catheter may be an intraventricular shunt. Other medical devices that can benefit from the present invention include blood exchanging devices, vascular access ports, cardiovascular catheters, extracorporeal circuits, stents, implantable prostheses, vascular grafts, pumps, heart valves, and cardiovascular sutures, to name a few. Regardless of detailed embodiments, applicability of the invention should not be considered limited with respect to the type of medical device, implant location or materials of construction of the device.

The invention also provides methods for coating a medical device with a zinc nanoparticles comprising a) immersing a medical device in a solvent comprising a zinc nanoparticle; b) drying the device; and c) washing off excessive composition. In some embodiments, the solvent used to immerse the device can be methylene chloride, methanol, or a combination thereof.

The invention also provides methods for preventing nosocomial infections in a subject comprising coating a medical device that the subject is required to use with a composition comprising zinc nanoparticles. The subject can be human or an animal model. The type of nosocomial infection that can be prevented by the methods of this invention include, but are not limited to, pneumonia, bacteremia, fungimia, candidemia, a urinary tract infection, a catheter-exit site infection, and a surgical wound infection.

The nosocomial infections that can be prevented may be caused by bacteria. In some embodiments the bacteria are drug resistant bacteria. Some non-limiting example of drug resistant bacteria include methicillin-resistant staphylococci, vancomycin-resistant enterococci, and resistant Pseudomonas aeruginosa.

The nosocomial infection may be caused by a fungus. In some cases the fungal agent is a drug resistant fungi. Examples of a drug resistant fungi include members of the Candida species. Other pathogenic organisms that can cause the nosocomial infections are cited elsewhere in this specification and coating devices and surfaces with the antiseptics of the present invention can prevent infections by these organisms as well.
Thus, the compositions of the present invention have broad uses including use in healthcare by providing sterile medical devices and surface sterilization and decontamination.

As used herein the specification and claim(s), the words "a" or "an" when used in conjunction with the word "comprising" may mean one or more.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.
BRIEF DESCRIPTION OF THE DRAWINGS

The following figures form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these figures in combination with the detailed description of specific embodiments presented herein.

FIG. 1 - Electron microscopy of zinc nanoparticles (ZNP) of approximately 100 nm size. Courtesy of Hefei Kaier Nanotech. Particles are of various different shapes and are electrodense.

FIG. 2 - Appearance of ZNP in solution. ZNP exist as a black/grayish powder in Solution, a optically dense gray solution forms. ZNPs aggregate in the absence of surfactant.

FIG. 3 - Solubility of ZNP in different solutions. ZNP are highly soluble in aqueous solutions. In all cases, dissolution of ZNP to zinc ions occurs within the first 10 min. ZNP dissolution is dependent on zinc supersaturation of the solution. Provided ample, volume, complete dissolution of ZNPs occurs. ZNPs are highly reactive to protein. A slight delay in ZNP dissolution is seen in solutions with large amounts of proteins such as media or serum.

FIG. 4 - Solubility of ZNP in BioGel formulations of Pluronic. Pluronic is a block co-polymer that shields the ZNP from dissolution in aqueous environments. ZNPs (10 mg/ml) were formulated in different percentages w/v of Pluronic gel. 100 µl of gel-ZNP was added to 5 ml of media and zinc was measured by TSQ fluorescence over time. ZNP dissolution was dependent on the shielding ability of the block co-polymer.

FIG. 5 - Cytotoxicity of ZNPs against cancer cells. ZNPs were added to 70% logarithmic growth cultures of PC3 (prostate cancer), CAL27 (Squamous Cell Carcinoma of the Head and Neck), and SKOV3 (Ovarian Cancer). Cancer cell viability was measured by MTT assay at various time points.

FIG. 6 - Dose dependent ZNP cytotoxicity against cancer cell lines. ZNPs were added to 70% logarithmic growth cell lines in culture. Cell viability was measured by MTT assay at 18 hrs. There is a threshold phenomenon. At low
doses, no cytotoxicity is seen. After a threshold dose, significant cell death is observed.

FIG. 7 - Synergy of ZNP with cancer chemotherapeutics. ZNPs were added to PC3 cells either alone (LD50) dose or in combination with the reported LD50 of the cancer chemotherapeutic drug on the X-axis. ZNPs potentiate the cytotoxic effects of chemotherapeutics in a number of different classes.

FIG. 8 - Efficacy of ZNPs against subcutaneous melanomas. Subcutaneous B16 melanomas were grown on the dorsum of immunocompetent C57B/6 mice. When tumors reached approximately 150 mm$^3$, they were directly injected with 100 µl volume of vehicle (PBS), 30% Pluronic, ZNPs (10 mg/ml in PBS), or 30% Pluronic with 10 mg/ml ZNPs. Tumor volume was determined by digital caliper measurement. Pluronic groups show a rapid increase in volume most likely due to the gel itself rather than tumor growth. This is supported by the initial increase in volume by Day 1 followed by a decline by Day 3. Non-Pluronic groups do not show a rapid increase in tumor size. ZNPs did not show anti-tumor effect likely due to rapid dissolution of the ZNPs. In contrast, Pluronic-ZNPs showed a significant decline in tumor size over time.

FIG. 9 - Survival of animals with melanoma. Subcutaneous B16 melanomas were grown on the dorsum of immunocompetent C57B/6 mice. When tumors reached approximately 150 mm$^3$, they were directly injected with 100 µl volume of vehicle (PBS), 30% Pluronic, ZNPs (10 mg/ml in PBS), or 30% Pluronic with 10 mg/ml ZNPs.

FIG. 10 - Cytotoxicity of Her2/Neu AB-conjugated ZNPs. Cell lines were plated in 6 well-plates at 70% confluence and treated as given on the X-axis. The Her2 AB concentration was 60 µg/ml. ZNP were added at 10 µg/ml and Her2-ZNP at 5 µg/ml. Cells were washed 1 hr after addition of treatment and then MTT assay performed at 18 hrs. Her2-conjugated ZNP show highly specific killing of Her2-expressing cells (SKOV3 and SKBR3) but not the Cal27 (Her2 non-expressing). Results demonstrate ZNP cytotoxicity can be controlled through the use of a targeting ligand. Cell killing was dose dependent.

FIG. 11 - Intracellular zinc ion concentration (Mean Fluorescent Intensity) levels are dependent on ZNP targeting. Mean Fluorescent Intensity (MFI, y-axis) representing intracellular zinc ion concentration was measured by FACS on cell
lines expressing HER2 (SKBR3 and SKOV3) or expressing high levels of transferrin receptor (PC3) after incubation with 1 mg of the designated treatment: Control - no treatment; ZNP - unconjugated ZNP; ZNP-Her2 - Anti-Her2 conjugated ZNP; ZNP-TNF - transferrin conjugated ZNP.

**FIG. 12** - Intracellular zinc ion concentrations rise over time and are directly proportional to cell death. Mean Fluorescent Intensity (MFI) was measured by FACS after staining cells with zinc ion specific fluorescent probes. MFI represents total histogram fluorescence of gated fluorescent cells. SKBR3 cells were analyzed for intracellular zinc ion concentration. Control (diamond) represent non-treated cells. ZNP (square) - cells treated with unconjugated ZNP (1 mg). ZNP-Her2 (diamond) - cells treated with Anti-Her2 antibody conjugated ZNP (1 mg). ZNP-TNF (cross marks) - cells treated with transferrin targeted ZNP (1 mg).

**FIG. 13** - Efficacy of conjugated ZNPs in killing cells is dose dependent. Cell viability (y-axis) was measured by MTT assay after 18 hours of incubation of the various cell lines (legend) with the treatment group (x-axis) represented.

**FIG. 14** - Efficacy of conjugated ZNPs in killing cells is dose dependent. Cell viability of SKBR3 cells was measured by MTT assay after incubation with treatment group (legend) at the dosage indicated (x-axis). Only Her2 specific killing is observed in a linear dose response.

**FIG. 15** - Release of zinc ions extracellularly is dependent on the hydrophobicity of the outer polymer coating. One mg of ZNP were coated with PEG polymers of various chain lengths (legend). The greater the chain length, the higher the hydrophobicity. ZNPs were placed in fetal calf serum and zinc ion concentration measured over time. Concentration adjusted for baseline serum zinc levels. Kinetics of zinc release could be altered and delayed based on the hydrophobicity of the polymer coating.
DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present invention provides novel pharmaceutical formulations comprising zinc nanoparticles having a core comprised of zinc and essentially free of other metals or metal oxides. The nanoparticles may advantageously comprise an outer shell or coating. This shell or coating can serve one or more purposes, namely, to provide a material to which ancillary agents (targeting, diagnostic, therapeutic) can be attached, and/or to shield the zinc from the environment and control the release of zinc ions.

A particular use of these particles will be in the treatment of cancer. As described in detail in U.S. Patent Publication 2007/0212331, the inventors previously determined that both cellular apoptosis and necrosis can be induced by increasing the intracellular concentration of zinc above a certain threshold. Furthermore, by selectively targeting specific cells or specific types of cells, such as cancer cells, and delivering killing-effective amounts of zinc to those cells, those cells can be killed. Liposomal delivery of zinc is provided as a particular embodiment. This present invention extends this work by introducing the use of the aforementioned zinc nanoparticles which have heretofor not been used in medicinal contexts.

Studies with various cancer cell lines (see Examples) show that zinc nanoparticle cytotoxicity was dose-dependent based on the amount of zinc nanoparticles added. The dose range was variable depending on the susceptibility of the cell line to zinc ion toxicity and the volume in which the zinc nanoparticles were dissolved to achieve threshold doses. As such, zinc nanoparticles did not need direct contact with cancer cells to exert cytotoxic effect. The cytotoxic effect was distance dependent from the area of zinc nanoparticle deposition. If threshold zinc ion levels were reached based upon zinc nanoparticle dissolution then all cells showed cytotoxic effect. In some cancer cells, zinc nanoparticle mediated cell death was directly dependent on contact of the nanoparticle with the cell. In studies using sub-therapeutic levels of zinc nanoparticles, only cells in contact with the nanoparticles die. Zinc nanoparticles showed no specificity of cell killing based on cellular genetic alterations or rate of growth, nor was there species specificity. The inventors believe that there are two mechanism of cytotoxic action for zinc nanoparticles. The first is through internalization into various cellular vacuoles and release of zinc ions resulting in disruption of cellular homeostasis. The second is through slow dissolution in aqueous solution resulting in release of zinc ions increasing
extracellular concentrations to a level that either forces internalization or disrupts cell growth. Moreover, studies with zinc nanoparticles revealed that there is a dose dependent release of zinc ions into aqueous solutions. This dose dependent release of zinc ions was dependent on both mass and surface area (and size) of the zinc nanoparticles. Zinc ion release could be impacted by coating of zinc nanoparticles with thiolic acids or encapsulation in bioinert gels. Use of bioinert gels reveals that zinc nanoparticle toxicity can be limited to a surface area consistent with the gel.

Another use of the zinc nanoparticles of the present invention will be to address the common occurrence of infections due to use of indwelling catheters and other similar implanted medical devices routinely used in hospitals on a diverse group of patients. Pathogens often attach to and proliferate in such devices and eventually invade the patient leading to nosocomial infections. Microorganisms usually migrate along the surfaces of devices to invade sterile environments, such as the bronchoalveolar space leading to pneumonia, the bloodstream leading to bacteremia, or the urinary bladder leading to urinary tract infections.

Finally, the zinc nanoparticles of the present invention will be also find use in diagnostic contexts by including a cell, tissue or organ targeting moeity. On their own, zinc nanoparticles can imaged based on their autofluorescent properties, and this can be enhanced by the addition of other detectable labels. Further, therapeutic agents can be added to achieve simultaneous imaging and therapy.

These and other aspects of the present invention are described in detail below.

I. Zinc Nanoparticles

Zinc nanoparticles (ZNP) or Zinc NanoDots discussed herein are comprised of a core of pure metallic zinc. They are spherical or multi-faceted particles of elemental zinc with high surface area. ZNP of 20-40 nm have a surface area of 30-50 m²/g and 100 nm particles have a surface area of 7 m²/g. There are important differences between ZNP and Quantum dots or other therapeutic nanoparticles in that they are purely aggregates of metallic zinc sized in the nano-range. They do not exhibit semiconductor like properties similar to quantum dots. Another important distinction between zinc nanoparticles and quantum dots which also contain zinc-sulfide caps around a metallic core is the difference in optical properties. Quantum dots exhibit fluorescent properties across a spectrum depending on energy absorption. The color
range exhibited is dependent on both size and shape. In contrast, zinc nanoparticles
do not exhibit the optical properties inherent of quantum dots.

