Review Article

Magnetic Nanoparticles: Magnetic Nano-technology Using Biomedical Applications and Future Prospects

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ABSTRACT

Magnetic nanoparticles are a class of nanoparticle which can be manipulated using magnetic field. Such particles commonly consist of magnetic elements such as iron, nickel and cobalt and their chemical compounds. While nanoparticles are smaller than 1 micrometer in diameter (typically 5–500 nanometres), the larger micro beads are 0.5–500 micrometer in diameter. The magnetic nanoparticles have been the focus of much research recently because they possess attractive properties which could see potential use in catalysis including nanomaterial-based catalysts, biomedicine, magnetic resonance imaging, magnetic particle imaging, data storage, environmental remediation, nano fluids[1] and optical filters. Nanoparticles play an increasing role in pharmaceutics and medicine. from physical and biological interest to applications in clinics. Magnetic Nanoparticles can be conjugated with any protein, drug and gene, and by that magnetic nano particles serve as contrast agent for MR imaging by changing the MRI signal. Additionally, they serve as a therapeutic tool by improving drug delivery to the target organ. This technology is of high interest for the pharmaceutical-industry. Magnetic nanoparticles enable in-vivo tracking of drugs, proteins and cells in many research fields, such as stem cell therapy, oncology, cordiovascular\atherosclerosis, inflammation etc.

Keywords: Bio-medical applications, Drug discovery, Future prospects, Magnetic nanoparticles, Methods.

INTRODUCTION

Nanotechnology plays an increasingly important role in the biomedical arena. In particular, magnetic nanoparticles (mNPs) have become important tools in molecular diagnostics, *in vivo* imaging and improved treatment of disease, with the ultimate aim of producing a more theranostic approach. Due to their small sizes, the nanoparticles can cross most of the biological barriers such as the blood vessels and the blood brain barrier, thus providing ubiquitous access to most tissues. In all biomedical applications maximum nanoparticle uptake into cells is required. Two promising methods employed to this end include functionalization of mNPs with cell-penetrating peptides to promote efficient translocation of cargo into the cell and the use of external magnetic fields for enhanced delivery. There is a great deal of inventiveness in the use of magnetic particles because one can tailor their dimensions, magnetic properties, and surface coatings.[6–8] They are unique probes for mechanotransduction because the diameters of the particles are at the same length scale as the biological structures to be interrogated. More importantly, their magnetization is not significantly diminished at the nanoscale. They can be synthesized as small as a few nanometres in diameter but still achieve good dimensional uniformity within a fabrication batch.[6] At this size, each particle possesses a single magnetic domain and super paramagnetic properties, in comparison with larger magnetic particles, which have multiple ferromagnetic domains and permanent magnetic properties.[6] The force that an external magnetic field exerts on the particle can range from 10-12 to 10-9 newtons, which are the typical levels that cells experience *in vivo*.[9] For mechanotransduction studies, iron oxide particles are used more commonly than other magnetic materials like cobalt or nickel because they are simpler to synthesize by co-precipitation from iron salts.[6] In fact, batches of synthesized iron oxide micro- or nanoparticles are available from commercial manufacturers and come prepared with reactive functional groups on the surface. By selecting the appropriate surface functional group, it is feasible to chemically attach a ligand to a particle, which enables it to bind to a chosen receptor type on a cell's surface.[7] However, a more straightforward manner is to adsorb matrix proteins from solution onto the particle's surface through hydrophobic interactions.[10,11] In general, proteins like collagen or fibronectin keep their native conformation when adsorbed and so cells can bind through their receptors' recognition of the protein's ligand domains that remain intact. Biocompatibility of magnetic materials is a concern, so iron oxide is a more favorable magnetic material than cobalt or nickel because iron homeostasis is tightly controlled by a cell to clear excess iron.[8] Magnetic nanoparticles offer some attractive possibilities in biomedicine. First, they have controllable sizes ranging from a few nanometres up to tens of nanometres, which places them at dimensions that are smaller than or comparable to those of a cell (10–100μm), a virus (20–450 nm), a protein (5–50 nm) or a gene (2 nm

wide and 10–100 nm long). This means that they can 'get close' to a biological entity of interest. Indeed, they can be coated with biological molecules to make them interact with or bind to a biological entity, thereby providing a controllable means of 'tagging' or addressing it. Second, the nanoparticles are magnetic, which means that they obey Coulomb's law, and can be manipulated by an external magnetic field gradient. This 'action at a distance', combined with the intrinsic penetrability of magnetic fields into human tissue, opens up many applications involving the transport and/or immobilization of magnetic nanoparticles, or of magnetically tagged biological entities. In this way they can be made to deliver a package, such as an anticancer drug, or a cohort of radionuclide atoms, to a targeted region of the body, such as a tumour. Third, the magnetic nanoparticles can be made to resonantly respond to a time-varying magnetic field, with advantageous results related to the transfer of energy from the exciting field to the nanoparticle. For example, the particle can be made to heat up, which leads to their us as hyperthermia agents, delivering toxic amounts of thermal energy to targeted bodies such as tumours; or as chemotherapy and radiotherapy enhancement agents, where a moderate degree of tissue warming results in more effective malignant cell destruction. Over the course of many hours, however, nanoparticles can be internalized through endocytosis, which may contribute to cytotoxicity by overwhelming the mechanisms of iron clearance.

In the human body, there is a constant movement of ions within and outside the cells as well as across cellular membranes. This electrical activity is responsible for magnetic fields, called biomagnetic fields, which we can measure using sensitive instruments placed outside the body. These, and many other potential applications, are made available in biomedicine as a result of the special physical properties of magnetic nanoparticles.

Magnetic nanoparticles technology could result in better biosensors; Handheld biosensors and diagnostic devices could be taking a huge step forward, thanks to recent advances made in the use of ferromagnetic iron oxide nanoparticles – also known as magnetic nanobeads. In the new system, the ferromagnetic iron oxide nanoparticles (essentially tiny particles of rust) would be attached to those biochemical probes. When specific chemicals were encountered, the resulting "ferromagnetic resonance" of the particles would be electronically relayed to a computer built into the detection device, which would in turn alert its human user to the presence of the chemicals. The researchers claim that the technology could be used to detect just about anything in air or water, without the need for special thin films or complex processing, while still offering great sensitivity and accuracy. "The particles we're using are 1,000 times smaller than those now being used in common diagnostic tests, allowing a device to be portable and used in the field. Just as important, however, is that these nanoparticles are made of iron. Because of that, we can use magnetism and electronics to make them also function as a signaling device, to give us immediate access to the information available.

