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Detoxification of blood using injectable magnetic nanospheres: A conceptual technology description

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Abstract

We describe injectable magnetic nanospheres as a vehicle for selective detoxification of blood borne toxins. Surface receptors on the freely circulating nanospheres bind to toxins. A hand-held extracorporeal magnetic filter separates the toxin-loaded nanospheres from the clean blood, which is returned to the patient. Details of the technology concept are given and include a state-of-knowledge and research needs.

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1. Introduction

We are developing a novel, integrated system based on superparamagnetic, biocompatible nanospheres for selective and rapid detoxification of biological, chemical, or radioactive toxins from humans. After intravascular injection, the circulating nanospheres would bind to blood-borne toxins due to selective receptors attached to the nanosphere surface. After circulation, a suitable artery

or vein is accessed with a small, hand-held magnetic filter unit. The blood is purified of the toxin-loaded nanospheres within the unit and the clean blood is returned to the body. The concentrated toxins can now be disposed or submitted for assay or forensics.

The impetus for technology development comes from the recognized limitations of current methods for removing deleterious components from the blood either for patient treatment or diagnosis. The clinically more important detoxification methods include hemodialysis and hemofiltration, plasmapheresis, extracorporeal immunoabsorption, and direct injection of chelators and antibodies.

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Hemodialysis applies an osmotic gradient across a semi-permeable membrane to dialyze/filter hydrophilic substances out of the blood. The major limitations are long procedure duration, extracorporeal circulation of large blood volumes requiring large-bore arterial access, non-selective substance removal, and effectiveness limited to hydrophilic substances with lower molecular weight. Plasmapheresis utilizes extracorporeal, non-specific exchange of plasma (which is cell-free blood) with albumin or saline solutions. This method removes most of the blood fluid phase and therefore can only be used for a limited period of time and in specific clinical situations. Extracorporeal immunoadsorption is a variation of hemodialysis in which circulated blood is exposed to a larger exchange surface saturated with immune absorbent materials (e.g., antibodies). It is a more specific removal method but less effective, requires the circulation of large blood volumes, and is restricted to specific antibody-antigen interactions. Direct injection of chelators and antibodies neutralize some actions of circulating antigen (e.g., medication or bacterial toxin interactions). However, complete antigen binding can often not be achieved and also relatively high antibody dosing is required increasing the risk of allergic (anaphylactic) and systemic (kidney failure, etc.) side effects. Furthermore, the antibody-toxin complex is not removed from the blood and remaining toxin can dissociate leading to rebound intoxication.

2. Conceptual description of the system

The system utilizes polymer-based magnetic nanospheres that are injected directly into the blood stream of exposed humans. There are several polymers from which to choose but the literature provides more information on lactide or lactide–glycolide-based polymer spheres than any other type. The nanospheres must circulate for prolonged periods in the vasculature to attain equilibrium with the toxin concentration and extracorporeal magnetic separation. Therefore, the surface must be conjugated to polyethylene glycol, which dramatically reduces opsonization

(protein adsorption) and phagocytosis (cell engulfment) mainly in the Kupffer cells of the liver. Nanocrystals of magnetic iron oxides are encapsulated within the polymer. Receptors (e.g., antibodies, chelators) are terminally-attached to the polyethylene glycol or encapsulated within the polymer sphere, depending on the application. In total, the chemical components of the spheres confer non-toxicity and biocompatibility, avoid rapid bioclearance and are biostabilized temporarily.

We are considering poly(lactic acid) and poly(lactic-co-glycolic acid) polymers. They are both approved by the US Federal Drug Administration for injection and are commonly used in sutures and prosthetics as well as in controlled drug delivery applications. The procedure by Gref et al. [1] describes the synthesis of co-block polymers of poly(lactic acid)-poly(ethylene glycol) via ring-opening polymerization.

The nanospheres are produced by solvent evaporation. The polymer or co-block polymer is dissolved in chloroform or other suitable volatile organic solvent. To this is added the nanocrystalline magnetic material. This is added to a water solution containing surfactant such as poly(vinyl alcohol). This mixture is stirred vigorously to create small emulsion droplets and drive off the volatile solvent. The hardened spheres are washed and lyophilized. The resulting size of the nanospheres is controlled by adjusting the nanosphere synthesis parameters, such as mixing speed and surfactant type and concentration, which affects the stable emulsion droplet size during hardening.