ZNP are also comprised of a different form of elemental zinc. As such, their
CAS No. is 7440-66-6. The appearance of ZNP is black. They have a molecular
weight of 65.37, a density of 7140 kg/m³ and a melting point of 419.53 ℃. The
purity is greater than 99%.

ZNP with the cytotoxic properties discussed herein can range from 5 nm to
200 nm in dimension. Size is an important aspect of this invention as it affects rate of
dissolution of zinc ions in solution, impacts cellular internalization, and limits
vascular permeability. ZNPs are shown in FIG. 1.

ZNP do not exist in any standard shapes. The inventors believe that the shape
may be varied to optimize binding of targeting ligands and to impact rate of
dissolution. The optimal shape for targeted ZNP is likely to be spheroids. ZNPs are
shown in FIG. 1.

ZNP described herein comprise of pure metallic zinc. As such they freely
dissolve in aqueous solution until there is super-saturation of zinc ions. The rate of
dissolution is dependent on the aqueous solution, pH, and the saturation of zinc ions.
Rate of dissolution can also be impacted by the coatings applied to ZNP (Described
later). The solubility of ZNP in aqueous solutions distinguishes them from other zinc
based nanoparticles such as zinc-oxide. Zinc-oxide nanoparticles have limited
solubility in aqueous solutions. The rate of zinc ion dissolution is given in FIGS. 3
and 4.

ZNP are highly reactive with proteins. A number of ligands can be bound to
the surface of ZNP including antibodies, peptides, proteins, aptamers, viral particles
and nucleic acids. The inventors have conjugated antibodies and proteins to the
surface of ZNPs. Efficiency of binding is dependent on size and shape of ZNPs.
ZNPs in solution are shown in FIG. 2.

ZNPs exhibit cytotoxicity against all organisms using nucleic acids for
replication. It has cytotoxic properties against enveloped and non-enveloped viruses,
bacteria, protozoa, molds and human cells including cancer cells. The mechanism of
ZNP cell death varies depending on the organism but includes apoptosis, necrosis and
inhibition of cell replication. Cytotoxic activity has been demonstrated against the cell
types shown in Table 1. Cytotoxicity is independent of the underlying molecular
pathway of transformation. Cell death and kinetics of cell death varied depending on
the cancer cell type. The LD$_{50}$ of each cell type is given in Table 1. Importantly, the rate of cell death was rapid for each cell line starting in as little as 2 hrs (FIG. 5). Dose response curves show sigmoid kinetics (FIG. 6). The linear range for cytotoxic activity depended on the rate of zinc ion dissolution which in term varied by characteristics of the solution and ZNP size and weight.

**Table 1 - Cytotoxic Activity of ZNPs**

<table>
<thead>
<tr>
<th>CANCER TYPE</th>
<th>CELL LINE</th>
<th>LD$_{50}$ (μg/ml)</th>
<th>Time to 50% Cell Death (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate Cancer</td>
<td>PC3</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>DU148</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>LNCaP</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Ovarian Cancer</td>
<td>ES2</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>SKOV3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>SKBR3</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>MCF7</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Skin Cancer</td>
<td>B16 (melanoma)</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>ASZ001 (basal cell carcinoma)</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Brain Tumors</td>
<td>9L (Glioblastoma)</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>RT2 (Gliosarcoma)</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>F98 (Glioblastoma)</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>GMB1 (Medulloblastoma)</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>GMB2 (Medulloblastoma)</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>GMB3 (Medulloblastoma)</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>SCC25</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Cancer</td>
<td>CAL27</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

ZNPs are believed to act synergistically with other pharmaceutical preparations with cytotoxic or cytostatic activity. ZNPs were combined with a number of chemotherapeutic drugs and showed either additive or potentiated cytotoxic cell killing (FIG. 7). The inventors have tested ZNPs with cancer chemotherapeutics of various different classes including nucleoside inhibitors, alkylating agents and topoisomerase inhibitors.

ZNP cytotoxic activity is associated with internalization of ZNP within cells and subsequent dissolution and zinc ion release or zinc ion release exterior to cells with subsequent zinc ion internalization. A RNA microarray analysis of zinc ion toxicity upon internalization into cells shows that the mechanism of cytotoxic activity is likely to be disruption of proteins with zinc ion motifs. Since zinc ion motifs are
rich in proteins with nucleic acid binding properties like DNA polymerases, RNA polymerases, and tRNAs, there is disruption of replication, mRNA production and amino acid incorporation into nascent growing proteins.

ZNPs were administered at 20-25 grams/mouse either subcutaneously, intradermally, or intratumorally. ZNPs were administered as high as 5% w/w without undo toxicity to animals. Histological examination of tissues including, brain, liver, spleen, kidneys, and heart did not show any cellular disruption or change in characteristics. Serum zinc levels increased depending on dose and were rapidly cleared within 24 hours. Maximum serum zinc levels were seen within the first 120 min with steady decline to normal levels between 8 and 24 hrs. Tissue ZNP accumulation was minimal. No tissue ZNP was detected 7 days after administration.

A. Core Structure

The present application relates to zinc nanoparticles previously unknown for their use in pharmaceutical contexts. These particles are unique from other zinc-containing nanoparticles that have been used medicinally in that their cores are substantially free of oxides or any other metals than pure elemental zinc. By substantially free, it is meant that at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9% or 100% of the core is element zinc.

In general, nanoparticles are defined as having sizes in the sub-micrometer range. In accordance with the present invention, it is contemplated that the zinc nanoparticles will range in size from 1 nm to 250 nm, more specifically 5 nm to 100 nm, and even more specifically, 10 nm to 50 nm. Sizes of 5 nm, 10 nm, 15 nm, 20 nm, 25 nm, 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 60 nm, 70 nm, 80 nm, 90 nm, or 100 nm.

B. Shell or Coating

In various embodiments, the zinc nanoparticles are covered, encapsulated or encased by a shell or coating. The ZNPs can be individually "coated" with a shell or outer layer with a chemical entity to protect against bio-erosion or dissolution, and may optionally contain a targeting ligand (antibody, peptide, nucleic acid). The shell in this embodiment is directly linked chemically to the surface of the ZNP. Alternatively, the ZNPs are suspended in a colloidal (biogel, inert gel) solution or
coating. The coating in this embodiment is a gel formulation and is not chemically
attached or linked to the ZNPs; the ZNPs are suspended in this material as a colloid. The gel/colloid may be formulated to protect the ZNPs from erosion or dissolution.

Thus, as stated, purpose of the shell or coating may be one or both of the following: (i) to shield the zinc from the immediate environment, and thereby regulate the introduction of zinc ions into the environment; and/or (ii) to provide a material to which other agents, such as targeting, diagnostic and therapeutic agents, can be attached. In general, the shell will be a bioinert gels, which may be synthetic or natural material that is polymeric or carbon-based and either biocompatible or biodegradable.

In one embodiment, the shell or coating is required to provide slow release of zinc ions through controlled bioerosion. The biocompatible polymers will permit a therapeutic range of zinc of ZNP released within a disease microenvironment. Additionally, the biocompatible polymer will have an appropriate viscosity for localize delivery into a specific disease compartment. In this system, the active agent, ZNP, are dispersed in a bioinert polymer. Diffusion through the polymer matrix and release rate is dependent on the choice of polymer. Two possible systems are envisioned. In the first system, polymer bioerosion is through the conversion of the polymer from a water insoluble to a water-soluble state. As such, the polymer surrounding ZNPs is degraded and ZNPs are released. In the second system, polymer chains are attached to ZNPs. The chains are degraded through interaction with water or enzymes resulting in release of ZNPs.

Both natural and biosynthetic polymers will need to be bio-degradable. Most of the polymers will have hetero-atom-containing polymer backbones. Bioerosion and rate of degradation can be controlled through the use of chemical linkages including anhydrides, esters or amide bonds. Additionally, the metabolic products of these biocompatible polymers will be bioinert meaning having no or limited toxicity and mechanisms for either excretion or degradation. Examples of biodegradable polymers for use with ZNPs include Poly-esters based on polylactide (PLA) polyglycolide (PGA) and polycaprolactone (PCL). Other potential polymers include modifications of polysaccharides including starch, cellulose, and chitosan.

ZNPs dispersed in a matrix of poly(ethylene glycol) (PEG) such that water dispersion into the matrix is limited over approximately 8 hours. This PEG-ZNP matrix is injected into a disease state (tumor bed). Through slow bioerosion of the
polymer, ZNPs are contacted with incoming water. Upon contact with water, ZNPs are activated and can contact tumor cells and be internalized to exert toxicity. An example of composition is:

ZNPs 5 mg/ml

Pluronic F127 30% weight/volume

ZNPs are conjugated with targeting antibody directed to disease state. Based on the conjugation efficiency of the targeted antibody, the remaining open space on the surface of ZNPs is filled with polymer cross-linkers consisting of PEG and/or fatty acids. The cross-linkers provide a hydrophobic surface to slow bio-erosion. The rate of biodegradation of the ZNPs can be controlled by the chain length and composition of the conjugated polymers. An example of composition would be:

ZNPs 5 mg/ml. Surface activated with NHS and EDC

Her2 AB at 60 µg/ml

PEG C8-C18 linker

C. Targeting Agents

Targeting agents, in accordance with the present invention, provide for the direction of zinc nanoparticles to various cells, tissues or organs within a subject. Targeting agents may be attached using standard technology to the matrices or polymers of the shell or coating.

In one embodiment, cross-linking reagents are used to form molecular bridges that tie functional groups of two different molecules. To link two different compounds in a step-wise manner, hetero-bifunctional cross-linkers can be used that eliminate unwanted homopolymer formation. Table 2 illustrates several cross-linkers.
<table>
<thead>
<tr>
<th>linker</th>
<th>Reactive Toward</th>
<th>Advantages and Applications</th>
<th>Spacer Arm Length after cross-linking</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMPT</td>
<td>Primary amines Sulfhydryls</td>
<td>· Greater stability</td>
<td>11.2 A</td>
</tr>
<tr>
<td>SPDP</td>
<td>Primary amines Sulfhydryls</td>
<td>· Thiolation</td>
<td>6.8 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Cleavable cross-linking</td>
<td></td>
</tr>
<tr>
<td>LC-SPDP</td>
<td>Primary amines Sulfhydryls</td>
<td>· Extended spacer arm</td>
<td>15.6 A</td>
</tr>
<tr>
<td>Sulfo-LC-SPDP</td>
<td>Primary amines Sulfhydryls</td>
<td>· Extended spacer arm</td>
<td>15.6 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Water-soluble</td>
<td></td>
</tr>
<tr>
<td>SMCC</td>
<td>Primary amines Sulfhydryls</td>
<td>· Stable maleimide reactive group</td>
<td>11.6 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Enzyme-antibody conjugation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Hapten-carrier protein conjugation</td>
<td></td>
</tr>
<tr>
<td>Sulfo-SMCC</td>
<td>Primary amines Sulfhydryls</td>
<td>· Stable maleimide reactive group</td>
<td>11.6 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Water-soluble</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Enzyme-antibody conjugation</td>
<td></td>
</tr>
<tr>
<td>MBS</td>
<td>Primary amines Sulfhydryls</td>
<td>· Enzyme-antibody conjugation</td>
<td>9.9 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Hapten-carrier protein conjugation</td>
<td></td>
</tr>
<tr>
<td>Sulfo-MBS</td>
<td>Primary amines Sulfhydryls</td>
<td>· Water-soluble</td>
<td>9.9 A</td>
</tr>
<tr>
<td>SIAB</td>
<td>Primary amines Sulfhydryls</td>
<td>· Enzyme-antibody conjugation</td>
<td>10.6 A</td>
</tr>
<tr>
<td>Sulfo-SIAB</td>
<td>Primary amines Sulfhydryls</td>
<td>· Water-soluble</td>
<td>10.6 A</td>
</tr>
<tr>
<td>SMPB</td>
<td>Primary amines Sulfhydryls</td>
<td>· Extended spacer arm</td>
<td>14.5 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Enzyme-antibody conjugation</td>
<td></td>
</tr>
<tr>
<td>Sulfo-SMPB</td>
<td>Primary amines Sulfhydryls</td>
<td>· Extended spacer arm</td>
<td>14.5 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>· Water-soluble</td>
<td></td>
</tr>
<tr>
<td>EDC/Sulfo-NHS</td>
<td>Primary amines Carboxyl groups</td>
<td>· Hapten-Carrier conjugation</td>
<td>0</td>
</tr>
<tr>
<td>ABH</td>
<td>Carbohydrates Nonselective</td>
<td>· Reacts with sugar groups</td>
<td>11.9 A</td>
</tr>
</tbody>
</table>
An exemplary hetero-bifunctional cross-linker contains two reactive groups: one reacting with primary amine group (e.g., N-hydroxy succinimide) and the other reacting with a thiol group (e.g., pyridyl disulfide, maleimides, halogens, etc.). Through the primary amine reactive group, the cross-linker may react with the lysine residue(s) of one protein (e.g., the selected antibody or fragment) and through the thiol reactive group, the cross-linker, already tied up to the first protein, reacts with the cysteine residue (free sulfhydryl group) of the other protein (e.g., the selective agent).

