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Remote magnetic actuation of cell signalling for tissue engineering

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Abstract

Magnetic nanoparticles (MNP) are extremely versatile tools in bioengineering and medicine with diverse uses ranging from magnetic resonance contrast agents to drug delivery vehicles. Recently, MNP have been adapted to target and regulate cell signalling pathways for control of cell behaviour. This approach has been applied to stem and progenitor cells to orchestrate tissue development in tissue engineering. This review introduces the bio-functionalisation mechanisms for MNP and highlights the recent advances in MNP-mediated cell signalling activation. We also explore how the application of this technology has novel uses for stem cell control in the context of tissue engineering and regenerative medicine.

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Magnetic nanoparticles, Mechanotransduction, Cell signalling, Tissue engineering.

Introduction

Tissue engineering and regenerative medicine (TERM) is a multidisciplinary field that aims to replace or regenerate damaged host tissue and restore organ architecture and function. This is principally achieved by combining biomaterials and stem cells with biochemical cues provided by growth factors. However, an often-overlooked aspect of tissue regeneration is the role of biomechanics. The mechanical environment may be replicated *in vitro* using bioreactor systems that impart physiologically relevant mechanical cues and provide optimal tissue growth environments that mimic the developmental niche *in vivo*. By combining these factors this enables implantable TE products to integrate with host tissue more effectively, promote stem cell differentiation, vascularisation, new tissue formation and ultimately repair of damaged tissue. Since the term 'tissue engineering' was introduced in the 1980's [1], the field has expanded to a point where new therapies are having an impact in the clinic with tangible benefits for patients, especially with conditions which previously had limited treatment options. As a result, the TERM industry is now estimated to be worth £3.6 billion and employs around 14,000 people [2].

TERM is underpinned by an array of enabling technologies that have developed in conjunction with these novel therapies. One such example is magnetic nanoparticles (MNP), which have emerged as innovative and adaptable tools in medicine. MNP have varied biomedical applications ranging from diagnostics and magnetic resonance imaging (MRI) contrast agents [3] to drug delivery vehicles [4]. A key factor in the use of the MNPs is the ability to remotely control the movement and tagging of these particles and cells [5,6]. A more recent application involves the deployment of these nano tools to remotely modulate cell signalling pathways, using external magnetic fields to induce a torque or pull on the MNP. The resulting force on the MNP is transduced to the target receptor or channel through the process of mechanotransduction [7].

Mechanotransduction pathways control fundamental cellular processes such as proliferation, differentiation, migration and apoptosis. As such these pathways are of special interest for manipulation in cell and tissue engineering [8]. Multiple proof-of-concept studies have demonstrated that MNP can be targeted to specific mechanosensitive receptors and ion channels and that mechanotransduction pathways can be activated using magnetic fields. In the field of TE this approach has been used to direct stem cell behaviour and augment tissue formation [7]. The potential opportunities and benefits of using MNP to promote optimal tissue regeneration are huge and represent a fast-growing area of research. The overarching aim of this approach is to create optimised tissue constructs, which can be complex and loaded with physiologically relevant forces

Abbreviations

MNP	Magnetic Nanoparticle
TERM	Tissue Engineering and Regenerative Medicine
TE	Tissue Engineering
NO	Nitric Oxide
EDAC	Ethyl-3-(3-dimethylaminopropyl)-carbodiimide
NHS	N-hydroxy succinimide
MRI	Magnetic resonance imaging
DNP	dinitrophenyl
ECM	Extracellular matrix
ROCK	Rho-associated protein kinase
ERK	extracellular-signal-regulated kinases
TREK1	Twik-related potassium channel 1
PDGFR	Platelet derived growth factor receptor
TGF-β	Transforming growth factor-β
RTK	Receptor Tyrosine Kinase
SMA	Smooth muscle actin
BMP	bone morphogenetic proteins
GDF	growth and differentiation factors
EGF	Epidermal growth factor
Cbfa1	core binding factor alpha1

whilst retaining the appropriate mechanical properties. This review will explore the application of magnetic nanoparticles for the control of cell signalling pathways, their roles in directing cell behaviour and will highlight their applications in cell and tissue engineering.

