Review

The fabrication of iron oxide nanoparticle-nanofibre composites by electrospinning and their applications in tissue engineering

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Abbreviations: **IONP,** Iron oxide nanoparticle; **SPION,** superparamagnetic iron oxide nanoparticles; **NP,** nanoparticle; **MNP,** magnetic nanoparticle.

Abstract

This paper reviews the use of iron oxide nanoparticle-nanofibre composites in tissue engineering with a focus on the electrospinning technique. Electrospinning is an established method of scaffold fabrication offering a number of key advantages which include its facile nature, with electrospun materials offering a high surface area to volume ratio, potential for the release of drugs and antimicrobials, controllable fibre diameters and high porosity and permeability. A number of different techniques for the preparation of iron oxide nanoparticles including their functionalisation are discussed along with their applications in the biomedical field. The review then focusses on the fabrication of nanoparticle-nanofibre composite scaffolds formed using electrospinning. The advantages and disadvantages of current fabrication techniques are discussed including the fabrication of nanofibres using pre-synthesised nanoparticles and post-treatment synthesised nanoparticles. We demonstrate that emerging *in-situ* synthesis techniques show promise by offering a reduced number of steps and simpler procedures for the production of magnetic scaffolds. These scaffolds have a number of applications in tissue engineering, allowing for improved bone and tissue repair.

1. Introduction

Tissue engineering has made great advances in recent years combining both engineering and biology to repair, replace or regenerate parts of or whole tissue(s). Naturally, the applications of such technologies in medical science have the potential to treat a wide range of conditions. Many fundamental components of biological systems, including the extracellular matrix, exist and function at the nanoscale with sizes measured in billionths of a metre. Thus, technologies that can create nanoscale materials are essential in shaping and controlling systems that are designed to mimic tissue chemical and physical environments.

Electrospinning is a fabrication technique that has become one the preferred jetting methodologies for the fabrication of tissue engineering scaffolds (1–3). It is an established method for fabrication of polymer constructs that offers many advantages to tissue engineering that include the formation of nanoscale fibres,

which are analogues to the extra cellular matrix (ECM), the molecular architecture that provides structure to the tissue. In addition, electrospinning methods allow the incorporation of biologically active moieties into the polymer construct rendering the scaffold 'smarter'. The improved functionality of scaffolds allowed by electrospinning coupled with recent advances in scale of manufacture have led to a resurgence of interest in electrospinning. The incorporation of nanoparticles within electrospun fibres is a further method by which improved functionality can be programmed into polymer nanofibers. A key example of such improved functionality is magnetic scaffolds, where iron oxide nanoparticles (IONPs) are incorporated into scaffold structures for applications in tissue engineering, drug delivery and wound healing. This review will firstly discuss tissue engineering scaffolds and the techniques used for their fabrication, and then review the applications of IONPs before focussing on the development of iron oxide nanoparticle-nanofibre composites using electrospinning and review their potential applications.

2. Tissue Engineering scaffolds

Tissue engineering scaffolds require four key properties to allow them to support the three-dimensional formation of viable tissue (4). These are biocompatibility, biodegradability, tensile strength and scaffold architecture. The scaffold must provide a biocompatible 3D matrix supporting or enhancing cell proliferation and migration while allowing the uninhibited diffusion of nutrients and other cell media throughout the structure. It must also be compatible with the human body, without triggering an immune response when implanted. The material used must be biodegradable to allow the scaffold to degrade as the cells begin to form their own matrix. The mechanical properties of the scaffold should match that of the tissue in which the scaffold will be introduced (5). For example, in tissue engineered cartilage and bone, the scaffold will require specific tensile and compression strengths to withstand the load placed upon it by the patient without "shielding" osteoblast cells causing bone resorption (6). The overall scaffold architecture is also key, for example the axons of neurons require directional

growth and therefore scaffolds comprised of uniaxial fibre arrays have been shown to result in improved axonal outgrowth parallel to the direction of the fibres (7). This is also true for myocytes (8). Finally, the overall nonwoven structure requires a high degree of porosity, with a sufficient minimum pore size to allow the proliferation of cells and diffusion of cell media, cell-signalling stimuli, waste products and other required substances (4). Cell signalling, the communication between proximal cells, is also of great importance for tissue growth. It has been reported that porosity, pore size, interconnectivity and tensile strength are all important influences of cell signalling and differentiation (9).