To successfully develop this technology we note several requirements for sphere size, PEGylation, magnetization, and receptors.

2.1. Size

The nanospheres must not be physically removed from free circulation. That is, they must not occlude the capillaries or be filtered by organs. Red blood cells are 3–5 μm wide but can deform to traverse the smallest capillaries. This restricts the maximum size to 1–2 μm . Moreover, splenic filtering occurs with particles >200 nm so the spheres should be <200 nm. On the other hand,

fenestrations in the liver sinusoids can filter particles < 100 nm in diameter. Therefore, we note that nanospheres 100–200 nm are most appropriate for circulation. Since variance in sphere size affects magnetization, vascular survival, receptor site density per unit mass of nanospheres, and computational model accuracy, we seek nanosphere synthesis methods that can produce monodisperse populations of nanospheres. Inherent with solvent evaporation, the resulting nanospheres are polydisperse. However, nanosphere size is not the dominant factor in prolonged circulation.

2.2. PEGylation

Success in liposome and nanoparticle systems identifies the importance of hydrophilic polyethylene glycol (PEG)-derivatives in prolonging intravascular survival. Particles were coated with PEG chains, offering steric and charge stability (near neutral) and to prevent antibody formation and opsonization and phagocytosis. Recent investigations found that the blood circulation times of particles increase as the molecular weight of covalently-linked PEG increases. Five hours after systemic injection, only one-third of 20 kDa PEG-conjugated poly(lactic-co-glycolic acid) nanospheres (140 nm) had been captured by the liver in comparison to uncoated particles [1]. This supports earlier work in the area [2–5]. Most recently, Gref [6] summarized the current state of knowledge in PEG biochemistry and the reporting of branched PEGs that increased the circulation half-life in the animal model to 20 h [7]. Several theoretical models are also discussed in Ref. [6]. Additional research projects are advancing our understanding of how to stabilize nanospheres. Indeed, our increased understanding of pathogenic agents and their polysaccharides surfaces offer a new perspective in achieving long circulation.

2.3. Magnetization

Magnetic separators in biology are implemented in immuno-magnetic cell sorting [8] where receptor-tagged magnetic nanoparticles bind to specific

cells and are separated using an applied magnetic field. Our nanospheres must be made sufficiently magnetic to overcome blood drag forces in the magnetic filter. However, a magnetic separator suitable for our application of clearing magnetic nanospheres from blood flow has not been designed. One can implement technically straightforward engineering for the design of the first prototype biomedical separators. Our filtration system utilizes small permanent magnets attached to the body of a specialized closed-loop catheter system. The actual design of the device has several plausible options given pre-defined conditions. Common to all design options, the blood will be diverted from the body to an array of tiny (few hundred micrometer diameter) flow tubes. These tubes will be immersed in a magnetic field gradient causing the magnetic nanoparticles to deflect towards and collect at the tube wall. The precise geometry of the tubing system (size, material, coating, length, shape etc.) will be defined as research progresses to minimize interactions with blood coagulation (i.e., thrombosis) and blood cells (i.e., destruction). In addition, our analyses will identify different design strategies for different modes of operation (i.e., in-field vs. unit based) and user level of training (i.e., self- vs. helper-applied).

The magnetic scalar force F is proportional to the magnetization of the nanosphere and the magnetic field gradient B . This can be expressed as

$$F \sim r^3 \rho M_{\text{sat}} f_{\text{magnetic}} \nabla B, \quad (1)$$

where r is the radius, ρ is the density of the nanospheres, M_{sat} is the magnetization assumed at saturation, and f_{magnetic} is the fraction of magnetic material in the nanosphere. We maximize the magnetic field gradient by our choice of magnet design and tube configuration. The typical magnetic field profile for simple magnet designs (Fig. 1) shows that the highest field gradients occur at the fringes of the magnets, the so-called fringe effect. Therefore, one can envision designs where the tube passes several times around the edge of the magnet or through small holes in the magnet face. Computational models aid in screening potential magnet filter designs and will be key to a successful prototype filter. In addition, by