Mechanotransduction in tissue engineering

Mechanotransduction is the process of converting a physical force into a biological signal. This process is instrumental in development and governs many processes during embryogenesis and tissue patterning. At the molecular level mechanotransduction occurs through mechano-sensitive proteins and receptors which include adhesion proteins, ion channels and growth factor receptors. The central premise behind mechanotransduction is that applied forces induce conformational changes in mechano-sensitive proteins. This leads to changes in their activation state and subsequent signalling through intracellular signalling cascades [9–11]. Activation of mechanosensors leads to downstream activation of second messenger systems and signal transduction pathways including Nitric oxide (NO), Calcium signalling and a wide range of growth factor signalling pathways [10,12]. Signalling activation ultimately leads to transcription of mechano-sensitive genes that leads to changes in cell behaviour such as cell proliferation, migration, differentiation and the production of signalling factors [13,14]. In the context of tissue engineering, mechanical forces such as shear stress, compression and tension are all fundamental physical stimuli which are required for physiologically relevant tissue formation, particularly for musculoskeletal tissues [15]. Mechanical conditioning of tissue constructs is achievable using bioreactor systems that replicate and convey these physical forces. However, these systems often require mechanically stiff materials for load applications. More recently MNP have been incorporated into these conditioning systems as tools to tag, track and guide cells, but also as agents to precisely stimulate mechanotransduction pathways without the need for strong scaffolds. In addition, complex loads can be applied spatially across a TE construct using MNPs of varying size and different forces applied to target receptors. This is not possible using other bioreactor systems.

Magnetic nanoparticles

The development of nano-scale technologies has opened up new fields in science and technology as well as new diagnostic tools and treatments in medicine [16]. One particularly versatile set of tools in the nanotechnology toolkit are MNP. In the broadest sense these are particles less than 1 μm in size with dimensions in the nanometre scale. MNP with an iron-oxide core volume in the low nanometre range (approximately 20 μm^3) display superparamagnetic properties, which is characterised by a net zero magnetisation of the magnetic core in the absence of an applied magnetic field. Superparamagnetic nanoparticles have proven to be useful in a wide range of biomedical fields including medical imaging, diagnostics and biotechnology [17]. The magnetic properties of MNP also aides their spatial manipulation, making them particularly useful as engineering tools and is enabling new applications in TERM [18]. The materials that make up the magnetic core of MNP can also be easily tuned to produce particles with different magnetic susceptibilities. In this way MNP can be easily tailored to the desired application.

Composition

The physical properties of MNP such as size, composition, surface functionality and biocompatibility can be easily tailored to the intended purpose during production [19]. Common methods to synthesize MNP include solution precipitation, co-precipitation, thermal decomposition, polyol synthesis, microemulsion, hydrothermal, and biological synthesis. These MNP synthesis methods have been described in detail by Gul et al. [20].

MNP contain a core of magnetic material for which iron oxide in the form of magnetite or maghemite is commonly used [21]. The use of iron oxide based compounds in medicine is advantageous as there are established metabolic pathways to metabolise and excrete these substances which also aids their biocompatibility when used for *in vivo* applications [22]. After synthesis of the MNP core, it is necessary to apply a surface coating in order to stabilise the particle and help prevent agglomeration. The surface coating can be modified to vary the physical properties of the MNP

such as the surface charge, topography and protein binding capacity. For *in vivo* applications it also becomes necessary to coat the MNP with biocompatible substances such as serum proteins (e.g. albumin), polysaccharides (e.g. dextran or pullulan), or materials such as silica, which helps to protect the magnetic core and reduce toxicity in the host [23–25]. The biocompatible coating can be further functionalised with biomolecules using a range of chemical reactions.

