Critical Review of Alcohol, Alcoholism and the Withdrawal Symptoms-III. An Introduction to Nanoparticles and their Applications in Alcoholism Treatment

Ashok K Singh*

Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, USA

Abstract

Alcoholism is a complex heterogeneous disease with many contributing factors that may vary from person to person, and are known to have a major impact on treatment outcome. Thus, a single treatment strategy may not work for everyone, stressing an urgent need to develop personalized treatments based on the person's genetic and environmental factors. Recent advancements in nanotechnology have allowed construction of unique Nanoparticles (NPs) having potentials for personalized treatments by: (i) Delivering Therapeutic Drugs (TDs) to specific sites, (ii) Releasing TDs on-demand by internal or external cues, and (iii) Serving as vectors for transfection of cDNA-plasmids into the host's gene to increase the gene expression and/or siRNA to inhibit the gene expression.

There are substantial, but not compelling evidence for application of Engineered NPs (ENPs) on screening and treatment of alcoholism. The key factor that confers the ENPs their unique therapeutic potency is that, irrespective of differences in their composition, ENPs exhibit some common unique physicochemical properties (such as high surface area to volume ratio, high surface reactivity that is inversely related to the size, and unique electronic, optical and magnetic properties) not found in bulk particles. The therapeutic potency/toxicity ratio of an ENP may determine its therapeutic index, possibly because the physicochemical characteristics that confer the ENPs their unique properties are also responsible for their toxicity. Therefore, the aims of this review are to discuss (1) The ENPs’ structure, physicochemical properties, beneficial properties and toxicity, and (2) Their relevance in development if individualized treatment against alcoholism.

Keywords

Nanoparticles, Clusters, Nanotechnology, Nanotoxicology, Bulk particles, Surface molecules, Surface area and volume ratio, Metals, Semiconductors, Magnetism, Dendrimers, Isotropic, Anisotropic, Precautionary principle.

Introduction

Alcoholism is a chronic, multifaceted disorder in which the gene-environment interactions play a key role in the disease’s etiology [1,2]. The consequences of alcohol abuse that results in development of alcoholism have been attributed to the alcohol’s toxic effects on the brain [3]. Figure 1 shows several neurobiological circuits that are central to different stages of addiction [4-11] (Figure 1).

Different neurotransmitter systems targeted by alcohol are GABA [12,13], glutamate [14], serotonin [15], nor epinephrine [16], neuropeptide Y [17], vasopressin [18], adenosine [19] and Dopamine (DA) [2,20-22]. Because of (i) The mechanistic complexities of alcoholism [23] and (ii) Diverse interaction of genetically differently people to their environment, the current common therapy consisting of a combination of psychological, behavioral and pharmacological approaches exhibits high individual variability in treatment outcomes including high incidence of relapse. Therefore development of an individualized, patient-specific treatment strategy is highly desirable.
Earlier attempts to design patient-specific treatment have failed because of lack of technology to establish alcoholism subtypes, inability to use the markers for identifying endophenotypes and a lack of diverse pharmacologic agents with proven efficacy at improving particular drinking outcomes [24-26]. Recent developments in genetic screening and transfection techniques as resolved some of the gaps listed above.

i. Genetic analysis may determine whether an alcohol-naïve person possess the genetic vulnerabilities associated with the risk of developing alcoholism.

ii. The diagnose techniques may decipher cell-signaling abnormalities that have already occurred in alcohol abusers.

iii. It is possible to modulate the expression of selective genes by administering viral vectors containing the gene’s cDNA for expression augmentation or siRNA for expression inhibition [27,28].

iv. Although these techniques may allow development of individualized therapy for alcoholism, technology to integrate all of the therapeutic components listed above is lacking. Recent development of novel Engineered Nanoparticles (ENPs) having unique electronic, physicochemical and biological properties bring hope to the development of patient-centric individual treatment of alcoholism and other addiction disorders.

ENP are $< 100$ nm at least in one dimension and behaves as a whole unit possessing unique electronic, physicochemical and biological properties distinct from those in bulk particles. As the ENPs become smaller, the proportion of atoms on the surface increases, thus they exhibit size- and shape-dependent properties such as electron confinement (transition from classic mechanics to quantum mechanics), metal to semi-conductor transition, an increase in mechanical adhesion and capillary forces, a drop in melting point, an increase in tunneling current, a blue shift in optical properties, and ferromagnetic to super paramagnetic shift [29]. The size-dependent unique properties of NPs are exploited by the industry to design and market new electronic, medicinal (diagnostics, prophylaxis, therapeutics and site-directed drug-transport), environmental, cosmetic, and food products that are growing every day [30]. Different ENPs are being designed and tested for screening and treatment of alcoholism and other substance addiction [31,32]. Unfortunately, the very properties responsible for ENPs’ commercial application also exponentially increase their toxicity and adverse effects. As ENPs becomes smaller, its surface reactivity and toxicity increase [33,34]. The therapeutic potency/toxicity ratio may determine the therapeutic index of ENPs. Therefore, it is important to understand possible relationship between the ENPs structure and properties to develop safe and effective medicinal products including novel treatment strategies for treatment of alcoholism and development...
of personalized treatments. The aim of this review article is to discuss the ENPs’ structural classification, physicochemical properties, surface functionalization - a critical step for NP functionality, nanoparticles applications, toxicity and risk assessment and applications in alcoholism treatment.

**Structure of Nanoparticles**

ENPs are the simplest form of diverse structures (Figure 2) with sizes in 1 nm to 100 nm range.

