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Biomedical applications of high gradient magnetic separation: progress towards therapeutic haemofiltration

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Abstract: High gradient magnetic separation is a well-established technology in the mineral processing industry, and has been used for decades in the bioprocessing industry. Less well known is the increasing role that high gradient magnetic separation is playing in biomedical applications, for both diagnostic and therapeutic purposes. We review here the state of the art in this emerging field, with a focus on therapeutic haemofiltration, the key enabling technologies relating to the functionalisation of magnetic nanoparticles with target-specific binding agents, and the development of extra-corporeal circuits to enable the *in situ* filtering of human blood.

Keywords: extra-corporeal loops; functionalised carriers; magnetic separation.

Introduction

High gradient magnetic separation (HGMS) is a long-established procedure in the mineral processing industry, where it is primarily used to separate out magnetic minerals and ores from mixtures containing substantial quantities of non-magnetic materials [56, 77, 79]. In a typical system, a suspension of particles in a liquid is passed through a filter comprising a magnetisable stainless steel wire mesh, to which is applied a magnetic field of sufficient strength $\boldsymbol{H}$ to saturate the magnetisation in the wires (see Figure 1). Near the wires, regions of high field gradient are established, resulting in a magnetic retaining force $\boldsymbol{F}$ that operates on any passing magnetisable particle. Assuming that the particles behave as point-like magnetic dipoles, this force is given by $\boldsymbol{F}=(\boldsymbol{m}\cdot\nabla)\boldsymbol{B}$, where $\boldsymbol{B}=\mu_0(\boldsymbol{H}+\boldsymbol{M})$ is the magnetic flux density in the particle, μ_0 is the permeability of free space, and $\boldsymbol{M}=\boldsymbol{m}/V=\Delta\chi\boldsymbol{H}$ is the magnetisation of the particle, where $\Delta\chi$ is the difference in magnetic susceptibility between the particle and the surrounding medium (which in most cases is water) [80].

HGMS has a similarly long history of application in the bioprocessing industry, particularly in the field of protein purification [28, 31, 47, 70]. Here the approach is different, in that biochemically functionalised magnetic particles are added to the fermentation broth (or another such crude bioprocess fluid) to act as an adsorbent species that can then be conveniently separated out by passing the broth through an HGMS filter. This is known to be a highly efficient way to achieve the same sort of purification that would normally require processing steps such as centrifugation, filtration and membrane separation [31].

Medical and biomedical applications based on the use of functionalised magnetic target agents coupled with HGMS have also been explored for many years [57, 88]. Indeed, a large number of different agents can be magnetically tagged using magnetic beads or nanoparticles [115, 80, 81], making HGMS an attractive and feasible method of both separation and concentration of a wide variety of target entities. Most of these applications are bench-top *ex vivo* applications, dealing with aliquots of extracted blood or other bodily fluids, or indeed, fluids formed from other tissue samples [36, 78]. There are relatively few therapeutic applications to date, i.e. ones that operate via *magnetic haemofiltration*, in which real-time separation and retention of targeted entities is performed directly on a subject's blood, as accessed via an extra-corporeal loop (see Figure 2). This is a field of biomedical research that has seen major advances over the last decade – albeit as yet mostly in the pre-clinical rather than the clinical phase – and it is this field that is the subject of this topical review.

The key enabling step in magnetic haemofiltration is the functionalisation of magnetic carriers, e.g. by conjugating antibodies to their surface, to activate their binding to specific moieties, which can then be magnetically actuated. These carriers are then mixed with the blood, either through prior injection into the patient, or

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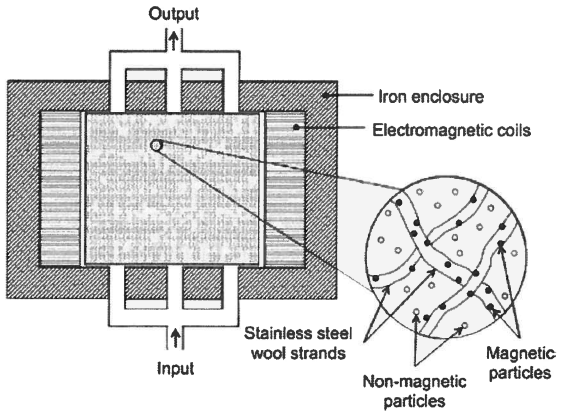

Figure 1: Schematic of a high-gradient magnetic filter, designed to extract magnetisable particles from a fluid or slurry input. Figure adapted from Pankhurst and Pollard [79].