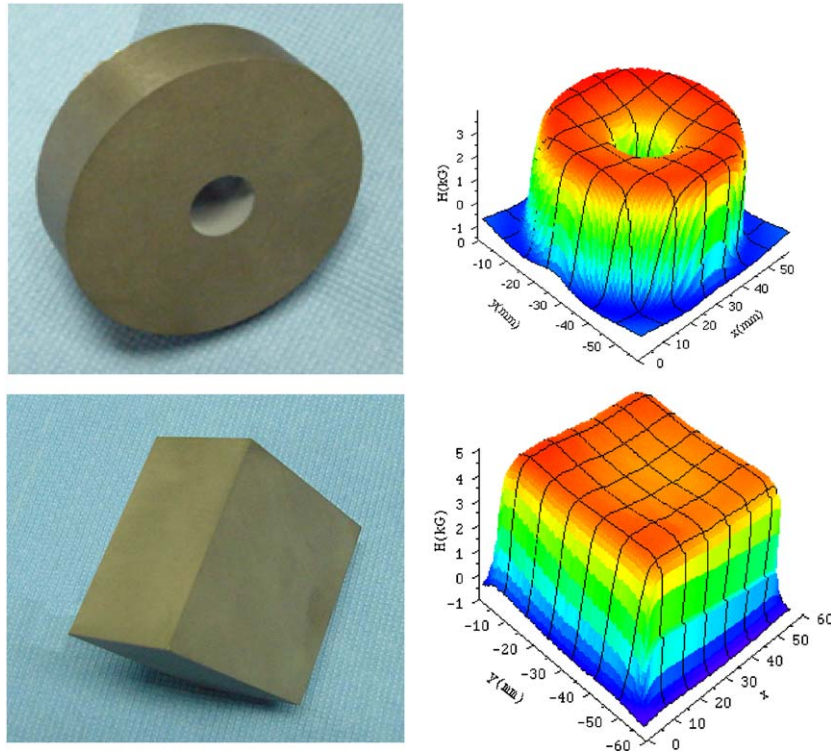


Fig. 1. Magnetic field strength (ordinate axis in kG) for donut and square NdFeB magnets. Note the dramatic reduction in field strength near the edges of the magnet surface.

imbedding ferromagnetic wires in the tube walls, large, local field gradients can be created, greatly improving separation efficiency. This approach is not unlike the approach described by Rosengart and Kaminski [9] and Chen et al. [10] where magnetic wires are used to increase trapping of magnetic nanospheres in circulation.

Magnetic separation using small, permanent magnets has been demonstrated by our group [11] (Fig. 2). Supplemental experiments and computational modeling show that even at high-flow velocities of 42 cm/s, 50% of magnetic cellulose particles (7 μm mean diameter, specific magnetization = 20 emu/g) are trapped in a 2×2 mm flow cell. From our laboratory tests, 400 nm spheres with a specific magnetization of 50 emu/g ($\sim 57\%$ $\gamma\text{-Fe}_2\text{O}_3$) can be easily separated in moderately flowing fluids. We envision our hand-held separator to reduce blood flow velocities to < 40 cm/s to improve efficiency. Based on

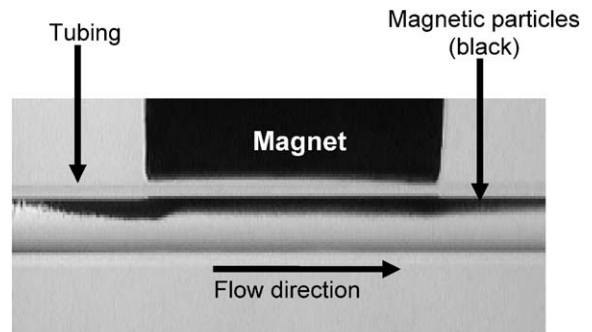


Fig. 2. Magnetic capture of magnetic microparticles in a tube under laminar flow (NdFeB magnet, 0.4 T at surface, saline fluid velocity = 40 cm/s).

this data and other experiments (assuming 400 nm particles, specific magnetization = 50 emu/g), we would require a tube > 20 cm in length to separate the particles from the flow. However, for flow rates

of 10 cm/s, easily achieved with microflow tubing design, only approximately 5 cm of tubing is necessary. In our design, multiple flow tubes will be used to compensate for the reduction in volumetric blood flow rates per tube and lower the pressure drop across the device. Moreover, smaller diameter tubes will facilitate separation and greatly reduce the length of the tube necessary for effective separation.