According to their shape, ENPs are classified as 0, 1, 2 and 3 dimensional particles [35,36]. The ‘0’ Dimensional (0D) ENPs are nanospheres and nanoclusters less than 100 nm in all dimensions (electrons are fully confined). The ‘1’ Dimensional (1D) ENPs such as nanotubes, nano-rods and nano-fibers are less than 100 nm in at least two dimensions (electrons are both confined and delocalized). The ‘2’ Dimension (2D) ENPs such as graphene, molybdenum disulfide and single-layer crystal composed of germanium are less than 100 nm in at least one dimension (electrons are confined and delocalized). The ‘3’ Dimensional (3D) materials such as dispersions of nanoparticles, bundles of nanowires, and nanotubes as well as multi-nanolayers that are not confined to the nanoscale in any dimension (electrons are delocalized). Structurally, ENPs can be classified as following:

- **Metal nanoparticles** (gold, copper, silicon, iron, etc.) are widely used in catalysis, electronics, sensors, photonics, environmental remediation and medicine. Porous silica nanoparticles contain microscopic reservoirs that can hold and protect sensitive drugs in a pH-sensitive manner. Acidic pH disrupts the drug-nanoparticle binding, thus releasing the drug load.

- **Polymeric nanoparticles** are prepared from synthetic polymers such as poly(2-hydroxy ethyl methacrylate), poly(N-vinyl pyrrolidone), poly(methyl methacrylate), poly(vinyl alcohol), poly(acrylic acid), polyacrylamide or natural polymers such as gums (Ex. Acacia, Guar, etc.), chitosan, gelatin and sodium alginate [29].

- **Biochemical nanoparticles** such as DNA, proteins and poly-amino acids such as poly-L-lysine and poly-L-serine are synthesized from biological precursors. DNA nanoparticles are three strands of DNA with a lipid and functional molecule attached to its ends. In water solution, the combination of hydrophilic DNA and lipophilic lipids causes the units to self-assemble into hollow spheres consisting of multiple layers of DNA, lipid and cargo.

- **Carbon Nanotubes (CNTs)** are formed from rolling-up graphite sheets. Depending on the direction of hexagons, carbon nanotubes can exhibit metallic or semi-conductor properties. CNTs are twice as strong as steel, but weigh many times less. In 1996, a new form of carbon - the Buckminster fullerene was discovered that looks like a nanometer-sized soccer ball made from 60 carbon atoms [33,37].

- **Nanoclays** are layers of mineral silicates nanoparti-
cles. Organically-modified or hybrid organic-inorganic nanomaterials have potential uses in polymer nanocomposites, as rheological modifiers, gas absorbents and drug delivery carriers.

**Physicochemical Properties of Nanoparticles**

As the ENPs get smaller, there is a gradual size-dependent transition in their physical (increase in hardness, strength and ductless), optical (color change and surface Plasmon) and surface related (greater surface area to volume ratio) properties [33,38]. Below certain size (usually < 10 nm), the electronic properties of nanoparticles switch from classical mechanics to quantum mechanics [39]. The goal of this sub-sections is to discuss (i) Size-dependent properties common to all chemically diverse ENPs and (ii) Chemical-composition related distinct properties.

**Common properties of all ENPs**

All ENPs, irrespective of their chemistry, exhibit comparable surface reactivity, thermodynamics, and electronic and mechanical properties described below.

The **surface reactivity**: ENPs exhibit exceptionally high Surface Area to Volume (S/V) ratio compared to their bulk counterparts. As the ENPs’ size decreases, the percentage of atoms at the surface increases relative to the core atoms, resulting in a decrease in the ENP’s melting point and an increase in the (valance-conduction) band energy gap, thus many metals may become semiconductor at nanometer level [40,41]. To understand the ENP’s surface effects, it is important to understand the characteristics of the core and surface atoms (Figure 3). The core atoms form stable covalent bonds with the nearest neighboring atoms. The surface atoms contain non-bonded electrons, exhibiting greater uncompensated spin. Therefore, the surface atoms exhibit relatively lower Nearest Neighbor (NN) number (Figure 3) and relatively greater free energy, anisotropy, bond defects and surface strain. These properties confer the surface atoms many unique physicochemical characteristics not present in the core atoms.

**Size dependent thermodynamic properties**: The Gibbs free energy of bulk particles \(G_b\) is defined as \(G_b = H - TS\) where \(H\) is change in enthalpy, \(T\) is temperature and \(S\) is the entropy [42]. Surface atoms have minuscule role in determining the bulk particle’s \(G_b\). In ENPs, the surface free energy begins to dominate the core free energy, thus size becomes the key determinant of their thermodynamic properties. For ENPs, \(G_{surface}\) is inversely related to the Diameter (D), Table 1 shows examples of surface free energy of different nanoparticles and corresponding bulk particles. In all cases, the free energy in bulk particles was lower than that in nanoparticles. Nanoparticles capped with functional groups or embedded in another particle exhibited lower free energy. This suggests that the free surface energy of nanoparticles also depends upon their functionalization and environment (Table 1).

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**Table 1**: Thermodynamic properties of bare and functionalized nanoparticles.

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<tr>
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<tbody>
<tr>
<td>Bulk</td>
<td>1.8</td>
<td>1.51</td>
<td>1.47</td>
<td>1.06</td>
</tr>
<tr>
<td>NP bare</td>
<td>6.0</td>
<td>2.5</td>
<td>2.45</td>
<td>6.4</td>
</tr>
<tr>
<td>NP-capped</td>
<td>1.74</td>
<td></td>
<td></td>
<td>1.3-5.9</td>
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<tr>
<td>Embedded</td>
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</tbody>
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**Figure 3**: The 3D structure of a nanocrystal. The core atoms such as atom-1 have six Nearest Neighbors (NN = 6). The surface atoms 2, 3 and 4 have NN numbers, 3, 4 and 5, respectively. Thus, depending on the location of an atom, different surface atoms may exhibit different electronic properties, commonly known as surface anisotropy. In addition, the surface and core atoms may exhibit different electronic properties, commonly known as structural anisotropy [29].
Electronic properties: In bulk particles, Electrons (e\textsuperscript{-}), bound with a positively charged Electron-Hole (e\textsuperscript{-}h\textsuperscript{+}) complex, are distributed in discrete energy bands called occupied orbital’s [43-45]. The number of electrons in Valance Orbital or Highest Occupied Molecular Orbital (HOMO) determines the electrical properties of an atom [46]. When an electron in HOMO is excited by absorbing energy (light or heat), it separates from the ‘hole’ and jump into the conduction band, resulting in creation of a positively charged ‘hole’ in HOMO [47,48]. The newly formed ‘hole’ attracts neighboring electron, resulting in propagation of a positively charged hole-current in HOMO, moving opposite to the direction of the electron current in the conduction bands [29]. In metals, since the HOMO and LUMO overlap, electrons from both HOMO and LUMO can bind the ‘hole’ (Figure 4).