Numerous types of disulfide-bond containing linkers are known that can be successfully employed to conjugate targeting and therapeutic/preventative agents. Linkers that contain a disulfide bond that is sterically hindered may prove to give greater stability in vivo, preventing release of the targeting peptide prior to reaching the site of action. These linkers are thus one group of linking agents.

Another cross-linking reagent is SMPT, which is a bifunctional cross-linker containing a disulfide bond that is "sterically hindered" by an adjacent benzene ring and methyl groups. It is believed that steric hindrance of the disulfide bond serves a function of protecting the bond from attack by thiolate anions such as glutathione which can be present in tissues and blood, and thereby help in preventing decoupling of the conjugate prior to the delivery of the attached agent to the target site.

The SMPT cross-linking reagent, as with many other known cross-linking reagents, lends the ability to cross-link functional groups such as the SH of cysteine or primary amines (e.g., the epsilon amino group of lysine). Another possible type of cross-linker includes the hetero-bifunctional photoreactive phenylazides containing a cleavable disulfide bond such as sulfo succinimidyl-2-(p-azido salicylamido) ethyl-1,3'-dithiopropionate. The N-hydroxy-succinimidyl group reacts with primary amino groups and the phenylazide (upon photolysis) reacts non-selectively with any amino acid residue.

In addition to hindered cross-linkers, non-hindered linkers also can be employed in accordance herewith. Other useful cross-linkers, not considered to contain or generate a protected disulfide, include SATA, SPDP and 2-iminothiolane (Wawrzynczak & Thorpe, 1987). The use of such cross-linkers is well understood in the art. Another embodiment involves the use of flexible linkers.

U.S. Patent 4,680,338, describes bifunctional linkers useful for producing conjugates of ligands with amine-containing polymers and/or proteins, especially for
forming antibody conjugates with chelators, drugs, enzymes, detectable labels and the like. U.S. Patents 5,141,648 and 5,563,250 disclose cleavable conjugates containing a labile bond that is cleavable under a variety of mild conditions. This linker is particularly useful in that the agent of interest may be bonded directly to the linker, with cleavage resulting in release of the active agent.

U.S. Patent 5,856,456 provides peptide linkers for use in connecting polypeptide constituents to make fusion proteins, e.g., single chain antibodies. The linker is up to about 50 amino acids in length, contains at least one occurrence of a charged amino acid (preferably arginine or lysine) followed by a proline, and is characterized by greater stability and reduced aggregation. U.S. Patent 5,880,270 discloses aminooxy-containing linkers useful in a variety of immunodiagnostic and separative techniques.

In one embodiment, the targeting agent may be an antibody, or antigen-binding fragment thereof. The antibody may be directed to a tumor cell surface antigen, or to an antigen expressed on the surface of a pathogen. The antibody may be bivalent or single chain (sc), or an Ab fragment, such as a Fab, an Fv, or scFv.

Particular antibodies for targeting of solid tumors include Bevacizumab (Avastin®; Genentech/Roche), Trastuzumab (Herceptin®; Genentech/Roche), Omnitarg (Genentech/Roche), Cetuximab (Erbitux®; Lilly/Imclone), Imatinib (Gleevec®; Novartis), Panitumumab (Vectibix®; Amgen), Nimotuzumab (YM Biosciences), Matuzumab (Merck KGaA) and Zalutumumab (HuMax -EGFR; Genmab).

Another targeting agent is a ligand for a cell surface receptor. Such ligands often are modified such that they do not activate their cognate receptor, i.e., a receptor-binding peptide.

Cancer cell markers suitable as targets for a target-directed zinc nanoparticle therapy include urinary tumor associated antigen (UTAA), fetal antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, estrogen receptor, laminin receptor, erb B and pl55, PSA, CEA, MART, MAGE1, MAGE3, gp100, BAGE, GAGE, TRP-I, TRP-2, PMSA, Mycobacterium tuberculosis soluble factor (Mtb), phenol soluble modulin (PSM), CMV-G, CMV-M, EBV capsid-EB nuclear antigen (EBNA), gp20, gp41, tat, rev, gag, toxan antigen, rubella antigen, mumps antigen, α-fetoprotein (AFP), adenocarcinoma antigen (ART-4), CAMEL,
Bacterial cell markers suitable as targets for zinc nanoparticles include bacterial outer membrane constituents, such as liposaccharide (LPS) and oligosaccharide cell surfaces, bacterial cell wall components, such as peptidoglycan (NAM, NAG, DAP), cell envelope, components, such as LPS and thioic acid, and outer envelope structures, including the pilus and fimbria. Viral markers suitable as targets for zinc nanoparticles include viral envelope components, including HIV p24, and viral core proteins, such as HCV NS3. Fungal markers suitable as targets for zinc nanoparticles include fungal cell wall and fungal hyphae structures. Parasitic markers are contemplated as suitable targets for zinc nanoparticles.

D. Nanoparticles Sources

Zinc nanoparticles can be produced using a number of different processes. In the first class of methods, zinc as a mined element exists as pure elemental zinc and needs to be rendered to nanoscale size. This size fractionation is performed by either milling (using very fine instruments) or through laser evaporation followed by condensation on a solid surface or substrate. A second class of method is a "bottom up" approach where the ZNPs are synthesized from zinc ions existing in either gas phase or solution. This methodology is more common for zinc oxide rather than ZNP, but is reported for both. In this methodology, an aqueous solution containing zinc ions is evaporated or otherwise treated to precipitate atomic zinc as a condensate. The initial starting "solution" may be zinc ions in some liquid substrate or may be zinc ions in gas phase.

Zinc nanoparticles can be obtained from commercial sources such as Sigma-Aldrich, American Elements, Xuzhou Hongwu Nanometer Material Company and NaBond. For example, product 578002-5G from Sigma-Aldrich is a 5 gram quantity of zinc nanopowder having the properties shown in Table 3.
TABLE 3 - Properties of Zinc Nanoparticles

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>vapor pressure</td>
<td>1 mm Hg (487 °C)</td>
</tr>
<tr>
<td>assay</td>
<td>&gt; 99% trace metals basis</td>
</tr>
<tr>
<td>form</td>
<td>nanopowder</td>
</tr>
<tr>
<td>resistivity</td>
<td>5.8 µΩ-cm, 20 °C</td>
</tr>
<tr>
<td>total impurities</td>
<td>≤ 10%</td>
</tr>
<tr>
<td>particle size</td>
<td>&lt; 50 nm</td>
</tr>
<tr>
<td>surface area</td>
<td>BET surf, area 35-50</td>
</tr>
<tr>
<td>bp</td>
<td>907 °C (lit.)</td>
</tr>
<tr>
<td>mp</td>
<td>420 °C (lit.)</td>
</tr>
<tr>
<td>Density</td>
<td>7.133 g/mL at 25°C</td>
</tr>
</tbody>
</table>

E. Pharmaceutical Formulations

One method for the delivery of a zinc nanoparticle according to the present invention is systemically. However, the zinc nanoparticle compositions disclosed herein may alternatively be administered parenterally, intravenously, intradermally, intramuscularly, transdermally or even intraperitoneally as described in U.S. Patent 5,543,158; U.S. Patent 5,641,515 and U.S. Patent 5,399,363 (each specifically incorporated herein by reference in its entirety).

Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Patent 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier
can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermolysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologies standards.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.
The compositions disclosed herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The phrase "pharmaceutically-acceptable" or "pharmacologically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared.

II. Cancer Applications

The zinc nanoparticles of the present invention find particular use in the treatment of cancer. Other than that the tumor will be a solid tumor, thereby permitting targeting of the treatment to a particular site within a subject, all cancers should be amenable to treatment with zinc nanoparticles given the non-specific nature of their toxicity.
A. Cancer Types