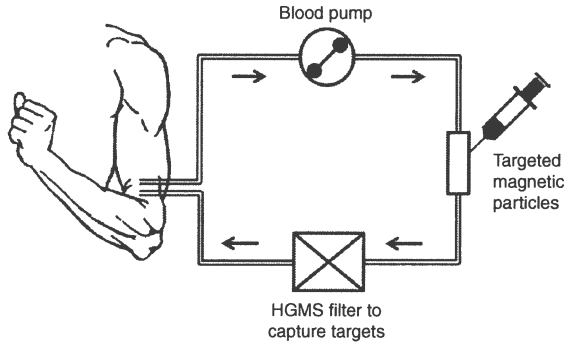

Figure 2: Simplified schematic of magnetic haemofiltration.

within the extra-corporeal loop. (The latter procedure is illustrated in Figure 2.) In principle, the functionalised coatings of the magnetic carriers then bind specifically to the target moieties, while remaining inert to all other moieties in the blood. The target might be an "undesirable" agent, such as a toxin, or a "desirable" agent, such as a stem cell that can then be used in research or a treatment.

The magnetic carriers, which are usually magnetic iron oxides such as maghemite, γ-Fe_2O_3, need to be biocompatible, able survive in the bloodstream, exhibit high magnetic susceptibilities, and bind to their targets with high efficiency and specificity. The specificity is particularly important to avoid false positive capture, while the biocompatibility is paramount: they must not cause any harm to the patient.

Combined with magnetic labelling techniques, magnetic haemofiltration could therefore be used to remove from the bloodstream any agent that can be labelled. This gives rise to a large number of potential applications. Many research groups are already working on magnetic nanoparticles (MNPs) or beads that target specific blood borne agents in order to deliver clinical benefits; some have designed their own magnetic separators. Others are working on magnetic particles for diagnostic or bench-top purification purposes (a well-established field), sometimes without considering their clinical applications.

We present here an overview of the state of the art of magnetic particle research as it might be applied to magnetic haemofiltration, outlining some of the many possible future clinical applications of the device. We break this down into four main sections which constitute potential applications of haemofiltration: (1) Sepsis: magnetic haemofiltration as a treatment for sepsis, through the removal of bacteria, fungi and other sepsis-causing pathogens; (2) Cells: the separation of undesirable cells from the bloodstream for treatment or diagnosis of a disease, or the separation of desirable cells for re-use in treatment or research; (3) Detoxification: the detoxification of blood, for the treatment of kidney disease and renal failure, drug overdoses or radiation exposure; and (4) Viruses: the diagnosis, treatment or control of viral infections through direct magnetic extraction of circulating virions.

Sepsis

Sepsis is caused by the body's immune response to pathogens in the bloodstream [12]. It is one of the most common deadly diseases and one of the leading causes of death in the developed world, exerting a huge human and economic toll. The mortality rate is 36%. In the UK, there are over 100,000 cases and 37,000 deaths annually; in the US, there are over a million cases and approximately 250,000 deaths. With an annual cost of $20.3 billion in 2011 (up from $4.4 billion in 1997), it is the most expensive condition in the US, making up 5.2% of total healthcare spending. Direct costs to the NHS in the UK are over £2.5 billion. Sepsis incidence has been increasing rapidly, by about 10% in the decade to 2013. Incidence is 50% greater than myocardial infarction (heart attacks) and 33% greater than stroke. Severity and mortality is strongly linked to the number of pathogens in the bloodstream [4, 39, 83, 87, 105, 108].

Bacteria and fungi

Recently, the Ingber group presented a microfluidic magnetic separation device designed to remove pathogens

and toxins directly from the bloodstream using functionalised MNPs (see Figure 3) [54, 114]. They used genetically engineered mannose-binding lectin as a targeting agent, binding it to the surface of 128 nm diameter MNPs via immunoglobulin-G and streptavidin. Mannose-binding lectin is able to bind a wide range of pathogens and bacteria, so is ideal for MNP functionalisation [74].

In their first study, the group using functionalised magnetic microbeads to extract 80% of *Candida albicans* fungi, a prominent sepsis pathogen. In a more recent *in vitro* study, the group simultaneously removed from whole blood 98% of anaerobic and 80% of aerobic bacteria in a single pass through the device, and over 90% of *Staphylococcus aureus*, *C. albicans* and *Escherichia coli* after five passes.

The group then tested their device on septic rats, achieving similar results to their *in vitro* experiments: 90% of *S. aureus* and *E. coli* were removed from the rat's bloodstream in 1 h. Furthermore, significant reductions of pathogens and $CD45^+$ inflammatory cells in the lung, spleen and kidney were achieved. After injection with a lethal dose of lipopolysaccharide, rats being treated with the device had significantly less of the endotoxin in those organs, and saw improved survivability when compared to untreated rats. The device appears to alleviate or prevent the symptoms of sepsis – the treated rats did not exhibit any signs of clinical distress while untreated rats did [54].