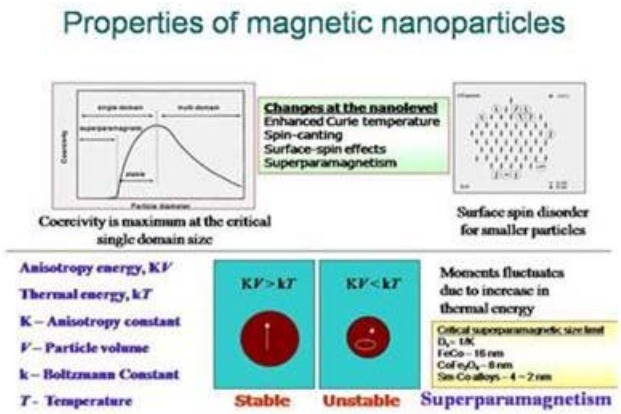

Figure 1: Properties of magnetic nanoparticles

Cobalt nanoparticle with graphene shell (note: The individual graphene layers are visible) Currently, three different kinds of magnetic nanoparticles are being produced and used.

Oxides-ferrite

Ferrite-nanoparticles are the most explored magnetic nanoparticles up to date. Once the ferrite particles become smaller than 128 nm.[10] they become super paramagnetic which prevents self agglomeration since they exhibit their magnetic behaviour only when an external magnetic field is applied. With the external magnetic field switched off, the remanence falls back to zero. Just like non-magnetic oxide nanoparticles, the surface of ferrite nanoparticles is often modified by surfactants, silicones or phosphoric acid derivatives to increase their stability in solution.[11]

Metallic

Metallic nanoparticles have the great disadvantage of being pyrophoric and reactive to oxidizing agents to various degrees. This makes their handling difficult and enables unwanted side reactions.

Metallic with a shell

The metallic core of magnetic nanoparticles may be passivated by gentle oxidation, surfactants, polymers and precious metals.[9] In an oxygen environment; Co nanoparticles form an anti-ferromagnetic CoO layer on the surface of the Co nanoparticle. Recently, work has explored the synthesis and exchange bias effect in these Co core CoO shell nanoparticles with a gold outer shell.[12] Nanoparticles with a magnetic core consisting either of elementary Iron or Cobalt with a nonreactive shell made of graphene have been synthesized recently.[13] The advantages compared to ferrite or elemental nanoparticles are:

- Higher magnetization

- Higher stability in acidic and basic solution as well as organic solvents
- Chemistry[14] on the graphene surface via methods already known for carbon nanotubes.

Table 1: Types of nanoparticles

Type of Nanoparticles	Description	Size (nm)	Advantages	Disadvantages	Applications
Magnetic	Superamagnetic iron oxide particles: In magnetic field usually shows large magnetic moments	5-100	External magnetic field shows magnetism allows control over distribution Loss of magnetism in absence of magnetic field lessens jeopardy of aggregation in vivo Ease of manufacturing Non-toxic in nature Acquiescent to surface functioning Chemically firm/stable Biocompatible	Thrombosis and Embolization are major consequences after aggregation of magnetic nanoparticles.	Cancer diagnosis and monitoring through Magnetic cell separation "Negative enhancer" MRI contrast agents Useful in target drug delivery of genes/drugs thermotherapy
Metallic	Gold or silver nanoparticles	<50	high drug doses can be beared by small particles with large surface area Safe; gold approved, useful in human treatments Acquiescent to conjugation to targeting ligands	Biocompatibility is poor indecisive in-vivo fate	Useful in control delivery of proteins, drugs, Encapsulated DNA in metal shells to the desired site on exposure of IR light, magnetic field. Also useful in Photoacoustic tomography, X-rays
Nanoshells	nanoshell plasmon is a type of spherical nanoparticle consisting of a dielectric core which is covered by a thin metallic shell. (usually gold) it absorb energy at near infrared wavelength. In this type the core shell thickness ratio is deciding factor in generation of heat/light.	10-300	Imaging/therapeutic potentials are as good as quanum dots.	Larger in size in comparison to quantum dots.	Used as contrast agent in optical coherence tomography. Useful in Photothermal tomor ablation therapy. Useful in bioassay of immunoglobulins as immunoconjugates.
Ceramic	Porous , inorganic particles comprises of silica, titania, alumina (Biocompatible materials)	<100	Ease of preparation Solubility in water Biologically satble		Efficient in carry DNA, proteins and heavy mol. weight compounds.

Dry-Magnetic-Particles

Dry magnetic particles can typically be purchased in red, black, gray, yellow and several other colours so that a high level of contrast between the particles and the part being inspected can be achieved.

The size of the magnetic particles is also very important. Dry magnetic particle products are produced to include a range of particle sizes. The fine particles are around 50 mm (0.002 inch) in size, and are about three times smaller in diameter and more than 20 times lighter than the coarse particles (150 mm or 0.006 inch). This makes them more sensitive to the leakage fields from very small discontinuities.

Wet-Magnetic-Particles

Magnetic particles are also supplied in a wet suspension such as water or oil. The wet magnetic particle testing method is generally more sensitive than the dry because the suspension provides the particles with more mobility

and makes it possible for smaller particles to be used since dust and adherence to surface contamination is reduced or eliminated. The wet method also makes it easy to apply the particles uniformly to a relatively large area. Wet method magnetic particles products differ from dry powder products in a number of ways. One way is that both visible and fluorescent particles are available. Most nonfluorescent particles are ferromagnetic iron oxides, which are either black or brown in colour. Fluorescent particles are coated with pigments that fluoresce when exposed to ultraviolet light. Particles that fluoresce green-yellow are most common to take advantage of the peak colour sensitivity of the eye but other fluorescent colours are also available. (For more information on the colour sensitivity of the eye...see the material on penetrate inspection.)

The particles used with the wet method are smaller in size than those used in the dry method for the reasons mentioned above. The particles are typically 10 mm (0.0004 inch) and smaller and the synthetic iron oxides have particle diameters around 0.1 mm (0.000004 inch). This very small size is a result of the process used to form the particles and is not particularly desirable, as the particles are almost too fine to settle out of suspension. However, due to their slight residual magnetism, the oxide particles are present mostly in clusters that settle out of suspension much faster than the individual particles. This makes it possible to see and measure the concentration of the particles for process control purposes. Wet particles are also a mix of long slender and globular particles.