Unlike the bulk particles, nanoparticles less than 100 nm gets quantized and acquire wave-particle duality [49,50]. Electrons are found within certain energy-states called the Density of States (DOS). Electrons cannot jump from HOMO to the Lowest Unoccupied Molecular Orbital (LUMO) if the DOS is occupied (the Pauli Exclusion Principle). The DOS of a system describes the number of states at each energy level that are available for occupation by electrons. In metals, the LUMO, HOMO and the Fermi level (Ef) overlap, thus electrons nearest to the Fermi level cross to the conduction band. In semi-conductors, there is an energy gap between HOMO and LUMO with the Fermi level situated in between (at 0 K, Ef = E gap/2). The electrons in HOMO must be energized to the Ef for its translocation from HOMO to LUMO. In insulators, electrons can’t absorb sufficient energy to cross from HOMO to LUMO.

Optical properties: Nanoparticles, along with ex-
Particle-specific physicochemical properties

**Dendrimers:** Dendrimers are hyper-branched synthetic polymers that can be engineered into well-defined structures for various biological and pharmacological functions (Figure 5). Dendrimers contain a Core group (C), branching Generations (G1 to G4) and end-groups that can be functionalized with functional groups such as an Antibody (Ab) or another dendrimer containing a fluorescing group. During synthesis, as dendrimers grow in size, different generations begin to show distinct features that are amplified with increasing generations [61,62]. Some of the unique properties of dendrimers are described below.

**Intrinsic viscosity (η):** Intrinsic viscosity characterizes the frictional contribution of polymers in dilute solutions [63]. Dendrimers exhibit a size-dependent increase in η values: the values increase as the dendrimer size increased from G0 to G4, then, further increase in the dendrimer size decreased the η values [64,65]. Unlike the η values, the hydrodynamic radius of the dendrimers increased linearly as their size increased [65].

**The dendrite-box concept:** The branched structure of dendrimers contains empty and defined-sized spaces surrounded by either hydrophilic or hydrophobic environment. These spaces can interact with and store guest particles. The hydrophilic particles accumulate in the sites surrounded by a hydrophilic surface, while hydrophilic particles accumulate in sites surrounded by the hydrophilic environment. Once entrapped, the particles are protected from the external environment. The entrapment-space is known as the dendrimer box [66].

**Biomimicry:** One of the outstanding properties of dendrimers is their ability to mimic biological particles, espe-
cally globular proteins such as insulin (3G dendrimer), cytochrome (4G dendrimer), and hemoglobin (5G dendrimer) [67-70]. Dendrimers also mimic histone clusters, thus they make stable complexes with the DNA and enhance gene expression [71,72]. Like proteins, dendrimers may respond to many external stimuli and adapt a tight-packed (resembling native proteins) or extended (resembling denatured proteins) conformation [73-75].

**Host-guest complex formation:** The unique dendritic topology allows their application in controlled delivery agents, DNA transporters and transfection agents [62,76-78].

**Properties of carbon nanotubes - metals or semiconductors:** CNTs are formed by folding of a hexagonal graphene sheet consisting of unit cells [33]. Figure 6A-i shows three different patterns of graphene sheet folding, resulting in formation of three electronically different nanotubes. When \( n = m \) (like 5,5 or 6,6), the nanotubes belong to the armchair family (Figure 6A-ia), whereas when \( n = 0 \) or \( m = 0 \), they are called zigzag tubes (Figure 6A-ib). All other combinations of \( n \) and \( m \) are called chiral nanotubes (Figure 6A-ic).

The chiral angle ranges from 0° (zigzag) to 30° (armchair). The armchair CNTs exhibit metallic properties, while the zigzag and chiral CNTs can be semiconductor (containing a band gap between HOMO and LUMO) if \( n - m \) is a multiple of 3, otherwise they are metallic [79-84]. Band gaps of 0.4 to \( > 1 \) eV have been reported for SWNTs (Figure 6B). The armchair CNTs exhibit metallic properties, while the zigzag and chiral CNTs can be semiconductor (containing a band gap between HOMO and LUMO) if \( n - m \) is a multiple of 3, otherwise they are metallic [79-84]. Band gaps of 0.4 to \( > 1 \) eV have been reported for SWNTs (Figure 6B).

**Magnetic ENPs:** Iron oxide nanoparticles, in addition to size-dependent surface characteristics, also exhibit size-dependent electron confinement and a transition from ferromagnetic (a high susceptibility to magnetization, the strength of which depends on that of the applied magnetizing field, and that may persist after removal of the applied) to super-paramagnetic (magnetization can randomly flip direction under the influence of temperature) field [85-88]. As shown in Figure 7, ferromagnetic bulk particles are multi-domain particles in which each domain’s local magnetization is saturated but not parallel to other domains’ local magnetization.
In the presence of a magnetic field, all domains exhibit parallel spin, resulting in development of magnetic field. As the particle size decreases to below 100 nm, transition from a Multi-Domain (MD) state to pseudo Single-Domain (mixture of multi-domain and single-domain properties, SSD), Single-Domain Para Magnetic (disordered atoms or electrons, SD-PM), Single-Domain Super Para Magnetic (spin reversal and loss of magnetic moment, SD-SPM), Single-Domain Sub-Super Para Magnetic (very high magnetic anisotropy showing freezing behavior, SD-SSPM) states may occur (Figure 7). The paramagnetic nanoparticles can be used in bio-imaging, while the super para magnetic nanoparticles can be used for separation processes in biochemistry.