Other research groups have focussed on the purification of bacteria to improve the diagnosis of sepsis, by increasing detection sensitivity with mass spectrometers. This could lead to applications in food quality control or water treatment [46, 82]. Bacteria targeted with functionalised MNPs include *Salmonella*, *Bacillus*, *Staphylococcus*, *E. coli*, *Bifidobacterium longum*, *Listeria monocytogenes* and many others [65, 66]. Others have targeted bacterial nucleic acids such as DNA and RNA [60, 86].

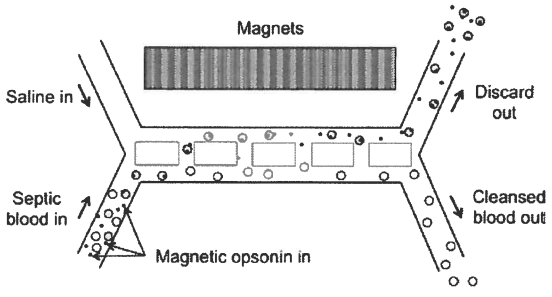

Figure 3: Magnetic labelling and capture of sepsis-causing bacteria *S. aureus* and *E. coli*. Figure adapted from Kang et al. [54].

Cytokines and endotoxins

The severe clinical manifestations of sepsis and septic shock can be attributed to the cytokines released by the immune system in response to pathogens as much as to the pathogens themselves. Some have therefore suggested treatments which target these cytokines rather than using anti-bacterial treatments to treat sepsis [94].

Weber and Falkenhagen, for example, used iron oxide MNPs functionalised with appropriate antibodies to target interleukin-1 and tumour necrosis factor α, two major sepsis causing cytokines [26, 27]. They successfully removed 80–90% of the pathogens from blood plasma [110].

Herrmann et al., another research group developing an extra-corporeal magnetic separator, used antibody functionalised MNPs to target other inflammatory mediators linked to sepsis, interleukin-6 and interleukin-1β, reducing their concentrations in blood by 38% in a single pass through their device [37, 40, 44].

Circulating bacteria-derived endotoxins are also believed to play a significant role in the clinical manifestations of sepsis, and their removal may be of significant benefit to patients [13, 25, 43]. Herrmann et al. have functionalised cobalt-iron MNPs with polymyxin B in order to remove *E. coli*-derived lipopolysaccharide from whole blood [43]. The group reported a significant reduction in inflammatory response in samples purified with the MNPs after an incubation time of 15 min.

Future potential

The extensive use of functionalised MNPs to target a wide range of bacteria and other pathogens responsible for sepsis indicate that the treatment of sepsis could be a very promising application for magnetic haemofiltration. The work of Ingber et al. has established the link between magnetic labelling and sepsis therapy, and their preclinical *in vivo* results are particularly promising. Their magnetic separation device however needs improvement before becoming a feasible medical device, particularly in terms of flow rate.

One significant challenge will be the development of MNPs able to target an extremely wide range of sepsis-causing pathogens while remaining unharmful to other blood components. As sepsis can be caused by almost any infection with bacteria or fungi, one solution may be to produce a "cocktail" of different MNPS, each targeting a different common pathogen known to cause sepsis. Together, the MNPs could be designed to target, for example, the ten bacteria responsible for 90% of sepsis

cases [7]. This cocktail could then be administered to any patient that succumbs to an infection in a hospital intensive care unit, even before the cause of the infection is identified. The patient could then be placed on a magnetic haemofilter similar to the one described by Ingber et al. It is feasible that such a method, if it were cost-effective (particularly in terms of the cost of the MNPs) could become common-place in intensive care wards throughout the developed world.

Cells

In the past 15 years, many functionalised MNPs have been developed with the intention of targeting and binding to cells and cell membranes. Indeed, targeted drug or treatment delivery is one of the main research areas for MNPs, typically using specific antibodies bound to their surface. In terms of blood-borne cells, research has focussed on the use of MNPs for the positive separation and concentration of rare cells for diagnosis or use in treatments. However, some applications, such as the isolation of circulating tumour cells (CTCs), could have potential as treatments themselves, for example by slowing or preventing cancer metastasis.

Before describing some of these applications, it is worth noting that as HGMS of cells may be motivated by "harvesting" rather than "destroying", it is important to consider whether any cytotoxicity is associated with the HGMS process itself. In this context there have been some interesting results published on the effect of static magnetic fields on the cellular uptake of MNPs [2, 6, 102]. In particular, Bae et al. [6] reported that pre-treating their MNPs by placing them in an 0.4 T applied field led to particle-to-particle aggregation, and a knock-on effect when the aggregates were added to the culture medium, in the form of enhanced uptake into the mouse hepatocytes they were studying. These increased levels of uptake led to increased cytotoxicities as the MNP load per cell became too much for sustained viability.