Several different methods exist to produce scaffolds for tissue engineering. Most common are jetting methodologies such as aerodynamically assisted jetting/threading, pressure assisted/driven jetting/spinning (10), laser guided writing (11), inkjet printing (12), electrospraying (13) and electrospinning (14). Laser guided writing and ink jet printing have historically dominated the field, contributing much to the recognition of jetting technologies and their application (10). However, due to their micron-scale size fabrication limits which are much larger than the natural ECM they are designed to mimic, these technologies do not translate easily into the development of constructs for clinical application. Other methods such as electrospraying and electrospinning are currently undergoing a widespread revival of scientific investigation of chemical, physical and biological outputs achievable and are able to produce structures more similar in size to the natural ECM. These techniques have generated much scientific and commercial interest in fields such as tissue engineering, regenerative devices and drug delivery.

3. Electrospinning

The advantages of electrospinning for the fabrication of non-woven fibrous structures has meant that it has been applied in a diverse range of fields from regenerative medicine to filtration and water treatment (10,14–19). An electrospun scaffold can offer a number of desirable properties such as a high surface area to volume ratio, potential for the release of drugs and antimicrobials,

controllable fibre diameters, high porosity and permeability. In more recent years the emergence of new electrospinning technologies has ensured that fabrication is scalable and economically viable for high volume production.

Figure 1 is a schematic representation of the needle based electrospinning process. Electrospinning uses a high voltage power supply to create a large potential difference between a grounded "collector" structure and a polymer solution or melt being delivered at a constant rate through an aperture, such as a blunt end needle. As the voltage is increased the body of the polymer fluid becomes charged and electrostatic repulsion directly opposes surface tension, resulting in the normally spherical droplet at the aperture distending into a conical shape. This cone is referred to as the "Taylor" cone, after Sir Geoffrey Taylor who first mathematically modelled the phenomenon (20,21). At a critical voltage the electrostatic attractive force between the solution and the collector causes a jet of polymer solution to be expelled from the cone tip towards the grounded collector surface. This jet then undergoes a whipping instability and dries in flight as the solvent evaporates, depositing the nanofibres on the collector (14).

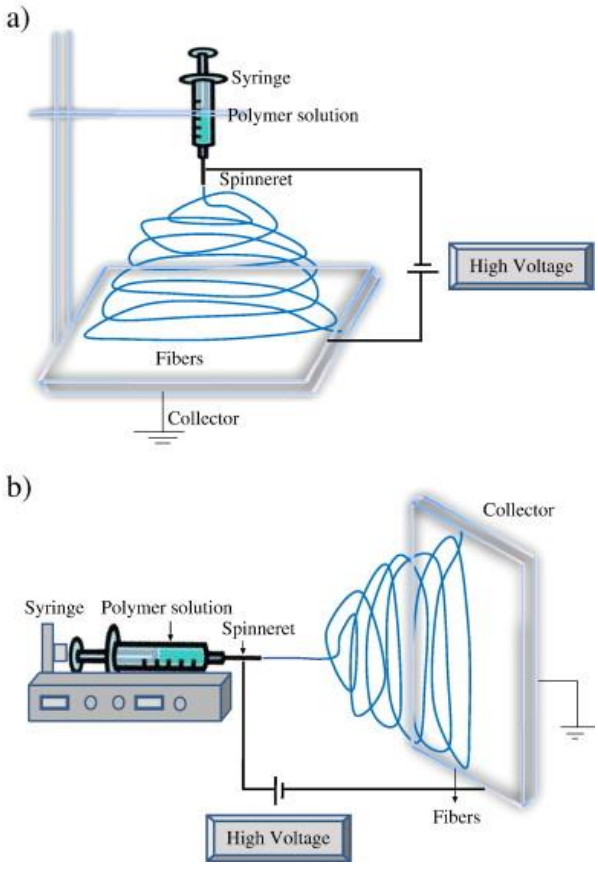

Figure 1. A Schematic diagram of electrospinning apparatus in (a) a vertical set up and (b) a horizontal set up. Reprinted from Biotechnology Advances, Volume 28, Issue 3, Nandana Bhardwaj, Subhas C. Kundu, Electrospinning: A fascinating fiber fabrication technique, Pages 325 - 347, Copyright (2010), with permission from Elsevier (14).