Cancers and pre-cancerous conditions which can be prevented and/or treated by the methods described herein include, but are not limited to: adenofibroma; adenoma; agenogenic myeloid metaplasia; AIDS-related malignancies; ameloblastoma; anal cancer; angiofollicular mediastinal lymph node hyperplasia; angiokeratoma; angiolymphoid hyperplasia with eosinophilia; angiomatosis; anhidrotic ectodermal dysplasia; anterofacial dysplasia; apocrine metaplasia; apudoma; asphyxiating thoracic dysplasia; Astrocytoma (including, for example, cerebellar and cerebral); atriodigital dysplasia; atypical melanocytic hyperplasia; atypical metaplasia; autoparenchymatous metaplasia; basal cell hyperplasia; bile duct cancer (including, for example, extrahepatic bile duct cancer); bladder cancer; bone cancer; brain tumor (including, for example, brain stem glioma, cerebellar astrocytoma glioma, malignant glioma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, ependymoma, medulloblastaoma, gestational trophoblastic tumor glioma, and paraganglioma); branchionia; breast cancer (male and female); bronchial adenomas/carcinoids; bronchopulmonary dysplasia; cancer or pre-cancerous growths or metastatic growths of epithelial cells; carcinoïd heart disease; carcinoïd tumor (including, for example, gastrointestinal); carcinoma (including, for example, unknown primary origin, adrenocortical, islet cells, adeno-, adeoncortical, basal cell, basosquamous, bronchiolar, Brown-Pearce, cystadeno-, ductal, hepato-, Krebs, papillary, oat cell, small cell lung, non-small cell lung, squamous cell, transitional cell, Walker, Merkel Cell, and skin carcinomas); cementoma; cementum hyperplasia; cerebral dysplasia; cervical cancer; cervical dysplasia; cholangioma; cholesteatoma; chondroblastoma; chondroectodermal dysplasia; chordoma; choristoma; chondroroma; cleidocranial dysplasia; colon cancer; colorectal cancer; colorectal/local metastasized colorectal cancer; congenital adrenal hyperplasia; congenital ectodermal dysplasia; congenital sebaceous hyperplasia; connective tissue metaplasia; craniocarpotarsal dysplasia; craniodiaphyseal dysplasia; craniometaphysial dysplasia; craniopharyngioma; cylindroma; cystadenoma; cystic hyperplasia (including, for example, cystic hyperplasia of the breast); cystosarcoma phyllodes; dentin dysplasia; denture hyperplasia; diaphysial dysplasia; ductal hyperplasia; dysgenninoma; dysplasia epiphysialis hemimelia; dysplasia epiphysialis multiplex; dysplasia epiphysialis punctata; ectodermal dysplasia; Ehrlich tumor; enamel dysplasia; encephalo-
ophthalmic dysplasia; endometrial cancer (including, for example, Ependymoma and endometrial hyperplasia); ependymoma; epithelial cancer; epithelial dysplasia; epithelial metaplasia; esophageal cancer; Ewing's Family of tumors (including, for example, Ewing sarcoma); extrahepatic bile duct cancer; eye cancer (including, for example, intraocular melanoma and retinoblastoma); faciodigitogenital dysplasia; familial fibrous dysplasia of jaws; familial white folded dysplasia; fibroma; fibromuscular dysplasia; fibromuscular hyperplasia; fibrous dysplasia of bone; florid osseous dysplasia; focal epithelial hyperplasia; gall bladder cancer; ganglioneuroma; gastric cancer (for example, stomach cancer); gastrointestinal carcinoid tumor; gastrointestinal tract cancer; gastrointestinal tumors; Gaucher's disease; germ cell tumors (including, for example, extracranial, extragonadal, and ovarian germ cell tumors); giant cell tumor; gingival hyperplasia; glioblastoma; glomangioma; granulosa cell tumor; gynandroblastoma; hamartoma; head and neck cancer; hemangioendothelioma; hemangioma; hemangio-pericytoma; hepatocellular cancer; hepatoma; hereditary renal-retinal dysplasia; hidrotic ectodermal dysplasia; histiocytoma; histiocytosis; hypergammaglobulinemia; hypohidrotic ectodermal dysplasia; hypopharyngeal cancer; inflammatory fibrous hyperplasia; inflammatory papillary hyperplasia; intestinal cancers; intestinal metaplasia; intestinal polyps; intraocular melanoma; intravascular papillary endothelial hyperplasia; kidney cancer; laryngeal cancer; leiomyoma; Leydig cell tumor; lip and oral cavity cancer; lipoma; liver cancer; lung cancer (including, for example, small cell and non-small cell); lymphangiomyoma; lymphaugioma; lymphopenic thymic dysplasia; lymphoproliferative disorders; malignant carcinoid syndrome; malignant mesothelioma; malignant thymoma; mammary dysplasia; mandibulofacial dysplasia; medulloblastoma; meningioma; mesenchymoma; mesonephroma; mesothelioma (including, for example, malignant mesothelioma); metaphysial dysplasia; metaphysial anemia; metaplastic ossification; metaplastic polyps; metastatic squamous neck cancer (for example, with occult primary); Mondini dysplasia; monostotic fibrous dysplasia; mucoepithelial dysplasia; multiple endocrine neoplasia syndrome; multiple epiphysial dysplasia; multiple myeloma/plasma cell neoplasm; mycosis fungoides; myelodysplastic syndrome; myeloid metaplasia; myeloproliferative disorders (including, for example, chronic myeloproliferative disorders); myoblastoma; myoma; myxoma; nasal cavity and paranasal sinus cancer; nasopharyngeal cancer; neoplasms located in the prostate, colon, abdomen, bone, breast, digestive system, liver,
pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital tract; neurilemmoma; neuroblastoma; neuroepithelioma; neurofibroma; neurofibromatosis; neuroma; nodular hyperplasia of prostate; nodular regenerative hyperplasia; oculoauriculovertebral dysplasia; oculodentodigital dysplasia; ophthalmomandibulomelic dysplasia; oropharyngeal cancer; osteoma; ovarian cancer (including, for example, ovarian epithelial cancer and ovarian low malignant potential tumor); pancreatic cancer (including, for example islet cell and exocrine pancreatic cancers); papilloma; paraganglioma, nonchromaffin; paranasal sinus and nasal cavity cancer; paraproteinemias; parathyroid cancer; periapical cemental dysplasia; pheochromocytoma (including, for example, penile cancer); pineal and supratentorial primitive neuroectodermal tumors; pinealoma; pituitary tumor; plasma cell neoplasm/multiple myeloma; plasmacytoma; pleuropulmonary blastoma; polyostotic fibrous dysplasia; polyps; pregnancy cancer; pre-neoplastic disorders including but not limited to benign dysplasias, fibrocystic conditions, tissue hypertrophy, intestinal polyps, colon polyps, esophageal dysplasia, leukoplakia, keratoses, Bowen's disease, Farmer's Skin, solar cheilitis, and solar keratosis; primary hepatocellular cancer; primary liver cancer; primary myeloid metaplasia; prostate cancer; pseudoachondroplastic spondyloepiphyseal dysplasia; pseudoepitheliomatous hyperplasia; purpura; rectal cancer; renal cancer (including, for example, kidney cancer, renal pelvis and ureter cancer, transitional cell cancer of the renal pelvis and ureter, ureter cancer); reticuloendotheliosis; retinal dysplasia; retinoblastoma; salivary gland cancer; sarcomas (including, for example, uterine, soft tissue, carcino-, chondro-, fibro-, hemangio-, Kaposi's, leiomyo, lipo-, lymphangio-, myo-, myxo-, Rhabdo-, sarcoïdosis, osteo-, and Ewing sarcomas as well as malignant fibrous histiocytoma of bone and clear cell sarcoma of tendon sheaths); sclerosing angioma; secondary myeloid metaplasia; senile sebaceous hyperplasia; septo-optic dysplasia; Sertoli cell tumor; Sezary Syndrome; skin cancer (for example, including melanoma and non-melanoma skin cancer); small intestine cancer; spondyloepiphyseal dysplasia; squamous metaplasia (for example, squamous metaplasia of amnion); stomach cancer; supratentorial primitive neuroectodermal and pineal tumors; supratentorial primitive neuroectodermal tumors; symptomatic
myeloid metaplasia; teratoma; testicular cancer; theca cell tumor; thymoma
(including, for example, malignant thymoma); thyroid cancer; trophoblastic tumors
(including for example gestational trophoblastic tumors); ureter cancer; urethral
cancer; uterine cancer; vaginal cancer; ventriculoradial dysplasia; verrucous
hyperplasia; vulvar cancer; and Wilms' tumor.

B. Additional Anti-Cancer Agents

Although the zinc nanoparticles of the present invention are themselves toxic
to cancer cells, it may prove useful to add a second therapeutic agent or "payload" to
the nanoparticle. Such agents, including chemotherapeutic and radiotherapeutics are
listed below. Others include cytokines, toxins (ricin, pertussis toxin, cholera toxin)
and hormones.

C. Routes of Administration

The present invention is contemplated to be used in both systemic and
localized administration. With respect to systemic administration, it will be desirable
to target the zinc nanoparticles to locations within the body using targeting or
"homing" molecules, such as antibodies or other ligands for cell surface receptors.

Administration of these compositions according to the present invention may
be via any common route so long as the target tissue is available via that route. This
includes oral, nasal, buccal, rectal, vaginal or topical. Alternatively, administration
may be by intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous
injection. Such compositions would normally be administered as pharmaceutically
acceptable compositions, described supra.

Of particular interest is direct intratumoral administration, perfusion of a
tumor, or administration local or regional to a tumor, for example, in the local or
regional vasculature or lymphatic system, or in a resected tumor bed. Injection of zinc
nanoparticle may be by syringe or any other method used for injection of a solution,
as long as the agent can pass through the particular gauge of needle required for
injection. A needleless injection system has been described (U.S. Patent 5,846,233)
having a nozzle defining an ampule chamber for holding the solution and an energy
device for pushing the solution out of the nozzle to the site of delivery. A syringe
system has also been described for use in gene therapy that permits multiple injections
of predetermined quantities of a solution precisely at any depth (U.S. Patent 5,846,225).

D. Combination Therapies

As discussed above, it may be desirable to combine zinc nanoparticles with other therapies in the treatment of cancers. An "anti-cancer" therapy is capable of negatively affecting cancer in a subject, for example, by killing cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth, reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer. Anti-cancer therapies are described below as "standard of care" therapies and include biological agents (biotherapy), chemotherapy agents (including anti-tumor antibiotics), and radiotherapy agents, surgery and immunotherapy. More generally, these therapies would be provided in a combined amount to achieve a clinically significant effect. This process may involve contacting the cancer cell with the therapies at the same time, such as by administering a single composition or treatment that includes both agents, or by administering two distinct compositions or treatments at the same time.

Alternatively, the zinc nanoparticle therapy may precede or follow the other treatment by intervals ranging from minutes to weeks. In embodiments where the other agent and zinc nanoparticle therapy are applied separately, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the therapy and zinc nanoparticle therapy would still be able to exert an advantageously combined effect. In such instances, it is contemplated that one may administer both modalities within about 12-24 h of each other and, more preferably, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several d (2, 3, 4, 5, 6 or 7) to several wk (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

Various combinations may be employed, where zinc nanoparticle therapy is "A" and the secondary therapy is "B":

A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B
It is expected that the treatment cycles would be repeated as necessary, including two, three, four, five, six, seven, eight, nine, ten or more cycles.

In one embodiment, a second anti-cancer therapy is radiotherapy. Radiotherapy includes, for example, fractionated radiotherapy, nonfractionated radiotherapy and hyperfractionated radiotherapy, and combination radiation and chemotherapy. Types of radiation also include ionizing (gamma) radiation, particle radiation, low energy transmission (LET), high energy transmission (HET), ultraviolet radiation, infrared radiation, visible light, and photosensitizing radiation.