**Surface Functionalization - A Critical Step for NP Functionality**

Bare (as synthesized) nanoparticles are nonfunctional because they agglomerate rapidly due to van der Waals force [89], Zeta (ζ) potential and/or the pH (close to the nanoparticles’ IEP for ‘zero charge’) of the dispersion liquid. A ζ values greater than +25 mV or less than -25 mV typically indicates a stable colloid because of charge repulsion between two ENPs. Surface functionalization with diverse ligands stabilizes ENPs and confer them specific functionality (Figure 8).

Studies have shown that mono- or poly-thiol ligands such as thiolated polyethylene glycol (x-PEG-SH where x is different reactive groups for attaching a functional group such as medicine, antibody, fluorescent groups, etc.) form stronger bond with gold and silver colloid’s surface, thus stabilizing the suspension [57,90-94]. Non-colloid nanoparticles can be chemically attached to Poly-L-Lysine (PLL), Poly-L-Glycone (PLG) or PEG for stabilization (Figure 7). The PEGylated ENPs, for performing specific functions, require specific end groups (-NH₂, -COOH, -OH, -N₃ and/or -SH) that interact, via either covalent or non-covalently binding, with drugs, imaging dyes, antibodies of specific proteins (Figure 9).

For achieving intracellular drug-release, acid- or enzyme-cleavable linkers that remain stable at physiological pH, but disintegrate at (i) pH less than 6.0 or (ii) in the presence of substrates, can be used [95-97]. For cancer treatment, the cathepsin B substrates such as Gly-Phe-Leu-Gly (GFLG) are used as the linker. Since cathepsin B is highly over expressed in cancer cells, the cathepsin B substrate-linkers are disintegrated, resulting in on-site release of drugs and/or
Nanoparticles Applications

NPs play a central role in recent technological advancements in the areas of disease diagnosis, drug design and drug delivery [105,106]. Multi-model magnetic nanoparticles contrast agents such as Super Paramagnetic Iron Oxide Nanoparticles (SPION) are anticipated to lead the way to advancements in understanding biological processes at the molecular level [107]. NPs improve efficiency of drug delivery by enhancing their bioavailability and reducing side-effects of a drug, and play an important role in development of bioassays, biosensors and biomedical devices, and bio fuel cells. Nano-chips may be a new paradigm for total chemical analysis systems [108,109]. Nanorobotics and nano-manipulation technologies will allow moving and manipulating nanoscale materials and nanoscale robotics [110]. Table 2 lists current applications of NPs in biology and medicine.

Application of nanoparticles in alcoholism treatment

Recently, nanoparticles-based therapeutic agents have acquired prominence in diagnosis and treatment of diseases including drug addiction and alcoholism, although they are not yet approved for clinical use. A review of literature provided substantial evidence for...
Table 2: Lists current applications of NPs in biology and medicine.

<table>
<thead>
<tr>
<th>NPs</th>
<th>Applications</th>
<th>Safety/Adverse effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold colloid</td>
<td>Medical diagnostics</td>
<td>LD: Relatively safe</td>
<td>[180-182]</td>
</tr>
<tr>
<td></td>
<td>Drug/gene delivery</td>
<td>HD: Cytotoxicity, kidney and liver damage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pharmaceuticals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silver colloid</td>
<td>Similar to Gold</td>
<td>LD: Oxidative stress</td>
<td>[183-185]</td>
</tr>
<tr>
<td></td>
<td>Antimicrobial agents</td>
<td>HD: Neurotoxicity and liver damage, antibacterial</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clothing odor resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TiO₂</td>
<td>Antimicrobial paint, tooth paste and cosmetics</td>
<td>Autistic disorders, epilepsy</td>
<td>[186-188]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alzheimer’s like plaques</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>Environmental remediation</td>
<td>Oxidative stress, inflammation liver toxicity</td>
<td>[189,190]</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>Magnetic nanoparticle - contrast agents for tumor imaging</td>
<td>Oxidative stress, membrane leakage of LDH, DNA damage, inflammation</td>
<td>[37,191]</td>
</tr>
<tr>
<td>CNTs</td>
<td>Diagnostics</td>
<td>Mitochondrial toxicity, cell cycle arrest, liver and lungs damage, frustrated phagocytosis</td>
<td>[192-195]</td>
</tr>
<tr>
<td></td>
<td>Consumer electronics</td>
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<td></td>
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<td></td>
<td>Sports equipment</td>
<td></td>
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<tr>
<td>Fullerenes</td>
<td>Drug delivery</td>
<td>Oxidative stress, inflammation liver damage</td>
<td>[196,197]</td>
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<tr>
<td>Linear polymers</td>
<td>Drug delivery</td>
<td>LD: Relatively safe</td>
<td>[198,199]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HD: CNS and liver damage</td>
<td></td>
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<tr>
<td>Dendrimers</td>
<td>Drug delivery</td>
<td>Relatively safe. Some dendrimers may mimic body proteins and enzymes</td>
<td>[200-202]</td>
</tr>
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</table>

Figure 10: Diagram of a simple AOx-AuNP/PANI biosensor for sensitive detection of alcohol. The biosensor was synthesized by incubating AuCl₄ and AOx under alkaline condition (pH 8.5). The AuNPs-AOx conjugate was encapsulated with Polyaniline (PANI) that was stabilized on a Glassy Carbon Electrode (GCE) by chitosan-Nafion mixture. The mechanism of ethanol oxidation is shown.