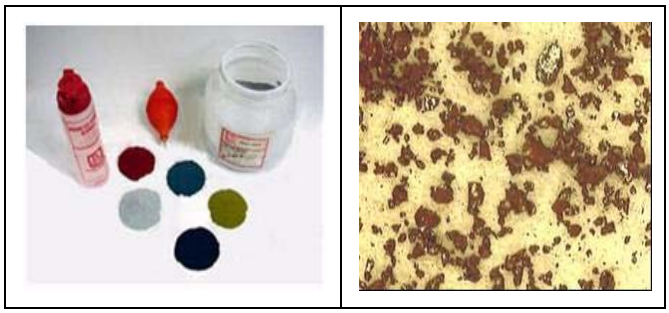

Figure 2: Dry magnetic particles

PHYSICAL PRINCIPLES

We now consider the physical principles involved in the applications of magnetic particles in bioengineering applications. In the case of targeted drug delivery we introduce drug coated magnetic particles into a blood vessel and then apply an external magnetic field. This field attracts and retains the particles at the site of the disease. The blood vessel will exhibit a paramagnetic response to the field from entities such as the haemoglobin. It will also exhibit a diamagnetic response because of proteins that contain carbon, hydrogen, nitrogen and oxygen atoms. These two responses are much smaller than the response by the magnetic particles. What kind of magnetic field should be applied to target the magnetic particles to the site of the disease?

A magnetic field that is uniform gives rise to a torque, but usually we wish to direct the particles in a specific direction, i.e., provide translation motion; this can be accomplished by means of a field gradient. If an appropriate magnetic field gradient is present, a force acts on the particles driving them in a direction which can be chosen to so that the particles are targeted to the site of the disease. Consider a magnetic particle in such a magnetic field gradient; for the case of magnetic nanoparticles suspended in water the magnetic force on the particle has been shown by Pankhurst to be

$$Fm = Vm\,(\chi m - \chi w)\,\nabla\,(12\ B.H)$$

Where Vm is the volume of the particle, χm and χw are the susceptibility of the particle and water respectively, and the quantity 12 *B.H* is the magneto static field energy density[5]. Assuming that the susceptibility of the particle is greater than that of water, this equation shows that the force is proportional to the differential in the energy density, and recalling the geometrical meaning of the operator, this magnetic force on the particle acts in the direction of steepest ascent of the energy density field. In the case of magnetic hyperthermia applications, a different principle is involved; we wish to raise the temperature to about 43 °C in a localized area in order to destroy cancer cells selectively.[3] This can be done by applying a magnetic field which varies with time; ferro- and ferri-magnetic material will be repeatedly cycled through the B-H loop, resulting in hysteresis and other losses which are then converted to thermal energy and result in an increase in temperature. Super paramagnetic materials can also be heated using this technique; the loss mechanisms differ from those observed in ferro- and ferri-magnetic materials.

FACTOR AFFECTING MAGNETIC PROPERTIES

From a materials standpoint it is useful to distinguish those properties that are structure sensitive from those that are relatively structure-insensitive; susceptibility and coactivity fall in the first category while saturation magnetization is an example of the second. Manipulation of the properties can be performed by altering the composition, crystal structure, stress state and size of the material. Since size plays an important part in many magnetic biomaterials applications, we now consider the effect of particle size on magnetic properties. In for example drug delivery, gene delivery and hyperthermia, small magnetic particles are used.[5] In large particles (greater than about 1μm) there are many magnetic domains; this leads to a narrow hysteresis loop. Such particles are useful in immunomagnetic separation of pathogenic microorganisms in microbiology. For smaller particle sizes (less than about 1μm) it is energetically more favourable for only one domain to exist. The response of such particles to a magnetic field is qualitatively different, resulting in a broader hysteresis loop. If the particle size is reduced further to about 20 nm (the exact size depends on the composition of the material), the material becomes super paramagnetic,

which means that the magnetic moment of the particle fluctuates because of the thermal energy (~kT); at the atomic level the individual atomic moments continue to be ordered relative to each other. Importantly, the remanence is zero, the result is a B-H curve showing no hysteresis; this property is important for reducing the tendency of the particles to agglomerate. The physical basis for the fluctuation of the magnetic moments can be understood as a battle between ΔE, the energy barrier to moment reversal and the thermal energy (kT). In the simplest approximation the energy barrier is the product of the anisotropy energy density K and the volume V. When the particle size is small (small V), the KV term is small and comparable to the thermal energy; this leads to flipping of the magnetic moment. The "blocking" temperature TB can be regarded as the temperature above which the material becomes super paramagnetic. Super paramagnetic particles are useful as magnetic biomaterials, some are, physiologically well tolerated; an example is dextran-magnetite. Iron oxide coated with dextran is commercially available for MRI, for cell separation and cell labelling applications.

SYNTHESIS OF MAGNETIC NANOPARTICLE

The established methods of magnetic nanoparticle synthesis include:

Co-precipitation

Co-precipitation is a facile and convenient way to synthesize iron oxides (either Fe_3O_4 or γ-Fe_2O_3) from aqueous Fe^{2+}/Fe^{3+} salt solutions by the addition of a base under inert atmosphere at room temperature or at elevated temperature. The size, shape, and composition of the magnetic nanoparticles very much depends on the type of salts used (e.g. chlorides, sulphates, nitrates), the Fe^{2+}/Fe^{3+} ratio, the reaction temperature, the pH value and ionic strength of the media[9], and the mixing rate with the base solution used to provoke the precipitation.[15] The co-precipitation approach has been used extensively to produce ferrite nanoparticles of controlled sizes and magnetic properties.[16-19] A variety of experimental arrangements have been reported to facilitate continuous and large–scale co–precipitation of magnetic particles by rapid mixing.[20,21] Recently, the growth rate of the magnetic nanoparticles was measured in real-time during the precipitation of magnetite nanoparticles by an integrated AC magnetic susceptometer within the mixing zone of the reactants.[22]

Thermal decomposition

Magnetic nanocrystals with smaller size can essentially be synthesized through the thermal decomposition of organometallic compounds in high-boiling organic solvents containing stabilizing surfactants.[9]

Microemulsion

Using the micro emulsion technique, metallic cobalt, cobalt/platinum alloys, and gold-coated cobalt/platinum nanoparticles have been synthesized in reverse micelles of cetyltrimethyl ammonium bromide, using 1-butanol as the co surfactant and octane as the oil phase.[9,23]

Flame spray synthesis

Using flame spray pyrolysis[13,24] and varying the reaction conditions, oxides, metal or carbon coated nanoparticles are produced at a rate of > 30 g/h .