In another embodiment, a second anti-cancer therapy is chemotherapy. When the second anti-cancer therapy is chemotherapy, the chemotherapy may comprise administration of one or more of: 20-epi-l,25 dihydroxyvitamin D3; (1aS,8S,8aR,8bS)-6-amino-8-(((aminocarbonyl)oxy)methyl)-1,la, 2,8,8a,8b-hexahydro-8a-methoxy-5-methylazirino(2',3':3,4)pyrrolo[1,2-a]in-dole-4,7-dione; (8S-cis)-10-((3-amino-2,3,6-trideoxy-alpha-L-lyxo-hexopyranosyl)oxy)-7, 8,-9,1,0-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5; (I)-mimosine; 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea; 12-naphthacenedione; 131-meta-iodobenzyl guanidine (1-131 MIBG); l,r,l"-phosphinothiolyldiynetris aziridine; 2-chloro-N-(2-chloroethyl)-N-methylthananmine; 2-deoxy-2-(((methylnitrosoamino)carbonyl)amino)-D-glucose; 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose; 2,3,5-tris(l-aziridinyl)-2,5-cyclohexadiene-1,4-dione; 2,4,6-tris(l-aziridinyl)-s-thiazine; 2,4,6-tris(l-aziridinyl)-s-triazine; 3-deazauridine; 3-iodobenzylguanidine; 4-(bis(2-chloroethyl)amino)benzenebutanoic acid; 4-(bis(2-chloroethyl)amino)-L-phenylalanine; 4-demethoxy-3-deamino-3-aziridinyl-4-methylsulphonyl-daunorubicin; 5-(3,3-dimethyl-1-triazenyl)-1H-imidazole4-carboxamide; 5-(bis(2-chloroethyl)amino)-2,4(lH,3H)-pyrimidinedione; 5-azacytidine; 5-ethynyluracil; 5-fluorouracil (5-FU); 5-FU and radiation; 5-FU plus leucovorin; 5,12-naphthacenedione; 6-azauredine; 6-mercaptopurine; 6-thioguanine; 8-azaguanine; 9-dioxamycin; 9-nitrocamptothecin; abiraterone; acivicin; aclacinomycin A; aclarubicin; acodazole; acodazole hydrochloride; acronine; acrornyline; actinomycin D; Actinomycin D (also called Dactinomycin); acylfulvene; adecypenol; adozelesin; aldesleukin (interleukin-2); alitretinoin; allopurinol; ALL-TK
antagonists; altretamine (hexamethylmelamine); altretamine (Hexylen); ambamustine; ambomycin; ametantrone; ametantrone acetate; amidox; amifostine; aminoglutethimide (cytadren); aminoimidazole carboxamide; aminolevulinic acid; amrubicin; amsacrine (also called "mAMSA"; m-AMSA or amsidine); anagrelide; angiogenesis inhibitors; annamycin; antagonist D; antagonist G; antarelix; anthracycline antibiotics; anthracyline; anthramycin; antiandrogen; antibiotic derivatives; anti-dorsalizing morphogenetic protein-1; antiestrogen; antiestrogens; antimetabolites; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; Ara C; ara-CDP-DL-PTBA; arginine deaminase; arifostine; Arimidex; Aromasin; arsenic trioxide; asparaginase; asparaginase (elspar); asperlin; asulacrine; atamestatine; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azacitidine; azacitidine (ladakamycin); azaguanine; azaserine; azasetron; azatoxin; azatyrosine; azauridine; azetepa; azirino(2',3':3,4)pyrrolo[l,2-a]indole-4,7-dione; azotomycin; baccatin III derivatives; balanol; batimastat; BCG (theraefs); BCG live; BCNU; BCNU chloroethyl nitrosoureas; BCR/ABL antagonists; benzamide; benzochlorins; benzodepa; benzoystauosporine; benzylguanine; beta lactam derivatives; beta-alethine; bexarotene; bFGF inhibitor; bicalutamide; bisantrene; bisantrene hydrochloride; bisaziridinylspermine; bischboroethyl nitrosourea; bisnafide; bisnafide dimesylate; bistratene A; bizelesin; bleomycin; bleomycin (blenozane); bleomycin sulfate; bleomycins; breflate; brequinar; brequinar sodium; bromodeoxyuridine; bropirimine; broxuridine; budotitane; busulfan; busulfan (myleran); buthionine sulfoximine; cachectin; cactinomycin; calcipotriol; calphostin C; calusterone; camptothecin derivatives; canarypox IL-2; carcacomide; carbamic acid ethyl ester; carbetimer; carboplatin (Paraplatin); carboxamide-amino-triazole; carboxamidotriazole; CaRest M3; carmustine (BCNU or BiCNU); CARN 700; carubicin; carubicin hydrochloride; carzelesin; casein kinase inhibitors (ICOS); castanospermine; CCNU; cecropin B; cedefmgol; celecoxib; cetrorelix; cetuximab; chlorambucil (leukeran); chlorins; chloroethyl nitrosoureas; chloroquinoxaline sulfonamide; chlorotrianisene; CHOP (cyclophosphamid, doxorubicin, vincristine, and prednisone including any combination of the components of CHOP); chorambucil; chorozotocin (DCNU); chromomycin A3; cicaprost; cireolemycin; cis-aminedichloro(2-methylpyridine)
platinum; cisplatin (also called cis-ddpl or platinol); cisplatin and radiation; cisporphyrin; cis-retinoic acid; clomifene analogues; clotrimazole; coformycin; coichicine; collistimycin B; combretastatin A4; combretastatin analogue; conagenin; CPT-I 1; crambeicin 816; crisnatol; crisnatol mesylate; cryptophycin 8; cryptophycin A derivatives; curacin A; cycloleucine; cyclopentanthraquinones; cyclophosphamide; cyclo-phosphamide; cyclophosphamide (Cytoxan); cyclophosphamide anhydrous; cycloplatam; cypermcyin; cytarabine; cytarabine HCl (cytosar-u); cytarabine ocfosfate; cytochalasin; cytolytic factor; cytosine arabinoside; cytostatin; dacarbazine; decitabine; dehydrodidemnin B; demecolcine; depsipeptide; deslorelin; dexamethasone; dexoramplatin; dexverapamil; dezaguanine; dezaguanine mesylate; dianhydrogalactitol; diarizidinylspermine; diaziquone; diazooxonorleucine; dibromodulcitol; dibrospidium chloride; dicarbazine; didemnin B; didox; diethylnorspermine; diethylstibestrol; diethylnorspermine; dihydro-5-azacytidine; diphenyl sariomustine; docetaxel; docetaxel (taxotere); docosanol; dolasetron; doxifluridine; doxorubicin; Doxorubicin (Adriamycin); Doxorubicin and Doxetaxel; doxorubicin HCl (adriamycin); droloxifene; droloxifene citrate; drosmostanone; drosmostanone propionate; drosmalinol; duazomycin; duocarmycin SA; ebselen; ecomustine; edatrexate; edelfosine; edrecolomab; eflomithine; eflornithine; eflornithine hydrochloride; elemene; elinafide; elsamitracin; emetine; emitefur; enloplatin; enpromate; epipodophyllotoxins; epipropidine; epirubicin; epirubicin hydrochloride; epristeride; erbulozole; erlinotib; erythrocyte gene therapy; esorubicin; esorubicin hydrochloride; estradiol; estramustine; estramustine analogue; estramustine phosphate sodium (emcyt); estrogen agonists; estrogen antagonists; ET-743; etanidazole; ethinyl estradiol; ethiodized oil; ethoglucid; ethyl carbamate; ethyl ester; ethyl methanesulfonate; etoposide; etoposide (VP 16-213); etoposide orthoquinone; etoposide phosphate; etoprine; exemestane; fadrozole; fadrozole hydrochloride; fazarabine; Femara; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; floxuridine; floxuridine (fudr); fluasterone; Fludarabine; fludarabine (fludara); fludarabine phosphate; fluorocitabine; fluorodaunorunicin hydrochloride; fluoxymesterone (halotestin); flutamide; flutamide (eulexin); fluxuridine; forfenimex; formentane; fosquidone; fosfrieicin; fosfrieicin sodium; fotemustine; fulvestrant; gadolinium texaphyrin; galarubicin; gallium nitrate; gallium nitrate (granite);
galocitabine; ganirelix; gefitinib; gelatinase inhibitors; gemcitabine; gemcitabine (gemzar); gemcitabine hydrochloride; gemcitabine; gentuzumab; genistein; glufosfamide; glutamic acid; glutathione inhibitors; goserelin (zoladex); GPX100; gramicidin D; hepsulfam; heptaplatin; heregulin; hexamethylene bisacetamide; hexestrol; human chorionic gonadotrophin; hydroxyurea; hydroxyurea (hydra); hypericin; ibandronic acid; ibritumomab; idarubicin; idarubicin (idamycin); idarubicin hydrochloride; idoxifene; idramantone; ifosfagemcitabine; ifosfamide; ifosfamide (iflex); ifosfamide with mesna (MAID); ilomastat; imatinib mesylate; imidazoacridones; imiquimod; immunostimulant peptides; improsulfan tosylate; insulin-like growth factor-1 receptor inhibitor; interferon; interferon α; interferon α-2a; interferon α-2b; interferon agonists; interferon α-n1; interferon β-1a; interferon γ-1b; interferons; interleukin II (IL-2, including recombinant interleukin II or rIL2); interleukin II (including recombinant interleukin II or rIL2); interleukin-2; interleukins; iobenguane; iobenguane iobenguane; iododoxorubicin; ipomeanol; ioproplatin; irinotecan; irinotecan (camptosar); irinotecan hydrochloride; irofulven; irinoplast; irsogladine; isobengazole; isohomohalicondrin B; isotretinoin (accutane); itasetron; jasplakinolide; kahalalide F; ketoconazole; lamellarin-N triacetate; lanreotide; lanreotide acetate; leinamycin; lenalidomide; lenograstim; lentinan; lentinan sulfate; leptolstatin; letrozole; leucovorin; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide; leuprolide acetate; leuprolide acetate (LHRH-analog); leuprolide+estradiol+progesterone; leuprolelin; levamisole; levamisole (ergamisol); liarozole; liarozole hydrochloride; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lometrexol sodium; lomustine (CCNU or CeeNU); lonidamine; losoxantrone; losoxantrone hydrochloride; lovastatin; loxoribine; L-serine diazoacetate; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannomustine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; maytansine; mechlorethamine; mechlorethamine HCl (nitrogen mustard); medroxyprogesterone; medroxyprogesterone acetate (also called provera or depo provera); megestrol; megestrol acetate (menace); melphalan (alkeran); MEN 10755; menogaril; mephalen; merbarone; mercaptopurine (purinethol); mercaptopurine anhydrous; MESNA; mesna (mesne); meterelin; methanesulfonic acid; methioninase; methotrexate (also called mtx); methotrexate sodium; methyl-ccnu; methyltestosterone; metoclopramide;
metoprine; meturedepa; microalgal; MIF inhibitor; mifepristone; miltefosine; mimosine; mirimostim; mismatched double-stranded RNA; misonidazole; mithramycin; mitindomide; mitoantrone; mitobronitol; mitocarcin; mitocromin; mitogillin; mitoguazone; mitolactol; mitomalcin; mitomycin (Mutamycin); mitomycin analogues; mitomycin C; mitonafide; mitosper; mitotane (also called o,p'-DDD or lysodren); mitotoxin fibroblast growth factor-saporin; mitoxantrone; mitoxantrone HCl (novantrone); mofarotene; molgramostim; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; mycophenolic acid; myriaporone; N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide; N-acetyldinaline; nafarelin; nagrestip; naloxone and pentazocine; napavin; naphterpin; nartogastim; nedaplatin; nelarabine; nemiobicin; neridronic acid; neutral endopeptidase; nicardipine; nilutamide (nilandron); nimustine; nisamycin; nitracrine; nitric oxide modulators; nitrooxide antioxidant; nitrullyn; N-methyl-bis(2-chloroethyl)amine; nocardazole; nogalamycin; novobiocin; N-substituted benzaamides; N,N-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide; 06-benzylguanine; octreotide (sandostatin); okicenone; oligonucleotides; onapristone; ondansetron; oracin; oral cytokine inducer; ornmelatin; osaterone; oxaliplatin; oxaunomycin; oxisuran; Paclitaxel (Taxol); pantamycin; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase (PEGx-I); peldesine; peliomyacin; pemtrexed; pentamustine; pentosan polysulfate sodium; pentostatin (2'-deoxycoformycin); pentrozole; peplomycin; peplomycin sulfate; peptic hemoglobin; perflubron; perflubron; perillyl alcohol; phenazinomycin; phenylacetate; phosphorylcholine; photophoresis; picamycin (mithracin); picibanil; pilocarpine hydrochloride; pinafide; pipobroman; piposulfan; pirarubicin; piritepin; piroxantrone hydrochloride; place tin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; Plicamycin (also called mithramycin); plomestane; podoflox; podophyllotoxin; porfimer; porfimer sodium; porfimer; prednimustine; prednisolone; prednisone; procarbazine; procarbazine HCl (matulane); profiromycin; propyl bis-acridone; prospidium; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; puromycin; puromycin aminonucleoside; puromycin hydrochloride; purpurins; PUVA (psoralen+ultraviolet a); pyran copolymer; pyrazofurin; pyrazoloacridine; pyridoxylated hemoglobin
polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ranimustine; rapamycin; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; rebeccamycin; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; riboprine; ribozymes; RII retinamide; rituximab; rogletimide; rohitukine; romurtide; roquinimex; rubiginone Bl; ruboxyl; safmgol; safmgol hydrochloride; saintopin; SarCNU; sarcophytol A; sartragrostim; satraplatin; s-azacytidine; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; sertenef; showdomycin; signal transduction modulators; single chain antigen-binding protein; sizofuran; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosate; sparfosate sodium; sparfosic acid; sparsomycin; spicamycin D; spiromustine; splenopentin; sponginstatin 1; stem cell inhibitor; stem-cell division inhibitors; steroids; stipamide; streptonigrin; streptozocin; streptozocin (zanosar); stomelysin inhibitors; sulfmosine; sulofenur; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; talisomycin; tallimustine; tamoxifen; tamoxifen citrate (nolvadex); tamoxifen methiodide; tasonermin; taumustine; taxanes such as taxol and taxotere; Taxol (paclitaxel); taxon; Taxotere (docetaxel); tazarotene; tecogalan; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; teloxantrone; teloxantrone hydrochloride; temoporfm; temozolomide; teniposide; teniposide (also called VM-26 or vumon); tenuazonic acid; TEPA; teroxirone; testolactone; tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiamiprine; thiocoraline; thioguanine; thiopenta (thioplex); thrombopoietin; thombopoietin mimic; thymotrinan; thyroid stimulating hormone; tiazofurin; tilorone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topotecan; topsentin; toremifene; toremifene citrate; totipotent stem cell factor; Toxotere; translation inhibitors; trantuzumab; trastuzumab; trestolone; trestolone acetate; tretinoin (vesanoid); triacetyluridine; triaziquone; trichodermin; triciribine; triciribine phosphate; triethylene glycol diglycidyl ether; triethylene melamine; triethylene phosphoramide; triethylenetriphosphoramide; trimetrexate (neutrexin); trimetrexate glucuronate; triptorelin; tris(l-aziridinyl)phosphine oxide; tris(l-aziridinyl)phosphine sulfide; tris(aziridinyl)-p-benzoquinone; trofosophamide; tropisetron; troxacitabine; tubuloxole; tubuloxole hydrochloride; tirosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; uracil mustard; uredepa; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists;
valrubicin; vapreotide; variolin B; velaresol; veramine; Vercyte; verdins; verteporfin; vidarabine; vidarabine phosphate; vinblastine; vinblastine sulfate (velban); vinca alkaloids; vincristine (Oncovin); vincristine sulfate (oncovin); vindesine; vindesine sulfate; vinepidine; vinepidine sulfate; vinglycinate; vinglycinate sulfate; vinleurosine; vinleurosine sulfate; vinorelbine; vinorelbine tartrate (navelbine); vinrosidine; vinrosidine sulfate; vinxaltine; vinzolidine; vinzolidine sulfate; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin; zinostatin stimalamer; zoledronate; zorubicin; zorubicin hydrochloride; and combinations thereof.