That said, this aggregation-enhanced transfection effect was observed under the prolonged conditions of a 3-day-long cell culture experiment [6], and cell-harvesting HGMS applications are likely to involve much less exposure to the static magnetic field environment. Nevertheless, it is an indication that attention does need to be paid to possible toxicity effects associated with the HGMS process, especially in cases where the harvested cells are to be retained in the separation chamber for significant lengths of time.

Circulating tumour cells

Cancer spreads from its primary site to other areas of the body through metastasis, during which CTCs may be found in the bloodstream. These settle in other areas of the body, forming new tumours and leading to extreme complications. Their isolation and concentration could allow for the earlier detection of metastasis, while some form of constant separation might help slow the cancer's progression.

Many groups have demonstrated the possibility of magnetically labelling CTCs using magnetic particles. One of the first was Enis et al. in 1997 [30]. They used MNPs functionalised with monoclonal antibodies to purify CTCs from colon cancer cell lines, facilitating detection with RT-PCR (reverse transcription polymerase chain reaction) methods [30].

Since then many other groups have targeted and concentrated CTCs using MNPs. Zigeuner et al. performed a similar study with prostate and kidney cancer cells, using commercial magnetic beads with a diameter of 2.8 μm. These were functionalised with antibodies specific to endothelial cells (BER-EP4) for positive selection of the cancer cells from mononuclear cells isolated from healthy volunteers. For samples with only one CTC per million mononuclear cells (an accepted CTC concentration typical of early-stage patients), the group increased positive detection of CTC from 23% to 93% of cases [118, 119]. Kularatne et al. used the same antibody to magnetically target lung cancer cells [61]. Georgieva et al. used a similar method but different antibodies to purify melanoma cells, using immunoglobulin-G coated magnetic beads [32].

More recently, Song et al. developed MNPs able to target several different cancer cell types, functionalising iron oxide MNPs with anti-CD3 monoclonal antibodies to target leukaemia cells, and prostate specific membrane antigen antibodies to target prostate cells [96]. The combined cocktail of MNPs were able to magnetically label up to 96% of cancer cells after a 15 min incubation time (see Figure 4). The group was able to detect the presence of CTCs at a minimum concentration of one cancer cell per 10,000 (0.01%) healthy cells [96].

Other groups have focussed on the use of microfluidics in combination with MNP technologies in an attempt to provide "lab on a chip" cancer diagnostic tests. Ingber et al. used 2.8 μm microbeads conjugated with epithelial cell adhesion molecule (EpCAM) antibodies to extract breast cancer cells from whole blood flowing through their device [53]. They were able to isolate nearly 90% of cancer cells from an initial concentration as low as 2 cells per ml of blood. The group also successfully used their

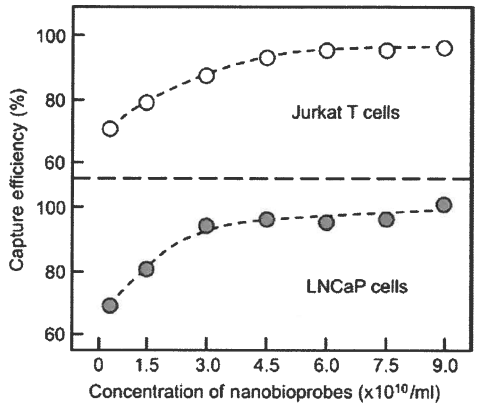

Figure 4: Capture efficiency of leukaemia cells (Jurkat T) and prostate cancer cells (LNCaP) using MNPs coated with monoclonal antibodies. Figure adapted from Song et al. [96].

method to extract CTCs from blood samples of mice with breast cancer, with high specificity (only 0.4% of the captured cells were leukocytes) [53]. Kim et al. have reported a very similar system, also using EpCAM functionalised magnetic particles and a microfluidic magnetic separator to isolated CTCs from blood taken from patients with lung and breast cancer [58]. Chang et al. have used MNPs functionalised with EpCAM antibodies to capture breast and lung cancer cells [14].

The rarity of CTCs, which can be as low as 1–10 cells per ml of blood, when a millilitre of blood would also contain 10^6 leukocytes and 10^9 erythrocytes, makes even their *in vitro* detection (and thus the detection of metastasis) very challenging. Hence, there is an understandable effort on-going in the purification and concentration of CTCs from *ex vivo* samples of blood [38]. Although this is outside the scope of the current review, there is nonetheless much work being done that may have future relevance to therapeutic applications, especially in the development of efficient MNP functionalisation strategies [20, 38, 73, 84, 95, 111].