Looking at Eq. (1), we also have the properties of the nanospheres to adjust to increase magnetic filtration efficiency. We can increase size of the nanospheres (changes r), the fraction of magnetic nanomaterial embedded in the nanospheres (f_{magnetic}) and the type of magnetic material (M_{sat}). In our experiment described above, the 400 nm particles could be separated. Reduction to 100 nm spheres reduces the specific magnetization by a factor of 64. Indeed, this may pose substantial engineering difficulties in designing an efficient filter while maintaining high volumetric flow rates. In most applications reported in the literature, iron oxides are the magnetic material of choice. They can be made nanocrystalline, dispersed (i.e., not agglomerated), superparamagnetic, and are commercially available or easily synthesized in the laboratory. Importantly, the US Federal Drug Administration has approved magnetite for in vivo use. Typical encapsulation levels are 10% Fe_3O_4 or $\gamma\text{-Fe}_2\text{O}_3$ and the upper limit to f_{magnetic} is not known. We do not expect, however, to obtain f_{magnetic} above 50–70%, due to phase separation during nanospheres synthesis or penetration of magnetic material through the polymer surface. We can change from Fe_3O_4 or $\gamma\text{-Fe}_2\text{O}_3$ to more magnetic phases such as pure metals. Iron saturates at 221 emu/g compared to 90 emu/g for Fe_3O_4 and 87 emu/g for $\gamma\text{-Fe}_2\text{O}_3$. Thus, all things equal we would gain a factor of 2.5 in force by use of pure iron nanoparticles over iron oxides. Cobalt metal is another highly magnetic material that has been formed into stable nanoparticles with magnetization of 90–110 emu/g [12,13]. Unfortunately, both these materials are not stable toward oxidation and efforts are ongoing to produce oxidatively stable pure metal nanodispersions [14].

2.4. Receptors

As this technology is a platform detoxification system, we would not be developing new types of anti-toxins. Instead, we borrow completely from existing technology and current R&D programs. For example, we do not expect the affinity coefficients for antibody-antigen to differ from those published in the biological literature. Similarly, the stability coefficients for chelator-radioisotope binding will not change.

For biological agents, the authors recognize that for truly efficient antibody-antigen binding in vivo improved antibodies are needed for treatment purposes. This, in fact, is the subject of ongoing research in programs such as the phage display technology being developed at Argonne National Laboratory [15], which uses robotics to genetically alter and assay great numbers of potential antibodies to improve binding coefficients and selectivity.

The antibodies or antibody fragments can be attached directly. We synthesize PLA-PEG with biotin end groups [16] following the procedure of Salem et al. [17]. After incubating the nanospheres in streptavidin solution, one has a direct manner of attaching a biotinylated antibody or antibody fragment to the nanospheres. It is possible that biotinylated antibody can be attached during the co-block polymer synthesis step but this procedure may denature the antibody.

With regard to blood-borne radionuclides, the chelators calcium diethylenetriaminepentaacetate (DTPA) and Prussian Blue ($\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$) are used in current decorporation practice. DTPA was developed in an effort that started shortly after World War II to bind radionuclides such as plutonium after accidental exposures. The DTPA–Pu complex is excreted from the body resulting in a reduction in the body stores. The reason that there are not more choices in decorporation therapy is due to the strict biological requirements. In addition to selectivity over benign elements such as calcium, the chelator must be acutely non-toxic and display a suitable biological half-life toward excretion. These requirements eliminated a number of chelators that displayed higher selectivity than DTPA and

Prussian Blue. We propose the use of our technology platform for decorporation and the need to revisit the chelator programs of the past. The nanospheres would act as the vehicle for circulation and physical magnetic filtration from the blood thereby modifying the biological half-life restriction. By incorporating the chelators within the protective PEG corona of the nanospheres, one may alleviate the acute toxicity restriction. We are now pursuing a generic attachment method for radionuclide chelators.

3. Summary

We are developing a detoxification system based on injectable magnetic nanospheres conjugated to selective receptors. We support our hypothesis with theoretical arguments and evidence from previous data and provide a conceptual summary of the technology. Our preliminary data is promising but key developments such as higher selectivity antibodies, new radionuclide chelators, better magnetic nanospheres, reproducible nanospheres synthesis techniques, and in vivo data are needed to realize this novel detoxification approach.

Acknowledgments

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