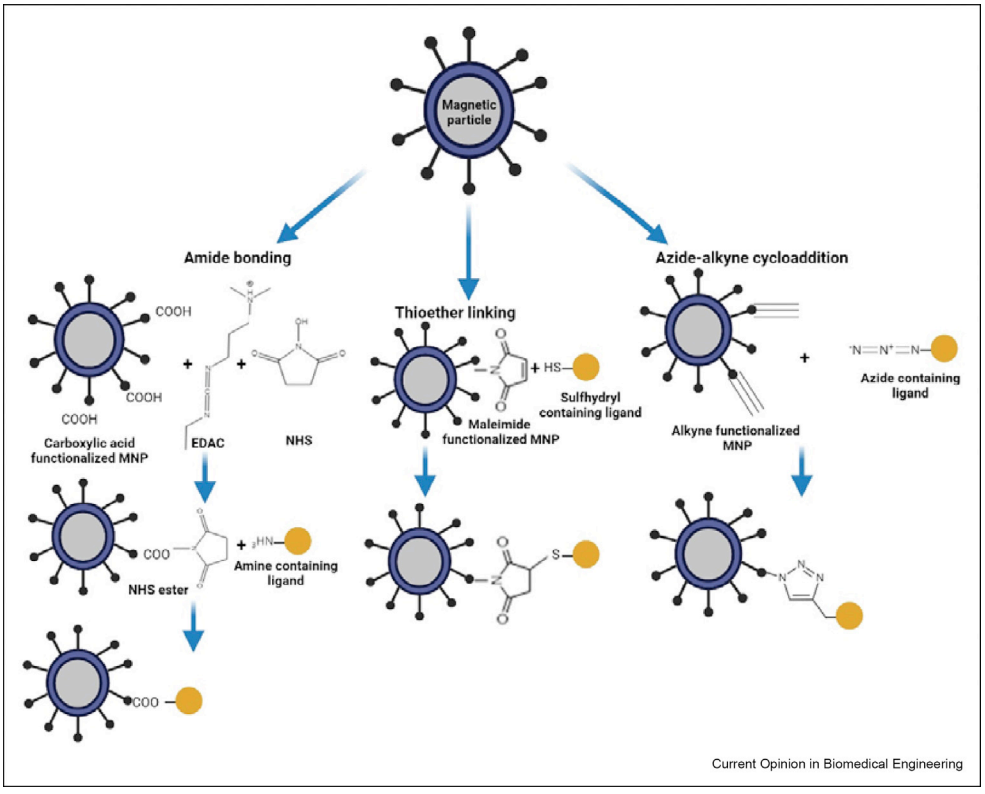
Magnetisation

MNP, due to their size, display superparamagnetic properties resulting in a net-zero magnetisation of the MNP core in the absence of an externally applied magnetic field (remnant magnetisation, $M_0 = 0$ kAm^{-1}). In the presence of an applied magnetic field the MNP core is readily magnetised and gains a total magnetisation equal to the saturation magnetisation of the material (typical values range between 320 and 350 kAm^{-1} for ferrite based MNP). Due to small applied magnetic field required (typically less than 1 mT), inducing a large magnetisation response the resulting susceptibility of MNPs is higher than that found commonly in other ferromagnetic samples.

Functionalisation

One of the main advantages of MNP is their tuneabilty for different biomedical applications. MNP may be functionalised with a wide range of biochemical ligands such as peptides, antibodies, drugs, small molecules, or DNA for targeted delivery of therapeutic drugs or genes [21,26]. These ligands are conjugated to the MNP via chemical groups present on the surface of the particle [27]. Reactive chemical groups such as carboxyl, amine, azide, alkyne, or sulfhydryl are all common functional groups for covalent ligand binding. Some common covalent binding mechanisms for MNP-ligand conjugation are illustrated in Figure 1. Non-covalent binding methods are also available and are based on affinity binding proteins, for example, avidin and biotin, or electrostatic interactions between oppositely charged species to promote MNP-ligand association. For a broader review of these conjugation methods see Conde et al. [28].

Figure 1



MNP covalent biofunctionalisation mechanisms. MNP may be conjugated to biological ligands by Amide bonding when using carboxylic acid functionalised MNP and amine containing ligands. EDAC is used to form an O-acylisourea intermediate (not shown), NHS is reacted with this intermediate to form an NHS ester which then reacts with primary amines contained within biomolecules to form a stable amide bond. Thioether linking can be used to link maleimide functionalised MNP to sulfhydryl groups contained within proteins to form a thioether bond. Click chemistry based reactions such as azide–alkyne cycloaddition may be used to bind alkyne functionalised MNP to azide functionalised ligands which results in a stable triazole. Figure created with BioRender.com.

MNP and magnetic fields as remote force providers

A key benefit of using MNP as mechanical actuators their strong response to external magnetic fields, which allows MNP labelled cells and tissues to be conditioned remotely. This helps to maintain physiological conditions and retains the sterile environment of the culture. In addition, parameters such as the speed, frequency and duration of magnetic stimulation can all be easily controlled and spatially delivered [29–31]. The non-invasive nature of magnetic field bioreactor systems also means that there are no obstructions in nutrient flow, as is common with other force generating systems that apply direct compression/shear stress. This aspect can be particularly problematic when translating mechanical stimulation regimes *in vivo*. Another benefit is the capability of altering force directions. Whilst traditional stimulation systems transmit force in one direction, magnetic field stimulation can conceivably be applied from multiple directions.

Having said this, there are some drawbacks of these systems. Currently, magnetic field stimulation has a limited range of tissue penetration and scaled up approaches for tissue engineered constructs are also restricted to multi-well tissue culture plastic applications [25,32], although magnetic devices for stimulation of MNP *in vivo* are being developed [33]. The limitation of magnetic field penetration depth arises due to the nature of exponential decay of magnetic fields at a distance. To overcome this different approaches can be taken in the construction of magnetic devices, including the use of halbach arrays [34,35] and tailoring the shape of the permanent magnets [36].

The magnitude of force exerted by MNP are relatively small in comparison to the forces imparted by other mechanical conditioning systems. However, MNP mediated forces are applied directly to distinct regions of proteins/cells which reduces off target effects and enables smaller forces to be sufficient to illicit biologically relevant changes.