Stem cells

Progenitor and stem cells such as hematopoietic stem cells (HSCs) may be used in treatments for various ailments. HSCs, for example, are extracted for the purposes of stem cell transplantation, principally as a treatment for leukaemia. This may include autologous stem cell transplantation, wherein the patient's own stem cells are used. These can be extracted from the bone marrow or peripheral blood, but are rare in both cases – about 1 in 10^5 nucleated cells in the bone marrow, and fewer in peripheral blood [116].

Bench-top magnetic separation of HSCs often involves the depletion of other cells, but markers such as CD34 and CD133 have been used to positively extract them. In terms of considering their potential extraction directly from the bloodstream using an extra-corporeal loop, negative depletion is not a feasible option. MNPs functionalised with anti-CD34 antibodies could be used to extract HSC directly from donors with high purity [1, 20, 51, 85, 112, 116]. Similar methods have been used to isolate more differentiated progenitor cells, which also have valuable clinical applications [45, 50].

Other rare cells

CTCs and stem cells are two examples of classes of "rare cells" that it could be beneficial to isolate from whole blood. Others include circulating endothelial cells and foetal cells [20, 84, 116]. Circulating endothelial cells, similarly to CTCs, are used for disease prognosis and personalised cancer treatment. They can be isolated using similar methods, for example using EpCAM antibodies conjugated to magnetic particles [103]. Foetal cells are used to diagnose prenatal diseases such as sickle cell anaemia or to perform genetic analyses [22]. Their bench-top magnetic separation has been possible for two decades; initial techniques required the depletion of other cells to ensure specificity, but more recently "one-step" techniques have been reported [10, 117].

Detoxification

Kidney disease

Stamopoulos et al. have used MNPs alongside dialysis in a technique they call magnetically assisted haemodialysis (MAHD). Their intention is to deliver clinical benefits to late-stage chronic kidney disease and end-stage renal disease patients by injecting functionalised MNPs into the patient to target toxic substances circulating in the bloodstream. The MNPs and targets are to be removed by adding a magnetic filter to a traditional dialysis circuit, and thereby removing toxins with higher efficiency and specificity than existing dialysis membranes, which rely on diffusion and convection through micro- or nano-sized pores [97–99, 101].

The Stamopoulos group advocates the use of iron oxides (maghemite, γ-Fe_2O_3, and magnetite, Fe_3O_4) as the

MNPs, principally because of their biocompatibility. They studied the effect that non-functionalised Fe_3O_4 MNPs and Fe_3O_4 functionalised with bovine serum albumin (BSA) conjugates had on blood cells. They did not find any interference between the MNPs and white or red blood cells, even when concentrations were high, or when the cells were matured in the presence of MNPs [100, 101]. They then used homocysteine and p-cresol as simulant targets *in vitro*, both of which occur in humans and are known to cause clinical symptoms. Elevated homocysteine levels in the bloodstream can cause cardiovascular disease and arteriosclerosis, while p-cresol has an impact on the metabolism. Binding efficiencies of approximately 40% and 20% were reported for homocysteine and p-cresol respectively [97]. They found that their method increased the first-pass removal of homocysteine to ca. 70%, 1.4 times better than the ca. 50% achieved by normal dialysis (see Figure 5) [98].

Interestingly, the Stamopoulos group discusses the possibility of producing MNPs functionalised to bind with multiple targets, citing "creatinine, urea, homocysteine, β-2-microglobulin, etc." [97]. The separation of β-2-microglobulin could be particularly valuable – its inefficient removal is a cause of hemodialysis-associated amyloidosis, which causes joint problems ranging from carpal tunnel syndrome to paraplegia, and can be fatal [33, 72, 104]. Indeed, other groups have investigated novel, non-magnetic methods to remove it and prevent amyloidosis [55].

The magnetic separator used by Stamopoulos et al. was simply a disc Nd-Fe-B magnet placed below the tubing of the dialyser. The MNPs are retained on the walls of the tubing. With this design, they were able to capture 80% of unfunctionalised MNPs from saline in a single pass. However, 15–20 passes of the BSA functionalised MNPs were required to achieve a similar separation efficiency [97]. The flow rates they used varied from 80 ml/min to 250 ml/min. Separation ceased to be efficient above about 150 ml/min [99]. Their work demonstrates that MNP technology has significant potential in terms of binding toxins and delivering clinical benefits to patients with kidney disease, but that their current magnetic separator is non-optimal. It is also worth noting that to date they have only analysed the separation in saline, and that previous research has demonstrated the difficulty of achieving magnetic separations at higher viscosities (i.e. blood rather than saline) [18].