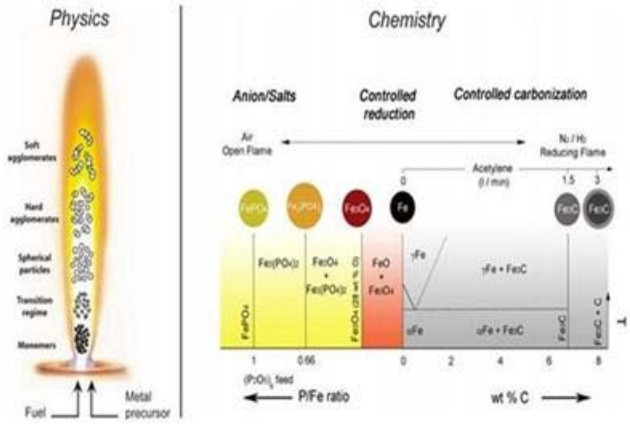

Figure 3: Flame spray synthesis for magnetic nanoparticles

Magnetic Particle Techniques for Cell Signal Activation

In general, the idea that physical force can act as a regulatory signal has parallelism to endocrine signalling. Force, like hormones or growth factors, can guide the development and function of cells through changes in gene expression. The process starts with mechanoreceptors that lead to changes in protein kinase or phosphatase activity inside the cell. In general, the signal is propagated forward to activate transcription factors that regulate the expression of target genes. As with mutations in extracellular signalling, mutations in mechanotransduction pathways can muddle the sensation of force and cause errors in signal interpretation. Dysfunction in mechano-transduction is hypothesized to drive cells to pathological outcomes like cancer, atherosclerosis, or asthma.[4,12] It should be pointed out that mechanotransduction changes do not always need to go through the nucleus; force can activate pathways that modulate the activation of cell functions like migration, contraction, or secretion. Although many phenomenological observations have been made, the mechanisms of how force initiates biochemical changes are not fully understood.[9,13] It may involve stretch induced conformational changes in proteins in the vicinity of the receptor,[14] force transmission from the receptor to specialized mechanosensors within a cell, [15,16] and/or changes in tensional integrity (tensegrity) of the cytoskeleton.[17] However, what is apparent is that there is not a single master switch that initiates cellular mechanotransduction.

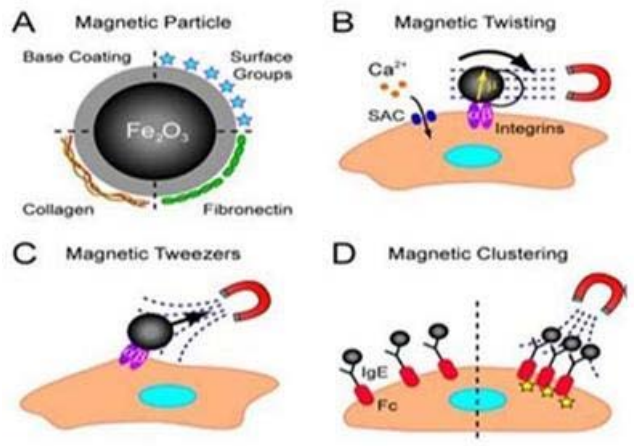

Figure 4: Magnetic nanoparticles for mechanical activation of cell receptors.

A. Nanosynthesized magnetic particles can be coated with a organic or nonorganic base coating that protects the magnetic core and provides a foundation for subsequent conjugation with reactive surface groups or adsorption of matrix proteins like fibronectin or collagen. **B.** Magnetic twisting cytometry imparts a mechanical torque through an applied magnetic field perpendicular to the particle's magnetic dipole moment . The induced shear stress, which can be as strong as 4 N/m2, mechanically activates integrin receptors or stretch-activated ion channels (SAC). **C.** Magnetic tweezers use a gradient field to pull magnetic particles toward the pole tip of an electromagnetic coil or permanent magnetic. With magnetic tweezers, forces up to 10 nN have been reported. **D.** Magnetic aggregation can be used to activate immunological responses in mast cell using super paramagnetic nanoparticles to induce clustering of IgE-bound Fc-RI receptors.

Magnetic particles can overcome the limits apparent in other techniques used for studying mechanotransduction. Approaches that subject cells to shear or stretch continue to pioneer the field of mechanotransduction, but they apply forces at multiple points on a cell, which obscures the identification of the mechanoreceptors involved in the process. Magnetic particles offer more control at the nanoscale because one can readily manipulate the strength, direction, and location of the magnetic force by the placement of the magnetic fields and the ligand coating on the bead. This isolates out the interactions from other receptors and sidesteps the convolution of stimulating multiple receptors types that could activate several pathways in parallel. The laws of physics allow for different ways to twist, pull, or cluster magnetic particles and so several magnetic technique platforms have been developed.[18,19] Magnetic twisting cytometry generates a mechanical torque at the particle-cell interface by applying a field in a direction perpendicular to the magnetic dipole of the particle.[10] The torque imparted by the applied field drives a particle to twist or roll on a cell's surface. Because the particle is physically restrained by the bonds at the receptor- ligand interface, the rolling action produces a shearing force at the cell's receptor. Force can be applied at high frequency by modulating the current passing through the electromagnetic coils that generate the field and thereby impart cyclic loading on the cell. The second main approach with magnetic particles is magnetic tweezers, which are able to pull on particles by gradients in a magnetic field.[20] Here one of the pole ends of an electromagnetic coil has a long, sharp tip that is placed using a micropositioner to be a close distance from a particle.

Magnetic Particle Testing (Mpt)

This testing method is called MT (Magnetic Testing) or MPT (Magnetic Particle Testing), and suitable for the surface / subsurface flaw inspection of ferromagnetic materials.
Principle of MPT is as follows:

1. When the work piece to be inspected is magnetized, magnetic flux is induced.
2. If there is a flaw on the surface, magnetic flux leaks into the air at the position of the flaw.
3. Then magnetic particles (dyed or fluorescent encapsulated) are applied to the surface.
4. These magnetic particles will migrate to the flaw where the magnetic flux leaks and forms a flaw indication that is several tens of times of actual flaw width.
5. The inspector visually identifies the flaw.