Other combination therapies include surgery, hormone therapy, cryotherapy and gene therapy.

III. Antibiotic Applications

In another aspect, the present invention provides for the treatment of various infections, including those caused by bacteria, viruses, fungi and parasites.

A. Organisms

The nosocomial bacterial infections result in diseases such as bacteremia, pneumonia, meningitis, osteomyelitis, endocarditis, sinusitis, arthritis, urinary tract infections, tetanus, gangrene, colitis, acute gastroenteritis, bronchitis, and a variety of abscesses, and opportunistic infections. Bacterial pathogens include Gram-positive cocci such as *Staphylococcus aureus*, coagulase negative staphylococci such as *Staphylococcus epidermis*, *Streptococcus pyogenes* (group A), *Streptococcus spp.* (viridans group), *Streptococcus agalactiae* (group B), *S. bovis*, *Streptococcus* (anaerobic species), *Streptococcus pneumoniae*, and *Enterococcus spp.*; Gram-negative cocci such as *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Branhamella catarrhalis*; Gram-positive bacilli such as *Bacillus anthracis*, *Corynebacterium diphtheriae* and *Corynebacterium species* which are diphtheroids (aerobic and anaerobic), *Listeria monocytogenes*, *Clostridium tetani*, *Clostridium difficile*, *Escherichia coli*, *Enterobacter species*, *Proteus mirabilis* and other spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella*, *Shigella*, *Serratia*, and *Campylobacter jejuni*. The antibiotic resistant bacteria that can be killed by the antiseptic coated devices of the present invention include *Staphylococci* (methicillin-resistant strains), vancomycin-resistant enterococci (*Enterococcus faecium*), and resistant *Pseudomonas aeruginosa*.
Fungal infections that may be prevented include fungal infections (mycoses), which may be cutaneous, subcutaneous, or systemic. Superficial mycoses include tinea capitis, tinea corporis, tinea pedis, onychomycosis, perionychomycosis, pityriasis versicolor, oral thrush, and other candidoses such as vaginal, respiratory tract, biliary, esophageal, and urinary tract candidoses. Systemic mycoses include systemic and mucocutaneous candidosis, cryptococcosis, aspergillosis, mucormycosis (phycomycosis), paracoccidioidomycosis, North American blastomycosis, histoplasmosis, coccidioidomycosis, and sporotrichosis. Fungal infections include opportunistic fungal infections, particularly in immunocompromised patients such as those with AIDS. Fungal infections contribute to meningitis and pulmonary or respiratory tract diseases.

Other pathogenic organisms that may be prevented from causing the infections include dermatophytes (Microsporum canis and other M. spp.; and Trichophyton spp. such as T. rubrum, and T. mentagrophytes), yeasts (e.g., Candida albicans, C. Parapsilosis, C. glabrata, C.Tropicalis, or other Candida species including drug resistant Candida species), Torulopsis glabrata, Epidermophytonflloccosum, Malassezia fuurfur (Pityrosporon orbiculare, or P. ovaie), Cryptococcus neoformans, Aspergillus fumigatus, and other Aspergillus spp., Zygomycetes (Rhizopus, Mucor), hyalohyphomycosis (Fusarium Spp.), Paracoccidioides brasiiliensis, Blastomyces dermatitides, Histoplasma capsulatum, Coccidioides immitis, and Sporothrix schenckii. Fungal infections include Cladosporium cucumerinum, Epidermophyton flloccosum, and Microspernum ypseum.

B. Additional Antibiotic Agents

Anti-bacterial antibiotics can be categorized based on their target specificity: "narrow-spectrum" antibiotics target particular types of bacteria, such as Gram-negative or Gram-positive bacteria, while broad-spectrum antibiotics affect a wide range of bacteria. Antibiotics which target the bacterial cell wall (penicillins, cephalosporins), or cell membrane (polymixins), or interfere with essential bacterial enzymes (quinolones, sulfonamides) usually are bactericidal in nature. Those which target protein synthesis such as the aminoglycosides, macrolides and tetracyclines are usually bacteriostatic. In the last few years, three new classes of antibiotics have been brought into clinical use. These new antibiotics are of the following three classes: cyclic lipopeptides (daptomycin), glycylcyclines (tigecycline), and oxazolidinones.
(linezolid). Tigecycline is a broad-spectrum antibiotic, while the two others are used for Gram-positive infections.

Antibiotics for use in conjunction with the present invention include amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paramomycin, geldanaymicin, herbimycin, loracarbef, ertapenem, doripenem, impenem, meropenem, cefadroxil, cefazolin, cefalotin, cefalexin, cefaclor, cefamandole, cefoxitin, cefprozil, cefixime, cefdinir, cefditoren, cefoperazon, cefotaxime, cefpodoxime, ceftazidime, cefibuten, ceftriaxone, cefepime, ceftobiprole, teicoplanin, vancomycin, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, troleandomycin, telithromycin, spectinomycin, aztreonam, amoxicillin, ampicillin, azlocillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, meticillin, nafcillin, oxacillin, penicillin, piperacillin, ticarcillin, bacitracin, colistin, polymyxin B, ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, lomefloxacin, moxifloxacin, norfloxacin, ofloxacin, trovafloxacin, grepafloxacin, sparfloxacin, mafenide, prontosil, sulfacetamide, sulfamethizole, sulfanilimide, sulfasalazine, sulfisoxazole, trimethoprim, demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, arsphenamine, chloramphenicol, clindamycin, lincomycin, entambutol, fosfomycin, fusidic acid, furazolidone, isoniazid, linezolid, metronidazole, mupirocin, nitrofurantoin, platensimycin, pyrazinamide, quinupristin, rifampicin, thiamphenicol and tinidazole.

C. Routes of Administration

The present invention is contemplated to be used in both systemic and localized administration. With respect to systemic administration, it will be desirable to target the zinc nanoparticles to locations within the body using targeting or "homing" molecules, such as antibodies or other ligands for pathogen surface receptors.

Administration of these compositions according to the present invention may be via any common route so long as the target tissue is available via that route. This includes oral, nasal, buccal, rectal, vaginal or topical. Alternatively, administration may be by intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection. Such compositions would normally be administered as pharmaceutically acceptable compositions, described supra.
D. Combination Therapies

As discussed above, it may be desirable to combine zinc nanoparticles with other therapies in the treatment of pathogens. An "anti-pathogen" therapy is capable of negatively affecting the pathogen infestation in a subject, for example, by directly killing pathogen or pathogen-infected cells, by reducing the growth rate or reproduction of pathogens, or by rendering the pathogen or pathogen-infected cells more susceptible to host defenses. More generally, these therapies would be provided in a combined amount effective to produce a clinically significant result in the subject. This process may involve contacting the host with the therapies at the same time, such as by administering a single composition or treatment that includes both agents, or by administering two distinct compositions or treatments at the same time.

Alternatively, the zinc nanoparticle therapy may precede or follow the other treatment by intervals ranging from minutes to weeks. In embodiments where the other agent and zinc nanoparticle therapy are applied separately, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the therapy and zinc nanoparticle therapy would still be able to exert an advantageously combined effect on the cell. In such instances, it is contemplated that one may administer both modalities within about 12-24 h of each other and, more preferably, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several d (2, 3, 4, 5, 6 or 7) to several wk (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

Various combinations may be employed, where zinc nanoparticle therapy is "A" and the secondary therapy is "B":

\[
\begin{array}{cccccccccc}
A/B/A & B/A/B & B/B/A & A/A/B & A/B/B & B/A/A & A/B/B/B & B/A/B/B \\
B/B/B/A & B/B/A/B & A/A/B/B & A/B/A/B & A/B/B/A & B/B/A/A \\
B/A/B/A & B/A/A/B & A/A/A/B & B/A/A/A & A/B/A/A & A/A/B/A \\
\end{array}
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It is expected that the treatment cycles would be repeated as necessary, including two, three, four, five, six, seven, eight, nine, ten or more cycles. Suitable agents that can be used as secondary therapies are described above.
IV. Coating of Medical Devices

The present invention provides compositions with activity against various nosocomial microorganisms that inhabit medical devices, including resistant bacteria and fungi. For example, the compositions can be used against resistant staphylococci, vancomycin-resistant enterococci, resistant *Pseudomonas aeruginosa* and *Candida* species. These compositions can be used to coat of various polymers, such as polyvinyl chloride, polyethylene, silastic elastomers, polytetrafluoroethylene, dacron, collodion, carboethane, nylon, polymers used in the formation of endotracheal tubes, silicone and polyurethane polymers used in the formation of vascular catheters and surgical silk sutures. Thus, they are suitable for coating a wide range of device surfaces.

The antiseptic compound can be applied on the surface of a device by simply immersing the device in solution containing the nanoparticles, air drying and washing out excessive antiseptic. Another method used to coat surfaces of medical devices with antibiotics involves first coating the selected surfaces with benzalkonium chloride followed by ionic bonding of the antibiotic composition (Solomon and Sherertz, 1987; U.S. Patent 4,442,133). Other methods of coating surfaces of medical devices with antibiotics are taught in U.S. Patent 4,895,566 (a medical device substrate carrying a negatively charged group having a pH of less than 6 and a cationic antibiotic bound to the negatively charged group); U.S. Patent 4,917,686 (antibiotics are dissolved in a swelling agent which is absorbed into the matrix of the surface material of the medical device); U.S. Patent 4,107,121 (constructing the medical device with ionogenic hydrogels, which thereafter absorb or ionically bind antibiotics); U.S. Patent 5,013,306 (laminating an antibiotic to a polymeric surface layer of a medical device); and U.S. Patent 4,952,419 (applying a film of silicone oil to the surface of an implant and then contacting the silicone film bearing surface with antibiotic powders).

The invention also provides methods to generate a wide variety of antiseptic medical devices. Some examples include antiseptic endotracheal tubes, antiseptic vascular catheters, including central venous catheters, arterial lines, pulmonary artery catheters, and peripheral venous catheters, antiseptic urinary catheters, antiseptic nephrostomy tubes, antiseptic biliary stents, antiseptic peritoneal catheters, antiseptic epidural catheters, antiseptic central nervous system catheters, including...
intraventricular shuts and devices, antiseptic prosthetic valves, orthopedic implants and antiseptic sutures.

V. **Diagnostic/Imaging Uses**

A. **Nanoparticles**

Yet another use of the zinc nanoparticles of the present invention involves diagnostics and imaging. Zinc nanoparticles have an inherent auto-fluorescent capacity and thus can be used, when coupled to a targeting agent, to image sites within a subject. Such sites may comprise focal infections or tumors.

In addition, it may prove useful to add a second or more powerful imaging agent to the zinc nanoparticle to improve its performance or widen its range of applicability. Non-limiting examples of diagnostic labels include enzymes, radiolabels, haptens, fluorescent labels, phosphorescent molecules, chemilluminescent molecules, chromophores, photoaffinity molecules, colored particles or ligands, such as biotin, paramagnetic ions, radioactive isotopes, fluorochromes, NMR-detectable substances, and X-ray imaging agents.

In the case of paramagnetic ions, one might mention by way of example ions such as chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and/or erbium (III), with gadolinium being particularly preferred. Ions useful in other contexts, such as X-ray imaging, include but are not limited to lanthanum (III), gold (III), lead (II), and especially bismuth (III).