Abbreviations: AOx: Alcohol Oxidase; AuNP: Gold Nanoparticles; GCE: Glassy Carbon Electrode; PANI: Polyaniline. (Adapted from Chinnadayyala, et al. [111]).
possible application of nanoparticles in diagnosis and treatment of addiction. Results from selected studies are presented below.

Alcohol Oxidase (AOx) functionalized Gold Nanoparticles (AuNPs) for development of alcohol biosensor: Chinnadayyala, et al. [111] have designed a simple AuNP-based biosensor for sensitive detection of alcohol. They synthesized AuNP using AOx protein in alkaline (pH 8.5) condition with simultaneous stabilization of the nanoparticles on the AOx protein surface. The AuNPs-AOx conjugate was encapsulated with Polyaniline (PANI) that was stabilized on a Glassy Carbon Electrode (GCE) by chitosan-nafion mixture (Figure 10). The biosensor was then utilized for detection of alcohol amperometrically using H₂O₂ as redox indicator at +0.6 V. The fabricated bio-electrode was successfully used for the selective determination of alcohol in beverage samples (Figure 10).

Gómez-Anquela, et al. [112] used AuNPs capped with thiolic acid to coordinate with the Zn (II) present in the catalytic center of Alcohol Dehydrogenase (ADH). The complex, in combination with Azure A (the NADH oxidation molecular catalyst) was electro grafted onto carbon screen-printed electrodes (Figure 11) that were efficient ethanol biosensor. The final bio-sensing device was highly efficient in ethanol oxidation with low over potential of -0.25 V with a detection limit of 0.14 ± 0.01 μM and a stable response for more than one month. Luo, et al. [113] constructed a disposable blood alcohol biosensor prepared by immobilizing Alcohol Dehydrogenase (ADH) and Nicotinamide Adenine Dinucleotide (NADq) coated by Nafion combined with AuNPs onto the surface of Meldola's blue modified screen-printed electrodes. The sensor was capable of detecting blood alcohol concentration in laboratory medicine and forensic medicine samples.

Anti-oxidative enzyme nano-complex enhance alcohol degradation: Luo, et al. [113] constructed a robust enzyme nano-complex (Figure 12) by assembling or conjugating selected enzymes (anti-oxidative enzymes) with synergic or complementary functions in a nano-complex, followed by its encapsulation within a cross-linked polymer nanocapsule.

Exemplified by the synthesis of a triple-enzyme nano-complex. Inhibitors for each enzyme were respectively conjugated to a single-stranded DNA with a designed sequence. Complementary assembly of the DNA molecules forms a DNA-inhibitor scaffold of a triple-enzyme nanocomplex. Subsequent in situ polymerization resulted in a thin layer of polymer network around each nano-complex, and the formation of nanocapsules containing a triple-enzyme core and a permeable shell (step II). Then, removal of the DNA-inhibitor scaffolds creates a highly robust enzyme nanocomplex (step III). The nano-
Enhancing the drug's bioavailability: Vyas, et al. [116] and Emerich, et al. [117] have shown that Nano-Functionalized Therapeutic Drugs (NFTDs) exhibited greater bioavailability than corresponding free therapeutic drugs possibly by reducing their biodegradation and/or increasing their intracellular accumulation. Yin, et al. [118] synthesized a hydrolysable cross-linked poly(ethylene glycol-graft-methyl methacrylate) di-block nano-polymer carriers for naltrexone that improved its bioavailability and site-directed release, resulting in reduced side effects. Banks, et al. [119] synthesized a nano-6-ß-naltrexol, the major active metabolite of naltrexone, and administered it via a transdermal patch for treatment of alcoholism. They designed a Micro Needle (MN) skin permeation enhancement system that provid-
and nucleus accumbens suppressed cocaine self-administration in rats. Furthermore, a cocaine dose response demonstrated that decreased lever response in rats that received GDNF-conjugated nanoparticles persisted after substitution with different cocaine doses. Carnicella, et al. [122] either an activation of the GDNF pathway or direct administration of a nano-GDNF formulation in the Ventral Tegmental Area (VTA) reduces moderate alcohol intake in a rat operant self-administration paradigm (receiving a 20% ethanol solution in an intermittent-access two-bottle choice drinking paradigm). They showed that microinjection of GDNF into the VTA 10 min before the beginning of an ethanol-drinking session.

Mouse group-c and group-d: Alcohol diet first followed by the enzyme preparation via tail vein (c: BAC and d: ALT levels in mice gavage with an alcohol diet followed by blank (no alcohol, not shown in ‘a’), PBS alone, native AOX, n(Cat), n(AOx), n(AOx)+n(Cat), n(AOx-Cat)). Data are presented as mean ± standard error of the mean (s.e.m.) and the significance levels are \( P < 0.05, \ P < 0.01 \) and \( P < 0.001 \). The BAC of the mice administered with n(AOx-Cat) was reduced by 10.1%, 31.8% and 36.8%, respectively, at 45 min, 90 min and 3 h of feeding (Figure 12a). Significantly smaller BAC reductions were observed in those with n(AOx) (< 8.5%) or with a mixture of n(AOx) and n(Cat) (< 10.6%). Insignificant BAC reductions were observed for those with n(Cat) or native AOX. All alcohol-fed animals showed an increased plasma Alanine Amino Transferase (ALT) level; nevertheless, the mice treated with n(AOx-Cat) had a tendency for lower ALT levels (Figure 4b). In c and d mice also, n(AOx-Cat) is more effective than the mixture of n(AOx) and n(Cat) for BAC and ALT reduction. They show that nanocomplexes containing alcohol oxidase and catalase could reduce blood alcohol levels in intoxicated mice, offering an alternative antidote and prophylactic for alcohol intoxication. (Adapted from Liu, et al. 2013 [209]).