Procedure of MPT

Procedure of MPT is "Pre-cleaning" - "Magnetization" - "Applying magnetic particle" - "Observation" - "Post-cleaning".

Pre-cleaning

Oil, paint, rust and other foreign materials on the surface to be inspected not only prevent attraction of magnetic particles to the flux leakage, but also lead to form a false indication. Therefore such materials will be cleaned chemically or mechanically before magnetization process.

Magnetization

The work piece is magnetized as direction of magnetic flux is orthogonal to direction of a flaw. Following method is applied for proper magnetization.

1. Axial current method: To pass electric current longitudinal direction of the work piece
2. Cross current method: To pass electric current cross direction of axis of the work piece
3. Prod method: To pass electric current between two prods contacted inspection area of the work piece
4. Through conductor method: To pass electrical current through the hole of the work piece
5. Coil method: Put the work piece in a magnetizing coil
6. Yoke method: Put the work piece between magnetic poles

7. Through flux method: To pass magnetic flux through the hole of the work piece

Applying magnetic particle

1. Types of magnetic particle

Easy magnetization and migration to flux leakage and discriminative flaw indication are required for performance of magnetic particle. There are two types of magnetic particle, one is non-fluorescent particle (white, red, black) for observation under visible light, and the other is fluorescent magnetic particle for observation under UV light. Dry method, magnetic particle is applied to the surface as it stands, and wet method, magnetic particle is dispersed in oil or water, are applied.

2. Applying timing of magnetic particle

There are two methods. Magnetic particle is applied during magnetization of the work piece, or applied after ceasing magnetization. In later case residual magnetism is utilized for forming flaw indication.

Observation

UV light is used for observation of the work piece under dark environment in case of using fluorescent magnetic particle for inspection.

Post-cleaning

Demagnetization, removing residual magnetic particle and rust proofing of the work piece are done if required.

Magnetic Nano Particles: Bio-Medical Applications

Micro- and nano-sized magnetic support is a recently developed revolutionary technology for bio separation, especially for ligand fishing, protein, enzyme, DNA, RNA and cell isolation or purification. Magnetic nanoparticles (MPs) are referred to by various synonyms, such as magnetic beads (MB) or micro- and nano-sized magnetic beads. They are also called ferrofluids or magnetic fluids, meaning colloidal suspensions of magnetic particles in a liquid carrier. Generally, these particles are part of nanotechnology, which can be defined as engineering of functional systems on a molecular scale. It has the potential to create many new materials and devices with a wide range of applications across the biomedical, chemical, electronic and mechanical fields. Their super paramagnetic properties have opened promising new perspectives for their application in several areas. The pioneering "medical" application in the treatment of lymphatic nodes and metastases based on injecting "metallic particles" preheated in a magnetic field was first published by Gilchrist *et al*. in 1957.[1] Since then, magnetic particles have been modified by coating with antibodies, enzymes, proteins or specific ligands that enable them to bind to other biologically active compounds or receptors on the cell surface. In the last decade, magnetic particles have been increasingly used as a promising technique for a wide spectrum of biomedical applications. The number of publications presenting original studies on using magnetic beads is increasing year by year. The growing interest in the magnetic carrier is focused on biochemistry, molecular biology and medical specialties. Because of the super paramagnetic properties and micro- and nano-dimensions, magnetic nanoparticles can be used to isolate any target and linked to various manual and automated applications.[2-4] Innovative research has produced a new application for magnetic nanoparticles that carries an exciting biomedical and bioengineering potential, for instance, cell molecule and nucleic acid separation, immunoassays, pathogen detection, protein purification, gene mutation analysis and magnetic-force-based tissue engineering.[5,6] Also, the interest in the potential application of the magnetic technique in pharmacy is significantly growing. It is currently being recognised that this magnetic nanotechnology could play an important role in this area. A wide variety of applications have been envisaged for this class of particles which include:

Medical diagnostics and treatments

Magnetic nanoparticles are used in an experimental cancer treatment called magnetic hyperthermia[25] in which the fact that nanoparticles heat when they are placed in an alternative magnetic field is used. Another potential treatment of cancer includes attaching magnetic nanoparticles to free-floating cancer cells, allowing them to be captured and carried out of the body. The treatment has been tested in the laboratory on mice and will be looked at in survival studies.[26,27] Magnetic nanoparticles can be used for the detection of cancer. Blood can be inserted onto a micro fluidic chip with magnetic nanoparticles in it. These magnetic nanoparticles are trapped inside due to an externally applied magnetic field as the blood is free to flow through. The magnetic nanoparticles are coated with antibodies targeting cancer cells or proteins. The magnetic nanoparticles can be recovered and the attached cancer-associated molecules can be assayed to test for their existence.

Magnetic immunoassay

Magnetic immunoassay[31] (MIA) is a novel type of diagnostic immunoassay utilizing magnetic beads as labels in lieu of conventional, enzymes, radioisotopes or fluorescent moieties. This assay involves the specific binding of an antibody to its antigen, where a magnetic label is conjugated to one element of the pair. The presence of magnetic beads is then detected by a magnetic reader (magnetometer) which measures the magnetic field change induced by the beads. The signal measured by the magnetometer is proportional to the analyte (virus, toxin, bacteria, cardiac marker,etc.) quantity in the initial sample.

Waste water treatment

Thanks to the easy separation by applying a magnetic field and the very large surface to volume ratio, magnetic nanoparticles have a good potential for treatment of

contaminated water.[32] In this method, attachment of EDTA-like chelators to carbon coated metal nanomagnets results in a magnetic reagent for the rapid removal of heavy metals from solutions or contaminated water by three orders of magnitude to concentrations as low as micrograms per Litre.

Chemistry

Magnetic nanoparticles are being used or have the potential use as a catalyst or catalyst supports.[33] In chemistry, a catalyst support is the material, usually a solid with a high surface area, to which a catalyst is affixed. The reactivity of heterogeneous catalysts occurs at the surface atoms. Consequently great effort is made to maximize the surface area of a catalyst by distributing it over the support. The support may be inert or participate in the catalytic reactions. Typical supports include various kinds of carbon, alumina, and silica.