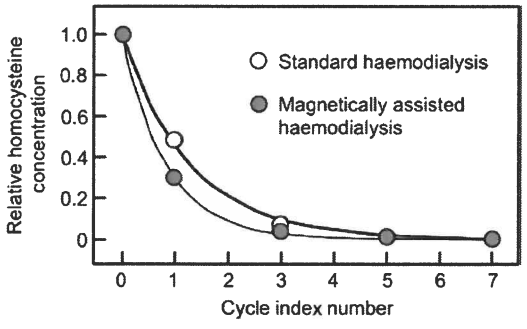

Figure 5: Comparison of reduction in homocysteine concentration from saline using standard haemodialysis (HD) and magnetically assisted haemodialysis (MAHD). (Note that the initial concentration in the HD case was 62 μmol/l, while in the MAHD case it was 100 μmol/l.) Figure adapted from Stamopoulos et al. [98].

Radionuclides

MNPs have been functionalised in order to treat nuclear waste by targeting radionuclides or actinides [3, 35, 75, 76]. Chen et al. have conducted various projects looking at using MNPs in the domain of blood detoxification, with a focus on radionuclides and blood-borne toxins. Their objective is to develop "a magnetically based detoxification system as a therapeutic tool for the selective and rapid removal of biohazards, i.e. chemicals and radioactive substances, from human blood" [18]. Their research has been partly funded by the Defense Advanced Research Program Agency (DARPA), the research arm of the US military, as the researchers believe that a portable version of their device could provide a convenient "in-the-field" treatment to troops exposed to biological or chemical weapons [8, 9, 16–19, 52, 69, 89, 90].

Most of the Chen group's work has focussed on the magnetic separator, which consists of several narrow tubes or capillaries placed between two ferromagnetic wires. A strong external magnetic field is applied, creating local field gradients close to the wires, pulling magnetic materials onto the surface of the tubes, where they are isolated. This design has been modelled extensively, showing a potential 90% MNP capture efficiency (although the design needs to be improved to be as effective for viscous fluids) [8].

Chen et al. have also suggested some MNPs that could be used to target radionuclides, including MNPs to separate caesium-137, a radioactive isotope of caesium. These are MNPs encapsulated in a poly(ethylene)glycolic acid matrix, coated in long-chain poly(ethylene)glycol (PEG), which is known to prolong lifetime in the blood by preventing clearance by the spleen, and finally the surface functionalised with Prussian Blue, a chelating agent that binds strongly to caesium [19]. They have also functionalised commercial magnetic latex particles with

streptavidin, demonstrating a binding affinity to horseradish peroxidase conjugated with biotin which was used as a model toxin. Successful binding was demonstrated via magnetic separation, albeit not under constant flow conditions, using the magnetic separator described above [69].

Wang et al. have removed radionuclides from blood, targeting uranyl ions, suggesting that the same method could be used to remove a number of other radioactive metal toxins from blood [109]. They used Fe_3O_4 MNPs functionalised with a novel conjugate of dopamine and bisphosphonate. Using a simple *in vitro* process, in which they spiked water and blood with uranyl ions and their MNPs, they removed 99% of the uranyl from water and 69% from blood by dipping a bar magnet into the solutions. The lower efficiency in blood is almost certainly due to their rather rudimentary magnetic separation technique, as the 99% removal from water indicates that the binding efficiency of the MNPs to the uranyl is high [109].

Digoxin and other drugs

Herrmann et al. have developed a magnetic separator for use in an extra-corporeal loop, targeting several model toxins, one of which, digoxin, is of particular interest as a model drug toxin [40–42, 44, 54]. Digoxin is a natural glycoside extracted from foxglove plants; it is the active ingredient for many drugs used in the treatment of some heart conditions [29]. Digoxin is toxic, and the accumulation of high levels in the bloodstream can be lethal [67]. Herrmann et al. used carbon-encapsulated platinum-spiked iron carbide MNPs, 20–40 nm in diameter. Antibodies were conjugated to their surfaces via cross-linking with PEG to render them specific to digoxin molecules. In their most recent study, published in 2013, they successfully reduced digoxin concentrations in rats by 50% in 10 min and 75% in 40 min (see Figure 6). MNP concentrations were reduced from 0.5 mg/ml to below the detection limit of 1 μg/ml [42].