Figure 13: Efficacy of n(AOx-Cat) as a prophylactic and antidote for alcohol intoxication.

* Engineeried nanoparticles facilitate targeted delivery of therapeutic agents: Sharma, et al. [120] have shown that microinjection of Glial-Derived Neurotrophic Factor (GDNF)-conjugated nanoparticles into the rat striatum and nucleus accumbens is able to block self-administration of cocaine in rats. Green-Sadan, et al. [121] showed that transplantation of a GDNF-expressing astrocyte cell line into the striatum and nucleus accumbens attenuated cocaine-seeking behavior in Sprague-Dawley rats. Then, they developed a nano-GDNF system as a safe and effective method for introducing GDNF into the brain. An administration of GDNF-conjugated nanoparticles microinjected into the striatum and nucleus accumbens suppressed cocaine self-administration in rats. Furthermore, a cocaine dose response demonstrated that decreased lever response in rats that received GDNF-conjugated nanoparticles persisted after substitution with different cocaine doses. Carnicella, et al. [122] either an activation of the GDNF pathway or direct administration of a nano-GDNF formulation in the Ventral Tegmental Area (VTA) reduces moderate alcohol intake in a rat operant self-administration paradigm (receiving a 20% ethanol solution in an intermittent-access two-bottle choice drinking paradigm). They showed that microinjection of GDNF into the VTA 10 min before the beginning of an ethanol-drinking session.

i. Significantly reduced ethanol intake and preference, but did not affect total fluid intake and
and especially on serotonergic systems that improve adaptation capacity of organism to the action of various deleterious factors [125]. Taking into consideration the wide range of biological activity of hydrated fullerene both at molecular and physiological levels, absence of any toxicity, and effectiveness even in super-small doses, aqueous solutions of C$_{60}$HyFn can be proposed as reliever of CNS dysfunctions induced by alcohol consumption and continual alcoholization.

**Gold nanoparticles synthesized by plant-extract attenuate alcohol drinking and withdrawal symptoms in alcohol preferring rats:** AuNPs are commonly synthesized using the traditional reduction of chloroauric acid (H[AuCl$_4$]) by reducing agent NaBH$_4$, N$_2$H$_4$, NH$_2$OH or (CH$_3$)$_2$NH·BH$_3$ [126,127] that required dispersants (such as polyethylene glycol) to prevent aggregation and complex surface functionalization for desired biological activity. Recent studies have proposed an alternative reduction method using plant extracts and gum for synthesis of AuNPs [128-134]. This process is also known as ‘green chemistry for synthesis of nanoparticles’ in which reactions occur at ambient temperatures (high temperatures may yield uniform size particles), neutral pH, low costs and environmentally friendly fashion [131]. AuNPs synthesized using plant extracts demonstrated procedure-dependent variations in size (Figure 14), low protein adsorption (low corona formation), poor aggregation into larger particles, and higher sta-

![Figure 14](image-url)
bility at physiological condition compared to chemical reduction and citrate capped nanoparticles [128]. AuNP size can be controlled by controlling temperature and/or the incubation composition.

A recent study from our laboratory (unpublished data) compared the effects of four AuNP preparations (KG@AuNP synthesized by kudzu extract and plant gum (7 to 10 mn, Figure 10), K@AuNP synthesized by kudzu extract alone, K + G + AuNP PEG that is a mixture composed of traditionally synthesized AuNP stabilized by PEG, kudzu extract and gum solution and AuNP PEG alone) on alcohol preference [(alcohol intake/total fluid intake) × 100] and the severity of the withdrawal symptoms in alcohol preferring rats. As shown in Figure 15, KG@AuNP administered rats exhibited smallest alcohol preference than K@AuNP, K + G + AuNP PEG or AuNP PEG administered rats allowed to self-administer alcohol as described previously [135]. In addition, the severity of the withdrawal symptoms showed the following patterns: KG@AuNP rats < K@AuNP rats < K + G + AuNP PEG < AuNP PEG rats. These preliminary observations suggest that KG@AuNP (AuNP synthesized by kudzu extract and plant gum solution) protected against, while the traditionally synthesized AuNP PEG augmented the adverse effects of ethanol drinking in alcohol preferring rats. This suggests that the plant-extract synthesized AuNPs may have therapeutic potential against development of addiction in humans (Figure 15).

Smart, multifunctional nanoparticles: As discussed above, alcoholism is complex, progressive, multifaceted disorder that cannot be efficiently treated with current therapeutic approaches. Although simple functionalized ENPs, described above, provide a unique approach in addiction treatment, they lack capacity to release cargo (TDs, siRNAs and/or plasmids inserted with specific cDNAs) in spatial-, temporal- and dosage-controlled fashions, on demand. Recent progress in nanotechnology and material chemistry has allowed construction of