$$F = \frac{1}{\mu_0}\chi V \boldsymbol{B}(\nabla B) \qquad \text{Equation 1}$$

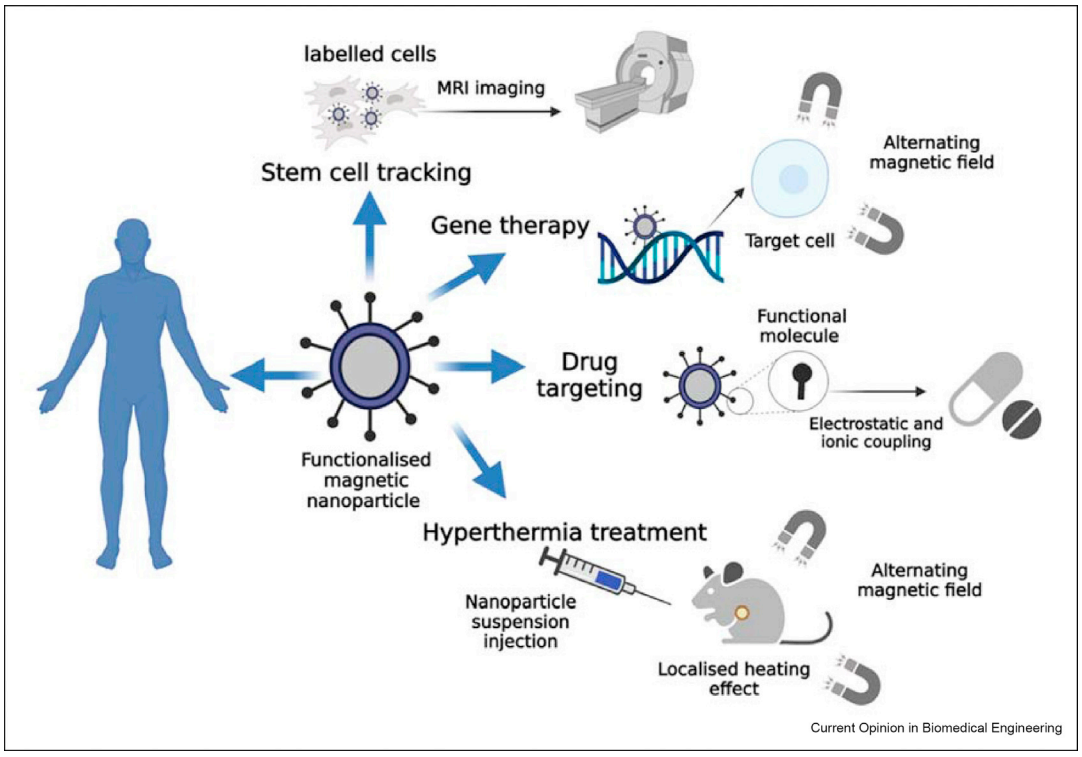
The forces provided by MNP can be modelled according to Equation (1) [37]. The total force is related to the volume (V) and magnetic susceptibility (χ) of the core material and the strength and gradient of the magnetic field ($\boldsymbol{B}$ and $\nabla\boldsymbol{B}$ respectively). Flux densities between 0.025 and 1T and a field gradient up to 10^4 T/m are typically required to generate the required force on MNP [33,38]. It has been shown that forces in the order of piconewtons (pN) are able to induce changes in cellular signalling activity. For example, both 10–15 nm magnetite MNP and larger 250 nm Ferric-based MNP have been estimated to exert forces between 0.2 and 2 pN per particle depending on the applied magnetic flux density and field gradient. These pico newton level forces have been shown to induce EGFR clustering, open and close TREK1 channels and activate PDGF receptors, respectively. The force transduced by larger microparticles has also been studied, both ferro-based and chromium oxide-based microparticles (4.0–4.5 μm diameter) have been shown to exert 6–30 pN forces on integrins, which was enough to activate BK channels, hyperpolarise MSC and induce osteogenic gene expression in human osteoblasts [9,30,39–41]. The obvious benefit of this strategy is that the force transduced by the MNP can easily be tuned for the mechanosensitive target by altering parameters such as magnetic field strength, particle size or MNP dosage [25].

Magnetic nanoparticles in regenerative medicine

MNP are particularly useful tools for biomedical applications due to the level of control that can be excised over particle size, charge, surface coating, magnetisation and dosage. This enables them to be tailored towards the desired target or procedure [19] and used for the growing field of regenerative medicine [42]. In the field of nano-medicine MNP have been utilised for diverse applications such as targeted gene or drug delivery and hyperthermia treatment of tumours. For regenerative medicine, the high relaxation times and slow renal clearance of MNP make them particularly useful for stem cell tracking by MRI after uptake by cells [27]. The magnetic properties of MNP tagged cells can also be exploited to direct cells to sites of injury using external magnets in order to direct repair [43,44]. The potential benefits of this strategy in regenerative medicine which requires targeted regeneration at precise sites are huge. At the cellular scale, cell migration and direction can also be controlled using internalised MNP. Bongaerts et al. recently demonstrated directional control over cell migration and neurite outgrowth towards arrays of magnetic pillars after passive uptake of MNP by differentiating PC12 cells [38]. MNP can also be localised to distinct cell compartments or organelles. Magnetic fields provided by magnetic tips have been used to accumulate MNP in neuronal fibres and influence the direction of their outgrowth [45,46]. Techniques such as this may become key tools in the development of cell therapies for Parkinsons disease.