In the case of radioactive isotopes for therapeutic and/or diagnostic application, one might mention astatine$^{211}$, carbon$^{14}$, chromium$^{51}$, chlorine$^{36}$, cobalt$^{58}$, copper$^{67}$, europium$^{152}$, gallium$^{67}$, hydrogen$^{3}$, iodine$^{123}$, iodine$^{125}$, iodine$^{131}$, indium$^{111}$, iron$^{59}$, phosphorus$^{32}$, rhenium$^{186}$, rhenium$^{188}$, selenium$^{75}$, sulphur$^{35}$, technicium$^{99m}$ and/or yttrium$^{90}$. $^{125}$I is often being preferred for use in certain embodiments, and technicium$^{99m}$ and/or indium$^{111}$ are also often preferred due to their low energy and suitability for long range detection.

Among the fluorescent labels contemplated for use as conjugates include Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy3, Cy5,6-FAM, Fluorescein Isothiocyanate, HEX, 6-JOE, Oregon Green 488, Oregon Green 500,
Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, Renographin, ROX, TAMRA, TET, Tetramethylrhodamine, and/or Texas Red.

B. Imaging Methods

Two forms of radiographic images are in use in medical imaging; projection radiography and fluoroscopy, with latter useful for intraoperative and catheter guidance. These 2D techniques are still in wide use despite the advance of 3D tomography due to the low cost, high resolution, and depending on application, lower radiation dosages. This imaging modality utilizes a wide beam of x rays for image acquisition and is the first imaging technique available in modern medicine.

Fluoroscopy produces real-time images of internal structures of the body in a similar fashion to radiography, but employs a constant input of x-rays, at a lower dose rate. Contrast media, such as barium, iodine, and air are used to visualize internal organs as they work. Fluoroscopy is also used in image-guided procedures when constant feedback during a procedure is required. An image receptor is required to convert the radiation into an image after it has passed through the area of interest. Early on this was a fluorescing screen, which gave way to an Image Amplifier (IA) which was a large vacuum tube that had the receiving end coated with cesium iodide, and a mirror at the opposite end. Eventually the mirror was replaced with a TV camera.

Projectional radiographs, more commonly known as x-rays, are often used to determine the type and extent of a fracture as well as for detecting pathological changes in the lungs. With the use of radio-opaque contrast media, such as barium, they can also be used to visualize the structure of the stomach and intestines - this can help diagnose ulcers or certain types of colon cancer.

Gamma cameras are used in nuclear medicine to detect regions of biological activity that are often associated with diseases. A short lived isotope, such as $^{123}$I is administered to the patient. These isotopes are more readily absorbed by biologically active regions of the body, such as tumors or fracture points in bones.

Computed tomography (CT) is a medical imaging method employing tomography. Digital geometry processing is used to generate a three-dimensional image of the inside of an object from a large series of two-dimensional X-ray images taken around a single axis of rotation. Computed tomography was originally known as the "EMI scan" as it was developed at a research branch of EMI, a company best
known today for its music and recording business. It was later known as computed axial tomography (CAT or CT scan) and body section röntgenography.

CT produces a volume of data which can be manipulated, through a process known as "windowing," in order to demonstrate various bodily structures based on their ability to block the X-ray/Röntgen beam. Although historically the images generated were in the axial or transverse plane (orthogonal to the long axis of the body), modern scanners allow this volume of data to be reformatted in various planes or even as volumetric (3D) representations of structures.

Positron emission tomography (PET) is primarily used to detect diseases of the brain and heart. Similarly to nuclear medicine, a short-lived isotope, such as $^{18}$F, is incorporated into a substance used by the body such as glucose which is absorbed by the tumor of interest. PET scans are often viewed alongside computed tomography scans, which can be performed on the same equipment without moving the patient. This allows the tumors detected by the PET scan to be viewed next to the rest of the patient's anatomy detected by the CT scan. Another 3D tomographic technique is SPECT but uses gamma camera-like method for reconstruction.

Magnetic resonance imaging (MRI) or nuclear magnetic resonance (NMR) as it was originally known, uses powerful magnets to polarise and excite hydrogen nuclei (single proton) in water molecules in human tissue, producing a detectable signal which is spatially encoded, resulting in images of the body. MRI uses three electromagnetic fields: a very strong (on the order of units of teslas) static magnetic field to polarize the hydrogen nuclei, called the static field; a weaker time-varying (on the order of 1 kHz) field(s) for spatial encoding, called the gradient field(s); and a weak radio-frequency (RF) field for manipulation of the hydrogen nuclei to produce measurable signals, collected through an RF antenna.

Like CT, MRI traditionally creates a two dimensional image of a thin "slice" of the body and is therefore considered a tomographic imaging technique. Modern MRI instruments are capable of producing images in the form of 3D blocks, which may be considered a generalisation of the single-slice, tomographic, concept. Unlike CT, MRI does not involve the use of ionizing radiation and is therefore not associated with the same health hazards. For example, because MRI has only been in use since the early 1980s, there are no known long-term effects of exposure to strong static fields and therefore there is no limit to the number of scans to which an individual can be subjected, in contrast with X-ray and CT. However, there are well-identified health
risks associated with tissue heating from exposure to the RF field and the presence of implanted devices in the body, such as pace makers. These risks are strictly controlled as part of the design of the instrument and the scanning protocols used.

Because CT and MRI are sensitive to different tissue properties, the appearance of the images obtained with the two techniques differ markedly. In CT, X-rays must be blocked by some form of dense tissue to create an image, so the image quality when looking at soft tissues will be poor. In MRI, while any nucleus with a net nuclear spin can be used, the proton of the hydrogen atom remains the most widely used, especially in the clinical setting, because it is so ubiquitous and returns a large signal. This nucleus, present in water molecules, allows the excellent soft-tissue contrast achievable with MRI.

VI. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 - ZNP as drug carriers

A significant advantage to ZNPs vs. quantum dots as drug delivery vehicles is their safety and complete dissolution in the body. As such they can be used to alter the pharmacodynamics of drugs conjugated to their surface. The surface of ZNPs can be protected from the aqueous environment through the use of surface polymers. Based on the level of surface protection and the composition of the coating the rate of dissolution and hence drug delivery can be altered.

Envisioned is a systemic method of increasing the half-life of pharmaceutical compositions. An example would be the conjugation of cancer chemotherapeutics such as doxorubicin to ZNPs. The chemical composition would consist of a specific molar ratio of ZNPs and doxorubicin. After doxorubicin conjugation to ZNPs, non-
drug bound surface of the ZNPs would be coated with polymers such as Poly-ethylene glycol. Upon administration of these drug conjugates, there would be increased anti-tumor activity or improved safety.

Example 2 - ZNPs as drug efficacy potentiators

Envisioned are cytotoxic drugs attached to ZNPs. Both the cytotoxic drug and ZNPs would accumulate within a tumor environment and act synergistically to improve efficacy by increasing cell killing. An example would be ZNP conjugated with Rapamycin. Cells highly responsive to mTOR signaling would be treated through the administration of ZNP conjugated Rapamycin. Both the ZNP and Rapamycin would exert cytotoxic effect.

In addition, it is anticipated that through the use of targeting ligands, highly specific delivery of both ZNP, Drug Conjugate or both can be used to improve efficacy with improved biosafety. The targeting ligand would result in highly specific delivery of drug conjugate directly to cells resulting in reduced metabolic breakdown and improved pharmacokinetics and improved efficacy.

Example 3 - BioGel formulations of ZNPs

Neoadjuvant therapy is used for the reduction of tumor size prior to systemic therapy. ZNPs are ideally suited as neoadjuvant agents for the reduction of tumor size. The inventors formulated inert biogels with ZNPs. Pluronic gels were mixed with ZNPs to make formulations between 1-30% w/v. Pluronic is a block co-polymer that can retard delivery of drugs. In this formulation, the rate of ZNP dissolution was altered to result in slow release of zinc ions. The rate of release was dependent on the w/v ratio of ZNPs and the percent formulation of the gel. In vitro kinetics of zinc ion release is shown in FIG. 4 (solubility of ZNP in BioGell formulations of pluronic). In vivo serum zinc levels upon intratumoral injection of BioGel-ZNP was measured and showed a maximum increase of serum zinc levels to 2X over baseline over a period of 3 days with return to baseline by day 5. The rate of serum zinc increase varied by animal likely related to differences in injection technique. The rate of increase was gradual compared to injection of non-gel formulations of ZNP which showed maximum serum zinc levels by 90 minutes with return to baseline by 8 hours. A 30% pluronic-50 mg/ml ZNP solution was injected into subcutaneous B16 melanomas
growing on the dorsum of immunocompetent C57B/6 mice. Tumor growth is shown in FIG. 8. A number of animals showed complete remission of tumors (cure) without relapse upon observation at least 1 month from the time of drug administration. Overall, Pluronic-ZNPs treated animals showed improved survival as shown in FIG. 9.

Example 4 - Targeted delivery of ZNPs

ZNP Cytotoxicity is enhanced by internalization within cells. Specificity of delivery to cells (bacterial, cancer, etc.) can be controlled through the conjugation of specific targeting ligands. The inventors used two cancer cell lines SKOV3 (ovarian cancer) and SKBR3 (breast adenocarcinoma) which overexpress Her2-neu. These cell lines were treated with ZNPs or ZNPs conjugated with antibody against Her2-neu (Herceptin analogue) Since ZNPs exhibit cytotoxic effect after 2 hrs to distinguish the effect of conjugated versus non-conjugated ZNPs, cells were treated for only 1 hr and then extensively washed to remove extracellular ZNPs and zinc ions (FIG. 10). Cytotoxicity was measured at 18 hrs by standard MTT assay. Conjugated ZNPs were internalized while non-conjugated ZNPs were not. Conjugated ZNPs showed 100% cytotoxic killing of both Her2-Neu expressing cell lines but no effect against non-Her2-Neu expressing cells showing that internalization and cytotoxic effect or specificity was dependent on the targeting ligand (FIG. 10). The Her2-Neu antibody showed no effect over this short period of time indicating that cell death required both cell receptor specific targeting through the use of a binding ligand and ZNPs.

Example 5 - Conjugation Data

To address the question of conjugation methodologies, the inventors used three different methods of conjugating antibodies and proteins to the surface of zinc nanoparticles. The first is ligand binding with zero-length cross-linkers. Using EDC, the inventors were able to bind two separate antibodies against Her2 and thiolated transferrin. EDC reacts with carboxyl groups to form reactive amines. In conjunction with Sulfo-NHS, ligands were bound to the EDC-coated zinc nanoparticles at the carboxyl group. The second is polymer coating. Through electrostatic binding, zinc nanoparticles were coated with PEG of various molecular weights 1000-20000. ZNP maintain a slight negative charge in acidic environments allowing for electrostatic
binding of reactive polymers. PEO and vinyl esters were bound in a similar manner. The third is ligand binding with monofunctional and bifunctional crosslinkers. Using both thiolated and amine terminated PEG, antibodies to Her2 were conjugated to the reactive groups to result in antibodies attached monofunctionally or heterobifunctionally. Heterobifunctional ligand attachment resulted in greater zinc deliver intracellularly to cells.

Methodology: To 5 mg of ZNP slurry n 100 ul of MES buffer solution (100mM, pH 4.5) 88.3 mg of NHS and 2.0 mg of EDC were added and solution incubated with stirring for 60 minutes at room temperature. The mixture was then centrifuged through Microcon YM-50 filter units and washed with 100 mM MES (ph 6.3) to remove unreacted coating. Either anti-Her2 antibody (ATCC) or thiolated transferrin 1.0 mg/ml was added and incubated for 2.5 hours. The final product was centrifuged to precipitate ZNP and washed twice with PBS prior to use in experimentation.

Polysaccharide polymer binding to ZNP was performed by physical adsorption under alkaline conditions. PEG (Sigma Aldrich), PEO (Sigma Aldrich) and polyacrylic acids b polystyrenes (Sigma Aldrich) were coated onto ZNP by ligand exchange. The PEG, PEO and vinyl-esters were purchased with functional amide groups for subsequent binding of antibody by chemical reduction. The chain length of the coatings varied between 1000-20000 and for the amphiphilic block co-polymers between 30-136 units. ZNPs (5mg) were suspended in neutral citrate buffer (ph 7.4) and then dried. Dry PEG, PEO, Esters were added and the mixture rested for 2 hours. The mixture was then hydrated with water, washed, centrifuged, and then resuspended in 100 mM MES buffer (ph 5.0) and stored prior to experimentation. Antibody or ligand attachment was performed as described above.