Figure 15: Effects of plant-extract synthesized AuNPs on alcohol abnormalities in Alcohol-Preferring (AP) rats. The AP rats (males, 100 g to 150 g) were allowed to self-administer 8% ethanol solution using (one bottle contained ethanol and the other pure water) a procedure described earlier [135]. Rats were divided into 5 groups and gavage PBS (group-1), AuNP PEG (synthesized as described by Singh, et al. (2013) [135]) solution -20 mg/kg (group-2), a mixture consisting of AuNP PEG, kudzu extract - Puerarin (PU) adjusted to 150 mg/kg and gum solution - adjusted to 10 mg/kg (group-3), K@AuNP synthesized using kudzu extract alone -20 mg/kg (group-4) and KG@AuNP synthesized using kudzu extract and gum -20 mg/kg (group-5). Water and alcohol intake was monitored daily when the samples were replaced. Alcohol was withdrawal at day-120 and resumed again at day-126. Rats were monitored for the development of the withdrawal symptoms as described by Benlhabib, et al. [211,212] and listed in this figure. This study showed that the PBS fed rats exhibited approximately 14% alcohol preference that did not differ significantly from the rats receiving AuNP PEG or AuNP PEG + kudzu + gum. Alcohol preference decreased slightly in rats receiving K@AuNP and decreased significantly in rats receiving KG@AuNP. Rats receiving PBS, AuNP PEG+ and AuNP PEG + kudzu + gum exhibited comparable severity of the withdrawal symptoms, except seizures. The severity were considerably lower in the rats receiving KG@AuNP. This suggests that the plant-synthesized AuNPs retained the medicinal properties of the extract.
Figure 16: Composition of ‘smart’ multifunctional nanoparticles for therapeutic, biomedical delivery and imaging applications. (A) Core: nature of the (core hydrophobic, hydrophilic, polymeric, metal, magnetic or QD) dictates the overall function of the NPs and type of the drug to be encapsulated. Magnetic NPs and QD core may facilitate imaging, while polymeric dendrimers may facilitate development of multifunctional NPs; (B) Functionalization: The surface is functionalized with a variety of ligands to enhance stability, prevent aggregation and corona formation, or attach functional groups; (C) Clusters of targeting moieties allow multivalent binding to receptors for enhanced cellular uptake. The use of various ligands (antibody, antibody fragment, peptides) allow specific binding of the ligands to a receptor, ion channel or enzymes; (D) Cargo: Nanoparticles can be loaded with a wide range of therapeutics ranging from small molecules to macromolecular cargos in various ways: covalently bound to a ligand such as PEG that interacts with the core, filled in a dendrimer’s hydrophilic or hydrophobic space, and cargo releasable via an internal or external triggers for on-demand release.

Figure 17: (i) Schematic representation of drug loading onto Magneto-Electric Nano-Carriers (ME-NCs) and on-demand-controlled drug release under the influence of external magnetic field. Surface functionalized super paramagnetic ME-NCs (A) binds with drug (or other ligands) via electrostatic interaction (B). On-demand drug release by ME-NP stimulated by a uniform alternate current magnetic field (C and D). (ii) Proposed schematic of ME-NCs-based ARV drugs delivery across the BBB (ARV: Anti-Retro Viral; BBB: Blood-Brain Barriers; ME-NC: Magneto-Electric Nano-Carriers). Nair, et al. [213] proposed that in an alternate current magnetic field, all bonds between drug and ME-NCs formulation breaks uniformly and efficiently, result in a rapid release of the cargo [214].
In general, there are three basic Toxicology Principles (TP) designed for bulk particles:

1. **The dose (mass-based) makes the poison** (Paracelsus theory). In general, dose is defined as the mass of a chemical per unit of body weight such as g/kg body weight. From Paracelsus’s time to the present, the mass-based dose has been used to determine a chemical’s beneficial effects and toxicity. The mass based dose-response relationship is the key determinant of a chemical’s toxicity and the risk it poses to humans and animals.

2. **The biological actions of a chemical are specific to the chemical’s structure**. In 16th century, Ambrose Paré recognized that each chemical may exhibit unique toxicity related to its structure.

3. **Humans are animals**. Therefore, protection against the toxicity of agents would be impossible without the ability to study the effect of toxins in laboratory animals.

Nanoparticles defy the 1st principle, that is the mass-based dose makes the poison [143-146], since dose in terms of size (diameter/kg), surface area/kg or particle number/kg is, if not more then, at least, as important as the mass-dose in correlating with toxicity [147]. This is because surface molecules, not the core molecules, determining the ENPs’ physicochemical, biological and adverse properties. As an example, for equal mass of 5 nm, 10 nm and 100 nm AuNPs, the particle enumeration, surface area, surface activity may increase with a decrease in their size (5 nm > 10 nm > 100 nm). Since the biomedical and toxicological properties of ENPs are dependent on their size and/or surface properties, their toxicity may increase with a decrease in size or an increase in particle enumeration (Figure 19).

The plots of mass-dose against toxicity, shown in Figure 20, revealed that different sized nanoparticles...
Figure 19: Size and surface-dependent toxicity of nanoparticles. At a fixed dose, toxicity of a nanoparticle is inversely related to the size and directly related to the particle number. Surface functionalization may decreases nanoparticle toxicity.

Figure 20: Nanoparticle dose (in terms of mass and surface area) and response curves for nanoparticle toxicity. Rats and mice were instilled with ultrafine (20 nm) and fine (250 nm) TiO$_2$ via their intra-tracheal instillation of (i) different mass doses (A, C) and (ii) different surface area dose (B, D). The Percentage of neutrophils in lung lavage of rats (A, B) and mice (C, D) at 24 hr after intratracheal instillation was used as indicators of inflammation. The 20 nm TiO$_2$ exhibited steeper dose response than 250 nm TiO$_2$ when the dose is expressed as mass (A, C). The two nanoparticles exhibited same dose response relationship with dose expressed as particle surface area (B, D). This indicates that particle surface area seems to be a more appropriate dose metric for comparing effects of different-sized particles having the same chemical structure (anatase TiO$_2$ in this case). Data show mean ± SD. (Adapted from Oberdörster, et al. [215]).
yielded different slopes and \( ED_{50} \) values. Thus, the mass-based dose response curve may not accurately describe the ENP toxicity, a departure from bulk particle toxicity that strictly follows the classic mass-based dose dependence. Many studies have shown that the dose as particle number, surface area (\( \text{nm}^2 \)), surface to volume ratio and/or surface reactivity may characterize the toxicity of nanoparticles [142,148-150]. Thus, diameter-response, surface area-response and/or enumeration-response relationships may be important in determination of the ENPs’ toxicity that can be used for risk assessment. The surface area is a better index than particle enumeration or mass in determining the inflammatory effects of nanoparticles (Figure 20) [143,151,152].