Spatial control of cell aggregation, formation of heterotypic cell layers and incorporation of growth factor conjugated MNP into scaffolds are also novel uses of MNP tagged cells to improve tissue formation [47]. The key applications of MNP in biomedicine are illustrated in Figure 2 and a comprehensive review on the development for cell therapies is provided by Harrison *et al.*, 2018 [48].

Figure 2



The applications of magnetic nanoparticles in regenerative medicine. Magnetic hyperthermia induced through injection of nanoparticle suspension, and application of a magnetic field to induce localised heating. Functionalised nanoparticles also allows electrostatic and ionic coupling to drugs, with magnetic field gradients utilised to provide localised treatment. MNP may be used for gene therapy, the coupling of MNPs with gene vectors, and thus amplifying gene transfer in the presence of a magnetic field. Cell labelling via MNP allows magnetic resonance imaging. Figure created with BioRender.com.

Magnetic actuation of cell signalling

An emerging application of MNP in biomedical research is magnetic activation of cell signalling pathways. Activation is achieved by first functionalising the MNP with biomolecules allowing targeting of the MNP to specific cell receptors coupled to certain signalling pathways. Signalling activity can then be modulated by alternating the magnetic fields. Under these conditions, high gradient magnetic fields induce a torque on the MNP resulting in a pull down, drag or oscillation in a given direction relative to the direction of the applied magnetic field. The translational force on the MNP results in tension and deformation of the cell membrane or mechanosensitive receptor to which the MNP is attached [25,49]. In turn, the conformational changes to the target protein/receptor initiate clustering of multiple receptors, open ion channels or open binding sites for regulator proteins that initiates downstream signalling pathways (illustrated in Figure 3). An example is the targeting of the activin receptor type IIA (ActRIIA) in human adipose stem cells (hASCs), using anti-ActRIIA functionalised MNPs, externally activated through an oscillating magnetic bioreactor. Upon activation, phosphorylation of Smad2/3 was induced which regulated tenogenic transcriptional responses [50].

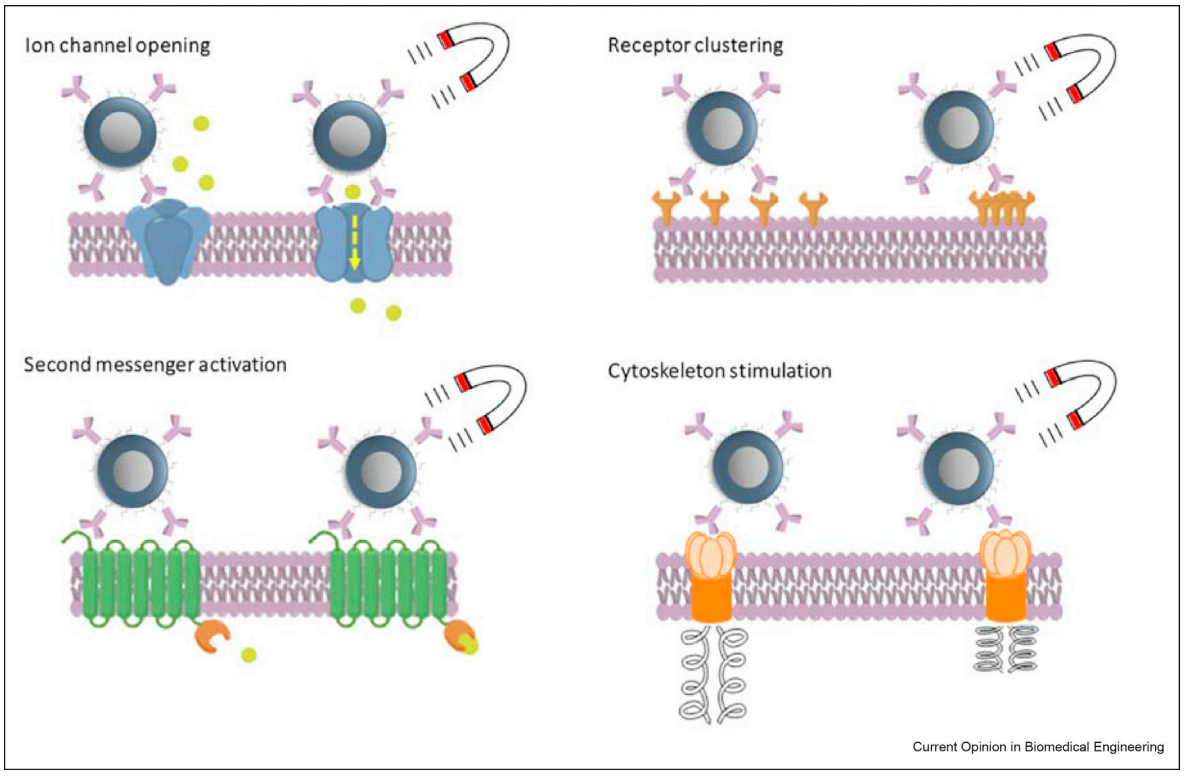
The example above demonstrates the proof of concept for using MNP to control rudimentary cell behaviour. The use of MNP to target distinct receptors for control of signalling pathways relevant to tissue engineering are summarised in Table 1.