Conclusion: ZNP can be functionalized using standard chemistries used for nanoparticle binding and conjugation. ZNPs show similar efficiencies in ligand binding to iron-oxide and zinc-oxide particles of similar size. (See Figures 1,2, 3, 4)

Example 6 - Uptake data

To address the question of ZNP uptake by cells, the inventors performed FACS analysis of ZNP treated cells followed by staining using zinc ion specific fluorophores.
Methodology: SKBR3 (Her2 expressing) and PC3 cells (non-Her2 expressing) were incubated with anti-HER2 conjugated ZNPs for 60 minutes in tissue culture plates. Excess ZNP was removed by three washes in PBS and then cells harvested by trypsinization. Cells were stained with TSQ or Fluozin3 (zinc ion specific fluorescent markers) by co-incubation in the dark at 4°C for 15 minutes. Cells were washed with PBS three times and then transferred to flow cytometry tubes and analyzed by FACS (Coulter EPICS). Cells were gated based on size distribution and mean fluorescent intensity measured. The fluorescent intensity reflects internal zinc ion concentration.

Conclusion: (FIGS. 11 and 12) Zinc ion levels rise intracellularly in a time dependent manner. Zinc levels rise faster in targeted ZNPs then with ZNPs alone or from the addition of zinc salts. Ligand conjugated ZNPs showed rises in intracellular zinc accumulation as fast as 20 minutes compared to 2-4 hours for zinc salts. This data inferentially suggests that ZNPs may be internalized in a receptor dependent manner.

Example 7 - Efficacy Data

To address the question of ligand specific cancer killing, the inventors treated prostate cancer cells, ovarian cancer cells and breast cancer cells with HER2 or transferrin-targeted ZNP (FIGS. 13 and 14). The prostate cancer cells, PC3, are known to express transferrin receptor but not HER2 while both the ovarian cancer cells (SKOV3) and breast cancer cells (SKBR3) are known to express HER2. Two separate HER2 antibodies were used. In experiment 1, the inventors evaluated cell specificity. Prostate cancer cells were only killed by transferrin-targeted ZNPs while the ovarian and breast cancer cells showed specificity for the HER2 targeted ZNPs. Cells were incubated for 90 minutes which based on uptake data was sufficient for internalization whereas non-targeted ZNPs showed no zinc accumulation and no toxicity over the same incubation period indicating that toxicity is dependent on both intracellular zinc delivery which is dependent on targeting. In experiment 2, they evaluated a dose response of targeted ZNP against susceptible target cells. Linear dose response kinetics were observed between 50 µg and 500 µg although we anticipate that this may not hold true depending on the physiochemical characteristics of the ZNP.
Methodology: Cells were incubated at 70% confluency in 6 well plates. In the first experiment, Conjugated ZNP or ZNP control were added to wells for 60 minutes followed by three washes of PBS to remove any excess ZNP. Fresh media was added and cells harvested at 24 hours for MTT assay (Promega). Wells were evaluated in triplicate and cell killing expressed relative to non-treated control wells.

Example 8 - ZNP Pharmacokinetics of zinc ion release

ZNPs were coated with different polymers including PEG and vinyl esters (see conjugation methodology). Based on the length of the polymer, the degree of hydrophobicity could be altered. Zinc ion release (half-life) of ZNPs were dependent on the degree of hydrophobicity of the polymer coating (FIG. 15).

Methodology: Zinc ion concentration was measured upon placing ZNPs in heat-inactivated fetal calf serum. Zinc ion concentration was measured by fluorescent spectroscopy of removed aliquots with the fluorescent molecular probe FluoZin3.

**************************

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.
VII. References

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

U.S. Patent 4,107,121
U.S. Patent 4,442,133
U.S. Patent 4,680,338
U.S. Patent 4,895,566
U.S. Patent 4,917,686
U.S. Patent 4,952,419
U.S. Patent 5,013,306
U.S. Patent 5,141,648
U.S. Patent 5,399,363
U.S. Patent 5,466,468
U.S. Patent 5,543,158
U.S. Patent 5,563,250
U.S. Patent 5,641,515
U.S. Patent 5,846,225
U.S. Patent 5,846,233
U.S. Patent 5,856,456
U.S. Patent 5,880,270
U.S. Patent Publn. 2007/0212331
CDC, 1993

1. A zinc nanoparticle composition comprising:
   (a) a zinc core substantially free of other metals or metal oxides; and
   (b) a non-zinc shell or coating.
2. The zinc nanoparticle of claim 1, wherein said non-zinc shell comprises a material permitting conjugation of an agent to said zinc nanoparticle.
3. The zinc nanoparticle of claim 2, wherein said material is a thiolic acid.
4. The zinc nanoparticle of claim 2, further comprising an agent conjugated to said zinc nanoparticle.
5. The zinc nanoparticle of claim 3, wherein said agent is a cell or tissue targeting agent.
6. The zinc nanoparticle of claim 3, wherein said agent is a diagnostic agent.
7. The zinc nanoparticle of claim 3, wherein said agent is a therapeutic agent.
8. The zinc nanoparticle of claim 1, wherein said non-zinc shell or coating comprises a biologically inert gel, polymer or matrix.
9. The zinc nanoparticle of claim 1, wherein said zinc nanoparticle is between about 5 nm and 100 nm.
10. The zinc nanoparticle of claim 9, wherein said zinc nanoparticles is between 10 nm and 50 nm.
11. A medical device coated with zinc nanoparticle composition comprising zinc nanoparticles comprising a zinc core substantially free of other metals or metal oxides.
12. The medical device of claim 11, wherein said device is an implanted or indwelling device.
13. The method device of claim 12, wherein said implanted or indwelling device is a catheter, stent, pump, or suture.

14. The medical device of claim 11, wherein said zinc nanoparticle further comprises a non-zinc shell or coating.

15. The medical device of claim 14, wherein said non-zinc shell or coating comprises a biologically inert gel, polymer or matrix.

16. The medical device of claim 11, wherein said nanoparticle is between about 5 nm and 100 nm.

17. The medical device of claim 11, wherein said nanoparticle is between about 10 nm and 50 nm.

18. The medical device of claim 11, wherein said non-zinc shell or coating comprises a material permitting conjugation of a therapeutic agent to said zinc nanoparticle.

19. The medical device of claim 18, wherein said material is a thiolic acid.

20. A method of treating a subject with a solid tumor comprising administering to said subject a zinc nanoparticle composition comprising:

(a) a zinc core substantially free of other metals or metal oxides; and

(b) a non-zinc shell or coating.

21. The method of claim 20, wherein said solid tumor is prostate tumor, basal cell carcinoma tumor, squamous cell carcinoma tumor, or melanoma tumor.

22. The method of claim 20, wherein said subject is a human, a non-human primate, a dog, a cat, a horse, a cow, a goat, a rat, a pig, a rabbit, or a mouse.

23. The method of claim 20, wherein administering comprises systemic administration.

24. The method of claim 23, wherein systemic administration comprises intravenous injection.
25. The method of claim 20, wherein administering comprises loco-regional administration.

26. The method of claim 25, wherein loco-regional administration comprises intratumoral injection, injection into tumor vasculature, or injection regional to said tumor.

27. The method of claim 20, further comprising administering to said subject a second anti-cancer therapy.

28. The method of claim 27, wherein said second anti-cancer therapy is radiotherapy, chemotherapy, immunotherapy, gene therapy, hormone therapy, and antibiotic therapy.

29. The method of claim 20, wherein said cancer is recurrent, metastatic or multi-drug resistant.

30. A method of treating a subject with an infection comprising administering to said subject a zinc nanoparticle composition comprising:

(a) a zinc core substantially free of other metals or metal oxides; and
(b) a non-zinc shell or coating.

31. The method of claim 30, wherein said infection is bacterial, viral, fungal or parasitic.

32. The method of claim 30, wherein said subject is a human, a non-human primate, a dog, a cat, a horse, a cow, a goat, a rat, a pig, a rabbit, or a mouse.

33. The method of claim 30, wherein administering comprises systemic administration.

34. The method of claim 33, wherein systemic administration comprises intravenous injection.

35. The method of claim 30, wherein administering comprises loco-regional administration.
36. The method of claim 35, wherein loco-regional administration comprises topical administration or injection regional to said infection.

37. The method of claim 30, further comprising administering to said subject a second anti-biotic therapy.

38. The method of claim 30, wherein said infection is caused by a drug resistant organism.

39. A method of imaging a site in a subject comprising:

(i) administering to said subject a zinc nanoparticle comprising

(a) a zinc core substantially free of other metals or metal oxides; and
(b) an agent that targets said zinc nanoparticle to said site, and

(ii) imaging said zinc nanoparticle in said subject.

40. The method of claim 39, wherein said zinc nanoparticle further comprises a detectable label.

41. The method of claim 40, wherein said detectable label is a fluorescent, chemilluminescent or radiolabel.

42. The method of claim 39, wherein said zinc nanoparticle further comprises a therapeutic agent.

43. The method of claim 42, wherein said therapeutic agent is an antibiotic, an anti-fungal, and anti-parasitic, an anti-viral, a chemotherapeutic, or a radiotherapeutic.

44. The method of claim 39, wherein said subject has a tumor or an infection.
Solubility of ZNP in Different Solutions

% Maximum TSQ Fluorescence vs Time (Minutes)

- Water
- Media
- Saline
- Serum

FIG. 3
Solubility of ZNPs in Pluronic Biogels

![Graph showing solubility over time with different Pluronic concentrations](image)

FIG. 4
Cytotoxicity of ZNPs

FIG. 5
Dose Dependent ZNP Cytotoxicity

% Viability

ZNP ug/ml

PC3
CAL27
SKOV3

FIG. 6
Synergy of ZNP with Cancer Chemotherapeutics

% Viability

Treatment

No Drug  Doxirubicin  Rapamycin  Etoposide

No ZNP  LD50 ZNP

FIG. 7
Efficacy of ZNPs against Subcutaneous Melanomas

FIG. 8
Survival of Animals with Melanoma

FIG. 9
Cytotoxicity of Her2-Neu AB Conjugated ZNPs

% Viability

No Treatment  Vehicle  Her2 AB  ZNP  Her2-ZNP

FIG. 10
FIG. 11
FIG. 12
FIG. 13

The figure illustrates the percentage of cell viability for different treatments in SKBR2, SKOV3, and PC3 cell lines. The treatments include Control, ZNP, ZNP-Her2, ZNP-Her2(2), and ZNP-TFN. The graph shows a comparison of cell viability across these treatments, indicating the effectiveness of each on cell viability.
FIG. 14
**INTERNATIONAL SEARCH REPORT**

**International application No**
PCT/US 10/45688

**A CLASSIFICATION OF SUBJECT MATTER**

<table>
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<th>IPC(8)</th>
<th>A01 N 59/1 6 (201 0.01)</th>
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<td>424/641</td>
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According to International Patent Classification (IPC) or to both national classification and IPC

**B FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

<table>
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<th>IPC(8)</th>
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<td>USPC</td>
<td>424/641</td>
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**B**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

| USPC   | 424/641, 977/906 (keyword limited, search terms below) |

Electronic database consulted during the international search (name of database and, where practicable, search terms used)

PUBWEST(PGPB,USPT,EPAB,JPAB), Google Scholar, Google Patents

Search terms zinc nanoparticle$2 cancer$5 thiol$3 thiolic core shell cell tissue targeting inert polymer$5 intravenous drug$2 biotic chemoluminescent$4 therapeutic

**C DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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<tr>
<td>US 2008/0089836 A1 (Hainfeld) 17 April 2008 (17 04 2008), especially, para [0001], [0003], [0012], [0014]-[0016], [0026]-[0030], [0069], [0202] and [0237]</td>
<td>1-10</td>
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<tr>
<td>US 2007/0293382 A1 (Santra et al.) 22 November 2007 (22 11 2007), especially, para [0019], [0036], [0038], [0042], [0045], [0047], [0069], [0089], [0101], [0105], [0108], [0109], [0119], [0120], [0134] and [0178]</td>
<td>11-44</td>
</tr>
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</table>

**D Further documents are listed in the continuation of Box C**

- Special categories of cited documents
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
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  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed

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- "X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search
3 October 2010 (03 10 2010)

Date of mailing of the international search report
08 OCT 2010

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