An important, but least studied, aspect of ENP toxicity is formation of protein corona. ENPs, when in contact with biological fluids, adsorb diverse type of biomolecules (e.g., proteins) called protein corona that dictates the ENPs interactions with the biological systems and ensuing biological fate, therapeutic efficiency and toxicity (Figure 21) [153]. Rapid corona formation is found to affect hemolysis, thrombocyte activation, nanoparticles uptake and endothelial cell death at an early exposure time [154]. In general, there are two types of corona: initially soft (reversible binding) followed by hard (irreversible binding and structure change) corona [155]. During soft corona phase, the physicochemical characteristics of corona may change spatially and temporally, resulting in highly heterogeneous population of ENP that may alter the particle’s properties and toxicity. The gold and silver

<table>
<thead>
<tr>
<th>Corona</th>
<th>Soft</th>
<th>Hard</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_d ) (dissociation constant)(^9,20,54)</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Adhesion to hydrophobicity(^35)</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Molecular weight(^25,32)</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Endosome–Lysosome trafficking(^13,21)</td>
<td>Low(^*)</td>
<td>Low(^*), High(^†)</td>
</tr>
<tr>
<td>Conformational changes (sheet)(^35,43)</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

**Figure 21:** Schematic illustration and characteristics of a hard and a soft protein corona encompassing the nanoparticles. Hard coronas exhibit high affinity and slow exchange (i.e., several hours) and lower abundance of proteins, whereas soft coronas exhibit low affinity but rapid exchange (i.e., several minutes) of proteins. There is a different response of cellular and biochemical factors by soft and hard corona formation.

\(^*\)Compared with serum-free condition; \(^†\)Compared with soft corona. (Adapted from Lee, et al. [161]).
nano-colloidal surface interact strongly with molecules containing monothiol or disulfide groups, partially due to the existence of several possible oxidation states of gold when it is bound to ligands (oxidation states from -I to +V are known). Interestingly, bulk gold is chemically inert and poorly reach to the thiol groups [156]. Gold or silver nanoparticles in biological samples rapidly interact with the thiol-containing proteins, fat or other chemicals, forming a soft corona that, in time, transform into hard corona [157]. Earlier studies have shown that sulpha- 
phidation decreases toxicity of silver nanoparticles [158-164]. Corona formation onto the silver nanoparticle surface (Figure 22) protects against inflammation (interleu-
klin-1beta, interleukin-6, interleukin-18, tumor necrosis factor alpha (TNFα), and macrophage inflammatory protein 2, Granulocyte-macrophage colony-stimulating factor) [154] (Figure 22).

Figure 22: Protein affinity of partially and completely sulphidated silver nanoparticles. Partially sulphidated and completely sulphidated AgNPs were pre-incubation in RPMI-1640 with 10% FBS. The TEM images (a-partially sulphidated, b-fully sulphidated) were captured and viability of J774 murine macrophages was measured (c) with MTT assays after 24h exposure to various concentrations (2, 5, 10, 15, 25, 50 and 100 μgml⁻¹) of Ag⁺ ions (black diamond’s), pristine Ag NPs (red triangles), partially sulphidated Ag NPs (blue squares) and completely sulphidated Ag NPs (orange circles). Error bars are provided as standard deviation; statistically significant differences as compared with the control are marked (**P < 0.005 or ***P < 0.0005, n = 6). (d) and (e) Release profiles of TNFα (d) and MIP-2 (e) after 24 h exposure of J774 macrophages to various concentrations (2, 5, 10, 15, 25, 50 and 100 μgml⁻¹) of pristine (red), partially sulphidated (blue) and completely sulphidated (orange) Ag NPs. This suggests that fully sulphidated nanoparticles inhibited corona formation, thus protected macrophages from nanoparticle toxicity. (Adapted from Miclaus, et al. [154]).
Mechanisms of nanoparticle toxicity

Humans and animals get exposed to toxins via dermal exposure, ingestion and/or inhalation. Toxin concentrations in the systemic blood depend upon (i) The route of exposure and transfer of the ingested toxin through the membrane barriers, (ii) Metabolism and excretion and (iii) Nonspecific distribution of toxins into different tissues. A very small fraction of the toxin may reach the target site. The toxin-target interaction initiates a series of mechanisms (Figure 23), resulting in an increase in oxidative stress, activation of pro-inflammatory pathways and inhibition of anti-inflammatory pathways, resulting in inflammation dysregulation and cytotoxicity such as necrosis [165-167] DNA damage [168-170] and membrane toxicity [171] (Figure 23).

Conclusions

Nanoparticles, because of their outstanding characteristics and properties, have potential for development of novel nanoparticle-based devices for individualized treatment of alcoholism. Nanoparticles exhibit (i) High surface area to volume ratio, (ii) Surface atoms influencing the particles properties and (iii) Size- and shape-dependent physicochemical properties, quantum confinement (semiconductors), surface Plasmon resonance (some metals) and super-paramagnetism (magnetic particles) [172,173]. Therefore, surface area and/or diameter may influence a nanoparticle’s beneficial effects and toxicity [174,175]. Because of the NPs’ unique surface and physicochemical properties, they may have therapeutic potential against alcoholism. Unfortunately, the very physicochemical properties that confer the NPs their therapeutic potency also increase their toxicity and adverse effects. Therefore, the therapeutic index of a NP preparation depends on its therapeutic potency/toxicity ratio.

References


Figure 23: The flow diagram showing mechanisms of action of nanoparticle toxicity.


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