Despite the relative simplicity of the equipment involved, by carefully controlling processing parameters the fibre's diameters, orientations, total mat porosity and other properties can be controlled, allowing optimisation of the mat for a given application. In addition, the technique's ability to work with a wide variety of materials allows a range of specific biological, mechanical or chemical properties to be achieved (15). Therefore by controlling solution properties such as the viscosity, conductivity, molecular weight and surface tension along with processing parameters such as the applied electrical field, distance from the syringe tip to the collector and flow rate of the polymer solution, a range of desirable characteristics can be attributed to the nanofibres (14).

Further to these controllable variables it has been shown that by modifying the collector architecture, for example by using two parallel conductive substrates of varying gap size, fibres can be aligned uniaxially into arrays (22). This alignment of fibres has led to anisotropic mat properties in terms of tensile strength as well as directional cell growth, as previously discussed. Further modifications to the collector have been illustrated to expand the possible fibre orientations including rotating drum electrode (23) and knife-edge collectors (24). Furthermore, "coaxial" electrospinning allows for a more complex fibre architecture, forming a fibre comprised of two non-mixed materials in a core-sheath arrangement (25,26).

Although needle based electrospinning allows excellent control over both fibre diameter and their composition it has an extremely low throughput where basic systems are limited to flow rates of less than 0.5 mL per hour. A free-surface electrospinning arrangement such as the El Marco NanoSpider™ system is capable of forming fibres with throughput many hundreds of times greater than the conventional needle based electrospinning set up (27,28). In the free-surface electrospinning approach the spinning solution is simply held in a bath, rather

than being delivered through an aperture, with the whole bath then being connected to a high voltage power supply (Figure 2). In the specific case of the NanoSpider™ a rotating metal mandrel is half-submerged in the bath to concentrate the electric field on the thin layer of polymer which coats the mandrel. In this process many Taylor cones are formed on the surface of the polymer solution, and electrospinning upwards onto a collector above the bath. This increases the throughput of the process many hundreds of times above the conventional needle-based system, however much higher voltages, up to 82kV in the case of the NanoSpider™ are required and solution properties such as viscosity, conductivity and surface tension must be more tightly controlled (29). This is due to requirement of the Taylor cone to form upwards and the inability to control the feed rate and subsequent Taylor cone formation. There is also an absence of a point at which to concentrate the electric field (such as in a blunt end needle) which results in a higher initiation voltage.