Biomedical imaging

Magnetic Co-Pt nanoparticles are being used as an MRI contrast agent for transplanted neural stem cell detection.[34] So far, we have only considered magnetic properties associated with the electrons in the material. However, protons also have a magnetic moment, and this can be utilized in the powerful imaging technique of magnetic resonance imaging (MRI).[5] The principle is as follows. We first apply a steady field of about 1 T to a material, causing a very small fraction of protons to line up parallel to the field. The net magnetic moment processes like a top around the direction of this field. In order to measure the signal produced as a result of this alignment, we now apply a transverse radio frequency magnetic field. The frequency is carefully chosen and its effect is to make the magnetic moment process in the plane perpendicular to the steady state field. When this second field is switched off, the amplitudes of the magnetic moments relax back to their initial values. This relaxation of the response is measured by pick-up coils. Typically, the relaxation time can be reduced by means of a magnetic particle. Thus if a region is tagged using the magnetic particles, the relaxation time will be lower compared to untagged regions; thus "contrast" is provided and the particle acts as a contrast agent. Usually paramagnetic Gd based materials are used; super paramagnetic iron oxide particles, usually coated with dextran, have also been used for this purpose.

Hyperthermia

The idea that a localized rise in temperature (typically about 43°C) can be used to destroy malignant cells selectively is called hyperthermia; this method of treatment can be affected by magnetic particles. The basic idea is that magnetic material can be heated by an a.c. magnetic field. The mechanisms of heating for ferromagnetic materials include hysteresis losses. In the case of super paramagnetic particles heating can occur by the rotation of the particles themselves or by the rotation of the atomic magnetic moments. Other non-magnetic methods of hyperthermia are also available. The advantage of using magnetic particles should by now be familiar, i.e., we can target the particles to the targeted region and then heat up the particles by using an external a.c. magnetic field. According to Pankhurst, typically a heat deposition rate of 100 mW/cm3 is required, and the frequency of the field should be in the kHz range with amplitude of a few kA/m.[5] Generally iron oxides are used for hyperthermia applications.

Information storage

Research is going into the use of using MNPs for magnetic recording media. The most promising candidates for high-density storage is the face-centred tetragonal phase Fe-Pt alloy. Grain sizes can be as small as 3 nanometres. If it's possible to modify the MNPs at this small scale, the information density that can be achieved with this media could easily surpass 1 Terabyte per square inch.[35]

Genetic engineering

Magnetic nanoparticles can be used for a variety of genetics applications. One application is the isolation of mRNA. This can be done quickly – usually within 15 minutes. In this particular application, the magnetic bead is attached to a poly T tail. When mixed with mRNA, the poly A tail of the mRNA will attach to the bead's poly T tail and the isolation takes place simply by placing a magnet on the side of the tube and pouring out the liquid. Magnetic beads have also been used in plasmid assembly. Rapid genetic circuit construction has been achieved by the sequential addition of genes onto a growing genetic chain, using nanobeads as an anchor. This method has been shown to be much faster than previous methods, taking less than an hour to create functional multi-gene constructs in vitro.[36]

Drug discovery

The process of drug discovery in the modern scientific aspect is very complex. It integrates many disciplines, including biotechnology, medicine and pharmacology. For years, drug discovery has also focused on identification of unknown bimolecular interactions with known targets. A number of established methods are used in evaluating the binding of ligands/drugs to receptors and proteins, such as equilibrium dialysis, ultra filtration, ultracentrifugation, bio affinity chromatography and other spectroscopic methods. Also, the BIAcore system and surface Plasmon resonance (SPR) are the two most popular recently technologies for real-time bio molecule interaction analysis. Because of the different modifications of multifunctional surface of the magnetic particles, these approaches are practically useful in this area.

Study has been done to adapt immobilized human serum albumin (HSA) onto the surface of magnetic beads for the purpose of "ligand fishing".[3] These beads correctly isolated a known binder from a mixture of known compounds, and the whole experiment was carried out

manually using a magnet and automatically using the Migration System. Furthermore, the magnetic separation technique was extended to the protein-ligand and protein–protein interaction using magnetic beads with immobilized heat-shock protein 90α (Hsp90α).[2] The advantage of this system is that the Hsp90α-coated magnetic beads can isolate interacting partners (ligand and proteins) from complex mixtures and cellular extracts (e.g. from KU-812 cells) in less than 15 min, unlike co-immunoprecipitation methods, which take several hours to recover the protein of interest. Presently, the extension of the magnetic beads method to multiple complex cellular extracts and complex plant extracts is being investigated. The most recent study demonstrates that ligand fishing based on biological functionalised MB is an effective and convenient way to identify and isolate bioactive small molecules from botanical extracts (*Camptotheca acuminata* and *Dioscorea nipponica*), and the process has a significant structure specifity.[7,8]

Using a similar concept, protein-coated magnetic beads were used as a tool for a rapid drug-protein binding study.[4] Knowledge of serum protein binding behaviour as a significant component of blood is very important for the rational use of drugs. An extensive understanding of the ligand/drug-protein interaction may be of special importance for the modelling of pharmacokinetics, including possible drug–drug interaction. In this context, magnetic beads with immobilized HSA have been successfully used to determine the affinity of known drugs for the protein.[4] Moreover, the preliminary competition experiments suggest that protein-coated magnetic beads can be employed in drug–drug interaction studies. However, the validation of the proposed method is being investigated.

Screening System for Drug Discovery Research: In drug discovery research, it is important to identify and purify the protein targeted by a medicine so functional analyses can be performed. However, the identification and purification of a target protein are quite difficult and require a lot of time and labour. We have developed a screening system (magnetic nanoparticles and automatic screening equipment) for drug discovery research, in collaboration with Professor Hiroshi Handa of the Tokyo Institute of Technology. The magnetic nanoparticles are carriers for affinity purification. They have the following superior characteristics, in comparison to existing carriers. Magnetic nanoparticles enable effective chemical bond formation, because they are nano-sized and have excellent dispersibility. Magnetic nanoparticles exhibit minimal nonspecific protein absorption. Magnetic nanoparticles are tolerant to organic solvents and can be used to immobilize ligands (molecules that trap target proteins). The magnetic nanoparticles for affinity purification can facilitate the one-step purification of target proteins. Moreover, the automatic screening equipment, which performs magnetic separation and dispersion, has enabled the automation of the affinity purification process and the simultaneous treatment of multiple samples, thus saving time and increasing efficiency. Magnetic particles for bio separation consist of one or more magnetic cores with a coating matrix of polymers, silica or hydroxyl apatite with terminal functionalized groups.