Integrins

Integrins are a family of cell surface receptors involved in cell adhesion that link the extracellular matrix (ECM) to the cell cytoskeleton via focal adhesions. These receptors facilitate mechanotransduction of external mechanical forces to the nucleus via the cell cytoskeleton. Integrin-mediated signalling has been implicated in the determination of Cell fate, polarity, proliferation, and differentiation through multiple intracellular pathways including Rho, Rho-associated protein kinase (ROCK), extracellular-signal-regulated kinases (ERK) and Akt signalling amongst others [64].

The RGD binding domain is prominently expressed by most integrin receptors. This enables proteins or

Figure 3



MNP mediated mechanoactivation mechanisms. A) MNP bound to ion channels can induce conformational changes in response to magnetic toque that results in ion flux. B) Receptor clustering at the cell membrane can be initiated by MNP binding and sequestration of multiple receptor targets. C) Magnetic stimulation of MNP bound receptors can alter the activation state of receptors that leads to second messenger signalling. D) Direct mechanotransduction through actin tethered proteins leads to activation of mechanotransduction pathways linked to the cytoskeleton. Figure created using ChemDraw (PerkinElmer).

peptides containing the RGD motif to bind integrins at an interface between the α and β subunits [65,66]. MNP can be easily functionalised with RGD peptides to allow attachment of MNP to integrin receptors and enable direct magnetic stimulation. This approach has been shown to enhance proteoglycan and extracellular matrix protein synthesis by hMSC [51] and increase endothelin-1 expression in endothelial cells [53]. In another study, RGD functionalised MNP tethered to glass substrates promoted MSC adhesion and led to increased RUNX and ALP expression when exposed to oscillating magnetic fields [52]. In human osteoblasts integrin stimulation was shown to induce rapid changes in intra cellular calcium signalling over short-term stimulation [54], whilst long-term stimulation upregulated osteocalcin expression and increased mineralised matrix production [29]. Furthermore, integrin stimulation using MNP functionalised with antibodies to α2 or β1 subunits has been shown to instigate changes in the actin cytoskeleton, formation of adhesion complexes, increased MAPK activity and generation of calcium fluxes in U-2 OS cells [55,56] and induce AKT phosphorylation in human tendon cells via integrin-linked kinase (ILK) [57]. However, one caveat to note when considering integrin targeting is the potentially limiting effects of MNP internalisation. Removal of MNP from the cell surface could offset the mechanical stimulus on the cells which may moderate stem cell differentiation [31].

Ion channels

Mechanotransduction is also mediated through stretch activated ion channels. Mechanical perturbation of the cell membrane causes conformational changes in ion channels, which results in opening and closing of the channels and ion flux across the cell membrane that results in changes to cell activity [10]. The Twik-related potassium channel 1 (TREK1), is a two pore domain outwardly rectifying potassium channel, expressed in a wide range of tissues, that regulates the resting membrane potential of the cell [67]. TREK1 is sensitive to a wide range of physical and chemical stimuli including heat, lipids, and pH changes. It is also known to be mechanoresponsive to forces such as stretch which

Table 1

Summary of MNP-mediated magnetic activation targets for signalling activation and tissue engineering.