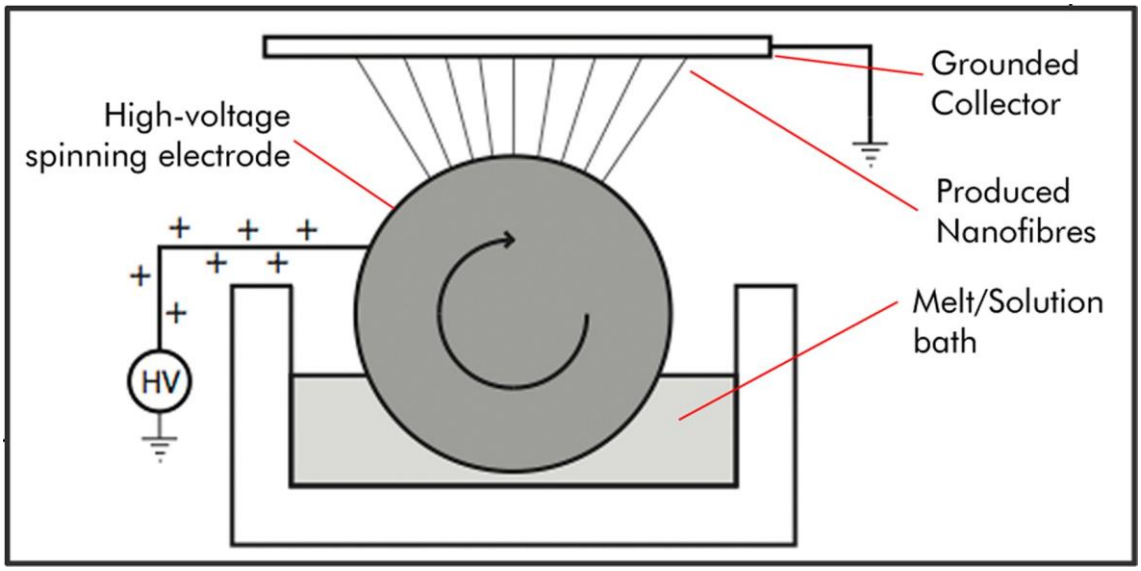

Figure 2. A schematic diagram showing a free-surface electrospinning set-up. A polymer solution/melt is held in a bath and a spinning electrode connected to a high voltage power supply is utilized to form multiple jets. Nanofibers are electrospun upwards and collected on a grounded collector plate.. Reprinted from Materials Science and Engineering: C, Volume 70, Part 1, 1 January 2017, Pages 512-519, Luke Burke, Chris J. Mortimer, Daniel J. Curtis, Aled R. Lewis, Rhodri Williams, Karl

Hawkins, Thierry G.G. Maffeis, Chris J. Wright, In-situ synthesis of magnetic iron-oxide nanoparticle-nanofibre composites using electrospinning, Copyright (2017), with permission from Elsevier (28).

Electrospun scaffolds have seen a vast amount of research in the tissue engineering field. This is due to the requirement of the scaffold to have an appropriate pore size and porosity to allow the proliferation of cells as well as a large surface area to volume ratio to promote cell adhesion, growth migration and differentiation (30).

In a recent review, we discuss the importance of understanding the interactions between not only eukaryotic cells but also bacteria for the development of tissue engineering scaffolds as cells need to compete with bacteria in many environments therefore the ideal tissue engineering scaffold will promote cell adhesion while inhibiting bacterial cell adhesion (31). Although there has been little research to date, interactions of bacteria with nanofibres and nano-structured surface have been shown to be similar. A smaller fibre diameter is favourable since scaffolds with nanofibre diameters smaller than the bacteria have been shown to be less susceptible to bacterial adhesion and fouling (31). Furthermore, the inclusion of an active antimicrobial ingredient can further inhibit bacterial colonisation and aid in the promotion of cell adhesion.

4. Iron Oxide Nanoparticles

Iron oxide is known to exist in sixteen forms as oxides, hydroxides or oxide-hydroxides (32). There are six non-hydrated crystalline iron oxide phases which have been identified so far, which are classified according to the valence state of iron in their crystal structure (33). Among these the most of interest in biomedical fields are magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) due to their functionality and favourable magnetic properties (33). Figure 2 shows the crystal structure and crystallographic data of hematite, magnetite and maghemite. Magnetite, Fe_3O_4, is a black ferromagnetic material with an inverse spinel structure. It contains both Fe^{2+} and Fe^{3+}. Maghemite, γ-Fe_2O_3, is a redish-brown ferromagnetic material which is

isostructural with magnetite, but with cation deficient sites. An important structural properties of both magnetite and maghemite is its crystal size. If the diameter of the nanoparticles are smaller than 20nm they display superparamagnetism, resulting in the particles showing no continuing magnetic interaction upon the removal of an external magnetic field (34). Furthermore iron oxide has been reported as non-toxic at low doses, biodegradable and biocompatible (35).