Several other groups have demonstrated the binding of nanoparticles to specific drugs for which overdoses are common [34]. Wang et al. for example, have functionalised MNPs with DNA polymers in order to target thrombinin and doxorubicin, used as models of large and small molecules respectively. They successfully bound 70% of thrombinin and 93% of doxorubicin [20]. Cai et al. used MNPs functionalised with β-cyclodextrin to target diazepam, a drug used to treat epilepsy, insomnia, anxiety and alcohol withdrawl, but also one that is sometimes used in attempted

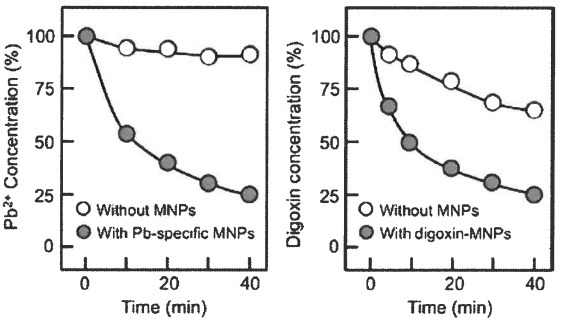

Figure 6: Removal of lead ions and digoxin from rats using MNPs. Figure adapted from Herrmann et al. [42].

suicides [11]. The group successfully removed ca. 50% of diazepam from rabbit blood after a 1.5 h incubation time. In his review, Leroux details a number of nanocarriers being investigated to target different drugs, although most are not MNP based [64]. However, the volume of research indicates that many different drugs could be targeted with MNPs, using different binding methods.

Heavy metal ions

Herrmann et al. also used their device to remove lead ions (Pb^{2+}). They functionalised the MNPs with physisorbed poly(ethylene imine) (PEI) and iminodiacetic acid to target Pb^{2+}, reducing concentrations in rats by 50% in 10 min and 75% in 40 min, the same results as they achieved with digoxin (see Figure 6) [42]. Lee et al. have targeted Pb^{2+} using MNPs functionalised with a new boron-dipyrromethene derivative to remove 94% of Pb^{2+} from human blood [63]. However, it should be noted that their method requires a 30 min sonication step, which might be a significant limitation to clinical adoption.

Jin et al. have similarly used PEI-coated MNPs to target cadmium ions. They synthesised their own iron oxide MNPs, with a diameter of about 50 nm, and used them to remove 98% of cadmium ions from water, as well as 50% of copper ions, demonstrating a high binding efficiency. After 10 min of incubation in cadmium ion spiked blood, the removal rate was 80%. This was achieved by simply placing a Nd-Fe-B magnet next to the sample. They demonstrated that their particles did not cause any haemolysis in blood, even at very high concentrations. They also coated their MNPs with 2,20-(phenyl azanediyl) diacetic acid and PEG to both improve dispersion and reduce uptake by the reticuloendothelial system [49].

Viruses

Several viruses have been successfully targeted with MNPs. Research has focussed on *in vitro* diagnostic techniques, typically by enabling the detection of very low viral loads through bench-top concentration of viruses from blood samples. These include "lab-on-a-chip" devices and bench-top magnetic separators such as the Miltenyi Biotec MACS columns. Although such methods have not yet been incorporated into extra-corporeal loop devices, it is conceivable that such a device could be used to isolate viruses directly from the bloodstream. This could then lead to novel diagnostic or therapeutic techniques. As such, we review here the work to date on *in vitro* targeting.

Human immunodeficiency virus

HIV type 1 virions have successfully been purified *in vitro* using MNPs [15]. The purpose of the method was to provide a novel diagnostic tool as a replacement for centrifugation or RT-PCR, purifying the virus from samples of whole blood. The idea was to use "lab-on-a-chip" magnetic separation to enable easy and cheap HIV diagnosis even from samples with very low viral concentrations. However, if the MNPs are biocompatible and can survive within the bloodstream for a sufficient length of time, they could be used with magnetic haemofiltration to extract HIV directly from a patient's bloodstream.

That said, it is not clear whether this would provide significant clinical benefits. HIV infection severity is linked to the viral load, or the concentration of virions in the blood [68]. Targeting these circulating virions could prevent further spread of the infection, and over time possibly help clear the virus from the body sufficiently to trigger remission. However, this would likely require very long treatment times, not least because only viruses that have not infected a cell would be separable, and achieving the required specificity may be a significant challenge.

Influenza

Various groups have successfully functionalised MNPs with influenza viral antibodies, to enable faster diagnosis by viral enrichment from blood samples – i.e. a sample is taken from the patient, incubated with functionalised MNPs, and enriched through bench-top magnetic separation. Sakudo et al. used 300 nm diameter ferrite MNPs functionalised with the anionic polymer poly(methyl vinyl ether-maleic anhydride) to magnetically label the avian flu viruses H5N1 and H5N2 [91]. Chou et al. used 100 nm diameter Fe_3O_4 MNPs functionalised with subtype specific monoclonal antibodies, which successfully conjugated to H5N2 (but not H5N1). They further suggested that hemagglutinin and nuraminidase, both abundant glycoproteins on the surface of influenza viruses, could be used as general influenza targets [24]. More recently, Hung et al. used 100 nm manganese ferrite MNPs functionalised with anti-influenza A nucleoprotein monoclonal antibodies to successfully label H1N1 [48].