Drug delivery

Advances in many fields are converging to make the commercialisation of advanced drug delivery concepts possible.[6] Innovative drug delivery devices should protect labile active ingredients, precisely control drug release kinetics, and minimise the release of the drug to non-target sites. The major advantage of ferromagnetic materials (Fe_3O_4) and magnetite (γ-Fe_2O_3) over other materials with a high magnetic moment is the possibility for *in vivo* applications.[6] Other materials, such as cobalt and nickel, can cause oxidative stress or long-term changes in enzyme kinetics. Hence, their use should be limited in biomedical applications. The use of magnetic nanoparticles as drug carriers in targeted therapy provides huge opportunities in cancer treatment, as the use of such carriers considerably reduces the side effects of conventional chemotherapy. For drug targeting, they are able to cumulate in desired locations within the body with the help of a magnet. In 1978, magnetic particles were first applied as a new class of drug target. The tool was based on albumin microspheres with entrapped Fe_3O_4 and doxorubicin. A novel carrier system allowed for the accumulation of local chemotherapeutic agent which was comparable to that achieved by the administration of a 100-fold higher dose of the drug. Other results indicate that the new targeting system of specific drug delivery based on the “magnetic granules” might be useful for local chemotherapy of oesophageal cancer in rabbits administered via the oral route. Another interesting application has utilised iron oxide nanoparticles covered by starch polymers which makes the ferrofluids tolerable to healthy tissues and cells. The time-dependent distribution of the ferrofluids as a carrier for mitoxantrone within the body was already investigated by labelling the nanoparticles with radioactive isotope ^{59}Fe.[9] Also, electron microscope investigations have shown that the ferrofluids do not only concentrate in the cancer tissue but also penetrate into the tumour cells. The use of magnetic beads can significantly improve hyperthermia cancer treatment. This therapy involves raising the temperature of the target tissue to 43–46°C, which increases its sensitivity to chemo- and radiotherapy and may additionally stimulate activities of the host immune system.[10] Magnetic hyperthermia relies on the introduction of ferro- or super paramagnetic particles into the tumour tissue. Under an applied magnetic field, magnetic energy is converted to thermal energy, which destroys cancer tissues. Cancer-specific binding agents could include antibodies, hormones, and other exo- and endogenous substances. Attaching these agents, such as estrogens, testosterone, and thyroid hormones, to magnetic nanoparticles has promising implications for magnetic fluid hyperthermia treatment (MFH) of breast,

prostate, and thyroid cancer, respectively. Recently, monoclonal antibodies to cancer cell surface antigens and modified viruses that are able to bind with receptors on certain types of cancer cells are being considered as novel potentially binding agents coupled with magnetic particles. This tool could be successfully used to locate cancer cells and deliver chemotherapeutics directly to "cancer" location at elevated temperatures.

A recent study published in nature nanotechnology reports for the first time target aerosol delivery to the lung achieved with aerosol droplets comprising super paramagnetic iron oxide nanoparticles in combination with a target-directed magnetic gradient field.[11] Standard therapy with cytotoxic drugs relies on the attainment of high drug concentrations at the affected site, which is particularly difficult in lung cancer.[12] Thus, the novel and promising method could be used to accumulate effective drug doses in the affected lung areas and reduce undesirable effects. As the *in vitro* study has illustrated the enhanced deposition of high aspect ratio aerosols in the small airways using magnetic field alignment, in a recent study, computational fluid dynamics (CFD) simulations of magnetic aerosol transport and deposition were conducted and compared with the experimental findings.[13] The demonstrated excellent predictive power of the CFD model suggests that inhalation delivery systems for *in vivo* selective respiratory drug delivery can potentially be designed based on observable magnetic and aerosol properties. The magnetic targeted carriers (MTCs) have also been proposed and developed to submicron-sized ferromagnetic particle preparation containing relaxant drugs for local anaesthesia and improve targeting efficiency of pharmaceutical agents in the treatment of carcinoma in rabbits.[14] Furthermore, recent clinical research shows that exposure to iron nanoparticles increases endothelial cell permeability caused by oxidative stress resulting from reactive oxygen material.[15] This phenomenon offers fascinating opportunities for magnetic beads in personalised cancer therapies.

Cell and gene therapy

Cell therapies are based on biological agents involving stem cells or immune cells to be administered to patients for the treatment of several diseases. The development of non-invasive imaging techniques to monitor cell migration to target tissues is of particular importance for cell-based therapies. Monitoring magnetically labelled cells with magnetic resonance imaging (MRI) has increased our understanding of cellular migration and the pathophysiology of diseases in experimental models and *in vivo.*[16] Recently, the potential clinical application of anionic magnetic nanoparticles (AMNPs), ultrasmall paramagnetic iron oxides (USPIOs) and super paramagnetic iron oxide nanoparticles (SPIONs) has been demonstrated to visualise cell migration during MRI monitoring of cellular therapies.[17-18] These agents cause a strong source of contrast resulting from the increased differences in signal intensities that each tissue produces in response to the applied radio frequency pulses. Recent reports describe utilising small contrast agents to track the migration of stem cells, immune cells and other cells in numerous animal models of malignancy, angiogenesis, ischemia, organ failure, autoimmune diseases and transplantation rejection.[17] Tracking labelled cells using magnetic particles with MRI has clearly demonstrated its usefulness in evaluating promising cell-based therapies in preclinical models. However, their translation to the clinical practice is being considered in the near future.[17]