Target	Cell type	MNP	Effect	Reference
Integrins	hMSC	250 nm silica MNP functionalised with RGD	Proteoglycan, ECM production	[51]
	hMSC	Amino-silica SPION	Increased RUNX2 and ALP expression	[52]
	HUVEC	4.5 μm Ferromagnetic nanoparticles functionalised with RGD	Endothelin-1 expression	[53]
	Human Osteoblasts	4–4.5 μm ferromagnetic CrO nanoparticles	Prostaglandin E2 production, osteopontin expression, matrix mineralisation	[29]
	Human Osteoblasts, MG63	4.5 μm ferromagnetic particles or 2.7 μm paramagnetic particles functionalised with RGD	Calcium signalling	[54]
	U-2 OS	2.8 μm streptavidin Paramagnetic particles functionalised with biotinylated anti-b1 or anti-a2	Tyrosine phosphorylation, MAPK activation, cytoskeleton rearrangement	[55]
	U-2 OS	Paramagnetic microbeads functionalised with anti-integrin	Calcium flux	[56]
	Human tendon cells	FerroMagnetic particles funtionalised with RGDS or anti-β1 integrin	AKT phosphorylation	[57]
TREK1	hMSC	250 nm silica MNP functionalised with anti-TREK1	Osteogenic gene expression, collagen 1 production	[51]
	hMSC	250 nm dextran MNP functionalised with anti-TREK1	Mineralisation in collagen hydrogels and ex vivo femurs. Collagen deposition and mineralisation in neighbour cells (paracrine effect)	[58,59]
	oMSC	250 nm dextran MNP functionalised with anti-TREK1	Mineralisation, bone formation, matrix protein, and ALP production	[33]
PDGFR	hMSC	250 nm dextran MNP functionalised with anti-PDGFRα or anti-PDGFRβ	Upregulated osteogenic genes, increased mineral/matrix ratio. PDGFR phosphorylation, increased SMA expression	[40,60]
Frizzled (Wnt pathway)	hMSC	250 nm dextran MNP functionalised with peptide UM206	Wnt signalling activation, frizzled clustering, matrix production, mineralisation in *ex vivo* femurs	[61]
	SH-SY5Y	250 nm dextran MNP functionalised with peptide UM206	Signalling activation, increased dopaminergic marker expression	[49]
Activin (TGF-B pathway)	hASC	250 nm dextran MNP functionalised with anti-Activin IIA	SMAD2/3 phosphorylation, increased tenomodulin expression, downregulation of non-tendon related genes, increased tenogenic differentiation	[50,62]
EGFR	A431	10–15 nm streptavidin MNP functionalised with biotinylated anti-EGFR	EGFR clustering, phosphorylation and Shc recruitment	[41]
Tie2/angiopoietin	HEK293 or HUVEC	15 nm zinc-doped ferrite MNP functionalised with anti-Tie2	HEK293: Tie2, Akt phosphorylation, ROS production HUVEC: Cell morphology changes, tubular structure formation	[63]

induce opening and closing of these channels and results in potassium flux across the cell membrane and polarisation of the cell [68,69].

Multiple proof of concept studies have demonstrated that TREK channels are also amenable to mechanical stimulation using MNP. Remote stimulation of mechanosensitive regions of TREK1 using MNP targeted to an extracellular loop of the channel were able to cause membrane depolarisation consistent with ion channel activation. In contrast, this response was not seen when control particles or magnetic field stimulation were applied alone [39]. Remote activation of TREK1 using MNP has been shown to induce MSC osteogenic differentiation *in vitro*, with synergistic increases when administered alongside BMP2 [51,58]. Stimulation of TREK has also been shown to exert paracrine effects in neighbouring cells. In one study increased collagen deposition and mineralisation in the non-stimulated cells surrounding the mechano-activated MSC population was observed. This suggests that mechano-activation of stem cells may lead to release of cytokines or growth factors that directs differentiation in nearby cells [59].

Growth factor receptor activation

Growth factor signalling pathways have also been implicated in mechanotransduction, under these circumstances physical cues can trigger signalling activity of many pathways. Some examples of the pathways that have been targeted and shown to be mechano-responsive through various mechanisms include the Platelet derived growth factor receptor (PDGFR) family, the Wnt pathway, transforming growth factor-β (TGF-β) and epidermal growth factor (EGF) receptors.

Platelet derived growth factor

PDGF receptors belong to the receptor tyrosine kinase (RTK) family and play crucial roles in embryonic development and regulate many cell processes including migration and proliferation. PDGF ligands signal through homo or hetero dimers of PDGF receptors alpha (PDGFRα) and beta (PDGFRβ) [70,71]. PDGF receptors have also been shown to be mechanoresponsive [72]. Magnetic stimulation of PDGFRα with antibody-functionalised MNP resulted in receptor phosphorylation and enhanced Smooth Muscle Actin (SMA) expression in hMSC [40]. This approach was also shown to enhance osteogenic marker expression and increase the mineral content and mineral to matrix ratio when hMSC were subjected to three weeks of magnetic stimulation *in vitro* [60].

Wnt pathway

Wnt signalling pathways play a central role in the regulation of cellular and biological functions. At the cellular level Wnts regulate cell polarity, asymmetric cell division as well as proliferation and differentiation in the stem cell niche. Wnt pathways therefore play important roles during development, embryogenesis, tissue formation and tissue homeostasis [73,74].

Recent studies have demonstrated targeting and remote activation of the primary Wnt receptor Frizzled using MNP functionalised with antibodies or synthetic peptides mimicking Wnt3a. Anti-Frizzled2-MNP mediated activation of Frizzled 2 was shown to initiate downstream activation of Wnt signalling in human Mesenchymal stem cells, which was enhanced with magnetic field stimulation. Signalling activation was subsequently shown to be an osteoconductive cue during osteogenic differentiation of hMSC that resulted in increased osteogenic markers and matrix production *in vitro* [61,75]. Recently, this approach has also been found to have applications in neuronal tissue engineering. Magnetic stimulation of Frizzled in the SH-SY5Y neuronal cell line using MNP coated with Wnt mimicking peptides had a synergistic effect on the expression of the dopaminergic marker tyrosine hydroxylase when cultured in neuronal differentiation media [49].