Hepatitis

Several research groups have investigated the use of functionalised MNPs to target hepatitis viruses (Hep). One of the main studies, by Uchida et al. targets Hep A, B and C with MNPs functionalised with PEI [106]. PEI-conjugated MNPs have in fact been reported to bind to several viruses [93]. Uchida's group demonstrated that the PEI-MNP successfully bound to and concentrated Hep A and Hep C viruses, but was less effective against Hep B; concentration was however successfully achieved by adding an anti-Hep B immunoglobulin-M antibody.

Meanwhile, Yassin et al. have successfully targeted Hep C using MNPs coated with protamine hydrochloride, claiming a 100% binding efficiency [113]. Arkhis et al. achieved similar results on Hep B and G using magnetic latex microparticles [5]. Leary et al. have used magnetic microparticles functionalised with specific monoclonal antibodies to target and purify Hep C [62], while Ko et al. avoided the use of expensive antibodies by using lectin bound MNPs to target Hep A [59]. Several other groups have successfully targeted hepatitis DNA and RNA using MNPs functionalised with specific antibodies or streptavidin [23, 71, 107].

Other viruses

In addition to HIV and influenza, a large number of other viruses have been targeted using MNPS, including yellow fever, dengue and herpes [21, 92, 107]. Indeed, thanks to the ability to conjugate specific antibodies to MNPs as well as the possibility of binding specific DNA or RNA, almost any virus could be targeted using MNPs. It may even be possible to synthesise generic MNPs that target many different viruses, although its lack of specificity could cause problems due to the unwanted separation of non-viral bodies from the bloodstream.

Conclusions

This review represents only a limited snapshot of the broad range of excellent research that is currently going into the magnetic labelling of agents using MNPs or magnetic beads. Magnetic separation is already an invaluable clinical technique, used principally for the bench-top purification of agents to improve the sensitivity of detection methods and improve diagnoses. However, there is clearly also a much wider future for magnetic separation for novel treatment and diagnostic techniques.

In some areas, such as rare cells and viruses, the research is focussed almost exclusively on diagnosis. However, several research groups have already proposed specific and general therapeutic applications for magnetic haemofiltration within an extra-corporeal loop, namely in the areas of kidney disease, blood detoxification and sepsis. Any of these applications, should they be fully realised, could save many lives throughout the world. In most cases, haemofiltration is proposed as a complement to existing therapies, such as using dialysis or antibitoics. However, magnetic haemofiltration also promises something new, such as the ability to treat drug toxins or to remove radioactive particles.

The most promising application is arguably as a treatment for sepsis. This is a disease that affects millions and kills hundreds of thousands throughout the world. Its impact in the developed world is high and growing, demonstrating not only the need for new treatments, but also the commercial potential of those treatments should they be viable. Magnetic haemofiltration also holds a number of potential advantages over antibacterials, as it does not require diagnosis of the sepsis-causing pathogen, and is immune to the resistance that can plague antibacterials. It is no understatement to say that a cocktail of biocompatible MNPs able to target 99% of sepsis-causing pathogens, combined with effective and efficient magnetic haemofiltration, could revolutionise healthcare.

Despite this potential, no extra-corporeal HGMS systems are either currently in use or commercially available. This could be due to a number of factors, not least the difficulty in developing the MNPs or beads appropriate for extra-corporeal use: these need to be effective, approved by the relevant regulatory bodies, and affordable. Each of these is a significant challenge. To date, those who have proposed extra-corporeal magnetic haemofiltration have focussed on microfluidic devices, which have their own complications. While they can be effective separators, they can be very expensive to manufacture and difficult to scale up. Flow rates are particularly challenging, given that any clinical therapeutic applications will have to be capable of filtering many litres of blood within an acceptable time-frame.

Magnetic haemofiltration is clearly a technology with a lot of potential. It is not possible to predict which, if any, of the potential applications presented in this review will eventually be clinically successful, or what new applications may be developed in the future. It is clear though that there is still a lot of work to be done, most of which will focus on the development and approval of appropriate magnetic particles, but some of which must also focus on the development of the haemofiltration technology.

Conflicts of interest: Although one of the authors (GF) is the founder and CEO of the magnetic haemofiltration company Medisieve Ltd., his role in this work has been purely academic.

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