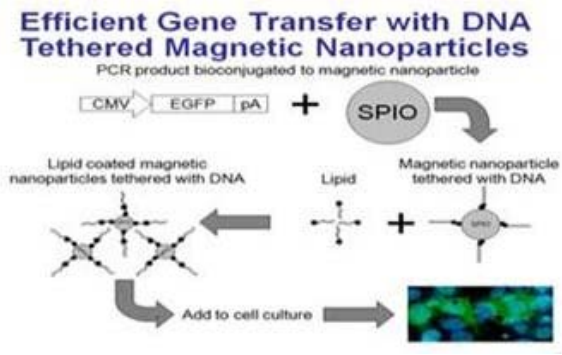

Figure 5: Efficient gene transfer with DNA tethered MNPs

The successful magnetic microsphere delivery approach has been used in gene therapy to reduce the incidence of adverse events, particularly in the case of viral and retroviral vectors. Gene therapy is a potential therapeutic tool that requires effective gene delivery. However, the most common methods for delivering genes into cell is associated with two potential dangers: oncogenesis and inflammation. This therapeutic strategy, based on magnetic technology, is an alternative method of viral gene delivery. Moreover, magnetic reporter genes inserted into cells result in expression of iron storage proteins that can be detected by MRI. The novel approach for improving viral DNA delivery system has been studied using modified cationic chitosan-coated iron oxide nanoparticles (*Nac-6-IOPs*).[19] The advantage of this system is the reduction of viral doses, time of infection and viral side effects. In another study, the enhancement of non-viral gene transfer was achieved by Lipofectamine™ 2000 or cationic lipid 67 (GL67)/plasmid DNA (pDNA) liposome complexes coupled with super paramagnetic particle.[20] A novel non-viral delivery system based on a polyethylenimine coated on the surface of bacterial magnetic nanoparticles (BMPs) has been developed with high transfection efficiency and low toxicity, which presents an attractive strategy for gene therapy and DNA vaccination.[21] The nanoparticle technology is highly novel and offers many possibilities for future development. Although the particles possess useful properties for biomedical applications, they also carry potential health risks. Generally, the nanomaterials pose many new questions on risk assessment that are not yet completely answered. Thus, a reliable risk assessment related to human health and environment and safety evaluation of these materials should be performed for all

in vivo studies. In 2007, the Food and Drug Administration issued a report to consider developing guidance for regulation of products that contain nanoscale materials.[22]

Radionuclide Delivery

Radionuclides (e.g., β–emitters, β is the symbol used to denote an electron) can also be attached to the magnetic particles and this system can then be targeted in the same way as described in the previous section on drug delivery, since the radionuclide does not have to be released in the same way as the drug; one restriction of drug delivery, i.e., control of drug release, is absent.

Magnetic Separation

Magnetic particles can be used to separate entities from their surroundings so that the surroundings can be purified or to concentrate the entities for further study.[37] This use is based on the difference in the susceptibility between a magnetically labelled entity and the surrounding medium. Examples of the use of this principle are magnetic cell sorting for cellular therapy and immunoassay (which is a process that measures and identifies a specific biological substance such as an antigen). Entities that can be labelled include cells, bacteria and some types of vesicles. The first step is to label the entities with the particles followed by the separation of the labelled entities by magnetic separation. Usually coated particles will be used; the coating will help to bind the particles to the entities such as cells. Specific sites on the cell surfaces can be targeted for attachment by antibodies; this works as a labelling procedure since antibodies bind to their matching antigen. In order to separate out these labelled entities we can use a magnetic field gradient which can attract and "hold" the entities in specific regions, followed by removal of these entities. This method has been applied to the selection of tumour cells from blood as well as to isolate enzymes, DNA and RNA from various sources including body fluids.

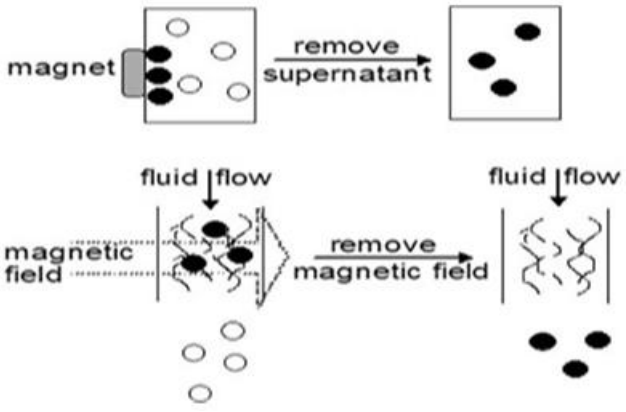

Figure 6: The standard methods of magnetic separation

(*a*) a magnet is attached to the container wall of a solution of magnetically tagged (•) and unwanted (◦) biomaterials. The tagged particles are gathered by the magnet, and the unwanted supernatant solution is removed. In (*b*) a solution containing tagged and unwanted biomaterials flows continuously through a region of strong magnetic field gradient, often provided by packing the column with steel wool, which captures the tagged particles. Thereafter the tagged particles are recovered by removing the field and flushing through with water.

Artificial Muscle

There have been various attempts to synthesize artificial muscles; attempts range from a robot-like metallic actuator to a more advanced soft actuator. Hydrogels, which are cross linked polymer networks swollen with water, can be used to make soft actuators. However, most gels are relatively homogenous materials which shrink or swell uniformly, with no dramatic change in shape. Therefore there is a need to improve the response of gels. Magnetic field sensitive gels in which magnetic particles of colloidal size are dispersed and incorporated into the gels have been developed. These ferrogels combine the magnetic properties of magnetic fillers and the elastic properties of hydrogel. Shape distortion occurs very quickly and disappears abruptly when the external magnetic field is applied and removed, respectively.

Future Prospects

Research is being conducted into magnetic twisting cytometry, a process in which ferromagnetic microspheres are bound to specific receptors on a cell wall. Changing the direction of an applied magnetic field twists the microsphere by a measurable amount, which can then be related to the mechanical properties of the cell membrane and cytoskeleton.[38–42] Magnetic nanoparticles are also being tested for tissue engineering applications, for example, in the mechanical conditioning of cells growing in culture.[43–45] In such systems magnetic particles are attached to either the cell membrane, or to mechanosensitive ion channels in the membrane, and a magnetic force is applied which activates the channels and initiates biochemical reactions within the cell, thereby promoting the growth of e.g. functional bone and cartilage. A third example is magnetic bio sensing: using magnetic nanoparticles coupled to analyte-specific molecules to detect target molecules via the crosslinking between coatings and the resultant aggregation of the magnetic particles, monitored by changes in proton relaxation times on a bench-top nuclear magnetic resonance system.[46-47]

Drug delivery via coating of nanoparticles is currently undergoing preliminary human trials, after successful tests in animals, with promising results, but it will be some time before it will be clinically available; and hyperthermia treatment of tumours is not yet accessible in humans, despite having been proven to be effective in animals. This is especially so if the goal is to transfer a procedure that has largely been the subject of *in vitro* or animal *in vivo* testing into a human *in vivo* therapy.[48]

In short, one of the biggest challenges in biomedical applications of magnetic nanoparticles lies in dealing with the issue of technology transfer. There are opportunities in this respect for more interdisciplinary approaches, for

example, to ensure that the laboratory based experiments can more explicitly emulate the expected conditions that would be encountered *in vivo*.[49] There is also scope for significant contributions via the mathematical modelling of complex systems, with the objective of understanding more specifically the full gamut of physical phenomena and effects that together determine whether, in the final analysis, a given application will be successful.

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