Transforming growth factor-β

The transforming growth factor-β (TGFβ) superfamily is a diverse group of signalling factors that includes TGFβs, activins, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) amongst others. These signalling proteins trigger SMAD2/3 activation that leads to transcription of a wide variety of genes that regulate cell fate. The activin A pathway has recently been targeted for mechano-activation for the purpose of tendon tissue engineering. In this approach MNP were first coated with activin receptor type IIA antibodies, cells were then mechanically stimulated using magnetic fields provided by a magnetic force bioreactor or by magnetically responsive polymer/MNP scaffolds. These activation strategies both led to SMAD2/3 phosphorylation, increased expression of tendon markers including tenomodulin and tenogenic differentiation of human adipose stem cells [50,62].

Epidermal growth factor

Epidermal growth factor (EGF) signalling is involved in the regulation of a host of cell functions including proliferation, apoptosis, differentiation, and migration. EGF receptor ligands signal through members of the EGFR family which together form another branch of RTK's. Ligand induced receptor dimerisation is a key part of pathway activation and is required to activate downstream mediators and signalling pathways that includes Ras/MAPK, PLC/PKC, PI3K/Akt, and STAT signalling [76]. Magnetic nanoparticles have also been used to cluster and activate EGFR signalling. Bharde et al. used MNP functionalised with ligand blocking anti-EGFR antibodies and used magnetic fields to locally cluster EGFR in A431 cells. This resulted in the characteristic early signalling events such as receptor phosphorylation and Shc recruitment that are seen during ligand dependent EGFR signalling [41].

Tie2/angiopoietin

The Tie2/angiopoietin signalling pathway is involved with regulation of angiogenesis during blood vessel development. Angiopoietin binds to multiple Tie2 receptors to form active signalling clusters that activate pathways including Akt, ERK1/2 and increased ROS production. Clustering of Tie2 has also been achieved using 15 nm zinc-doped ferrite magnetic nanoparticles, which were coated with an anti-Tie2 antibody and aligned using permanent magnets. In Tie2 over-expressing HEK293 cells the approach was shown to trigger Tie2 and Akt phosphorylation and ROS production. Interestingly when this was applied to HUVEC cells, magnetic clustering caused changes in cell morphology with cells eventually forming tubular structures reminiscent of tubulogenesis [63].

Progress towards *in vivo* applications of magnetic particles in TERM

Remote activation TREK1 or Wnt signalling have both been shown to have beneficial effects on bone formation *in vitro*. Recent studies have also demonstrated the translatable potential of modulating these pathways within complex 3D and pre-clinical models. For example, in an *ex vivo* foetal chick femur model of bone development, magnetic activation of Frizzled receptors or TREK channels on hMSC that were injected into the epiphyseal regions of the femurs were shown to induce localised bone formation and secondary mineralisation areas at the injection sites [58,61]. This remote activation strategy has also been tested in *in vivo* models of bone/cartilage formation. Subcutaneous implants of MNP labelled hMSC encapsulated in hydrogels were performed in rats. Subsequent magnetic stimulation of the hydrogel constructs via TREK1 ion channels or integrins via RGD-MNP resulted in increased proteoglycan matrix, expression of core binding factor alpha1 (Cbfa1), extracellular matrix production and elevated expression of collagen type 1 and type 2 after 21 days both *in vitro* and *in vivo*. Importantly, the MNP labelled hMSC remained viable in this model [51]. The translation of this approach to large animal models has also been recently demonstrated. Remote magnetic stimulation of TREK1 channels on implanted MSC using a magnetic cuff attached to the limbs of sheep was shown to improve bone fill in an osteochondral defect model [33]. Whilst further extensive pre-clinical studies are required to push the technology towards the clinic, these current studies highlight the unique benefits that targeted MNP and remote magnetic activation strategies can provide for tissue engineering and bone tissue engineering in particular.

Conclusions and future perspectives

MNP have undoubtedly opened new avenues of research and are providing new clinical tools in medicine. In the burgeoning field of TERM, MNP now have a variety of previously unforeseen and unique applications. Recent work has demonstrated the value of MNP for cellular control, where their properties can be easily tuned, functionalised with varied biomolecules and targeted to a range of mechano-sensitive receptors. This unique approach allows for remote activation of signalling pathways to influence cell fate. The advantages of MNP to TERM are clear and include control of stem cell fate, direction of cell organisation and regulation of tissue formation at a distance which are all possible using this approach. The possibility now exists for these nano-tools to be incorporated alongside injectable cell therapies in an approach that is readily transferable to the clinic.

Author contribution

Michael Rotherham: Conceptualization, Tasmin Nahar: Writing – review & editing. Thomas Broomhall: Writing – review & editing. Neil Telling: Writing – review & editing, Supervision. Alicia El Haj: Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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