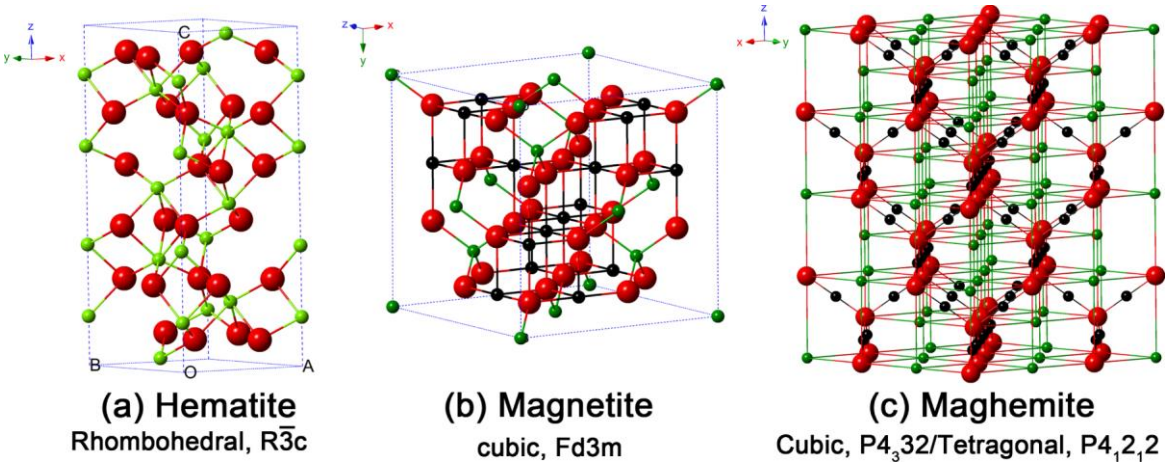

Figure 3. Crystal structure and crystallographic data of hematite, magnetite and maghemite (the black ball is Fe^{2+}, the green ball is Fe^{3+} and the red ball is O^{2-}). Reprinted with permission from (36).

Both magnetite and maghemite exhibit ferrimagnetism at room temperature. Many of the properties of IONPs are dependent on both their size and shape (32).

4.1.1 Nanoparticle Synthesis

A number of different synthesis techniques have been applied to produce magnetic iron oxide nanoparticles (IONPs) including co-precipitation, thermal decomposition, hydrothermal and solvothermal syntheses, sol-gel synthesis, microemulsion, ultrasound irradiation and biological synthesis. These methods include both aqueous and non-aqueous techniques with the former usually preferred due to the lower cost and sustainability (37).

Co-precipitation and thermal decomposition are the most commonly applied methods for preparing iron oxide nanoparticle-nanofibre composites, particularly for post-treatment and *in-situ* synthesis techniques.

4.1.2 Co-precipitation

Co-precipitation is considered the most conventional method. Generally, a basic solution is prepared containing a 1:2 molar ratio of ferric and ferrous ions. A suitable reducing agent can be used leading to the reaction shown in equation 4.1.1.

$$Fe2+ + 2Fe3+ + 8OH- \leftrightarrows Fe(OH)2 + 2Fe(OH)3 \rightarrow Fe3O4\downarrow + 4H2O \qquad \text{Equation 4.1.1}$$

Due to the nature of the technique, particle aggregation is a major drawback (38). To overcome this problem researchers have introduced surfactants and biomolecules into the process to reduce the likelihood of aggregation and maintain a small particle diameter. Salavanti-Niasari *et al.* used a co-precipitation method in the presence of octanoic acid, a surfactant, obtaining magnetite nanoparticles of average diameter 25nm (39). Magnetic chitosan coated magnetite nanoparticles were prepared by Liu *et al.* using a co-precipitation method (40). Suh *et al.* presented an *in situ* synthesis technique for the production of non-spherical magnetic iron oxide nanoparticles in a carboxyl functionalised polymer matrix (41).

4.1.3 Thermal Decomposition

Thermal decomposition is a particle formation technique with slower production rates than co-precipitation but offers more control over particle size and size distribution. The thermal decomposition technique can generally be sub-divided into hot-injection strategies where the precursors are injected into a hot reaction mixture, and conventional reaction strategies where a reaction mixture is prepared at room temperature and then heated in a closed or open reaction vessel (37). IONPs formed using thermal decomposition have a narrow size distribution and high crystallinity when compared to particles formed using a co-precipitation technique. A number of different ferric salts are used including iron pentacarbonyl (42), iron (III) acetylacetonate (43), iron oleate (44), ferrocene (45) and triiron dodecacarbonyl (46). Organic molecules are often added to the reaction process as

stabilisers which can slow down the nucleation process and favour the formation of small IONPs.

4.2 Biomedical Applications

The biocompatibility and low toxicity of iron oxide nanoparticles makes them ideal candidates for applications in the biomedical field. It is established that the iron oxide nanoparticles themselves are biocompatible and are excreted naturally by the liver when released into the body at low doses (47). There are a number of *in vivo* applications which can generally be grouped into three categories; (1) magnetic vectors guided to a certain location using a magnetic field (2) contrast agent in MRI and (3) hyperthermia agent for thermoablation (Figure 3). Of important interest is their superparamagnetic properties which allow them to be used for drug delivery and also improved tissue and bone repair in tissue engineering. Growth factors can be attached to a magnetic carrier and guided to the site of a tissue engineering scaffold containing iron oxide nanoparticles which can improve tissue and bone repair (48–50). It has also been shown that a static magnetic field can improve bone repair in rabbit models. This will be discussed in more detail in section 6.1.

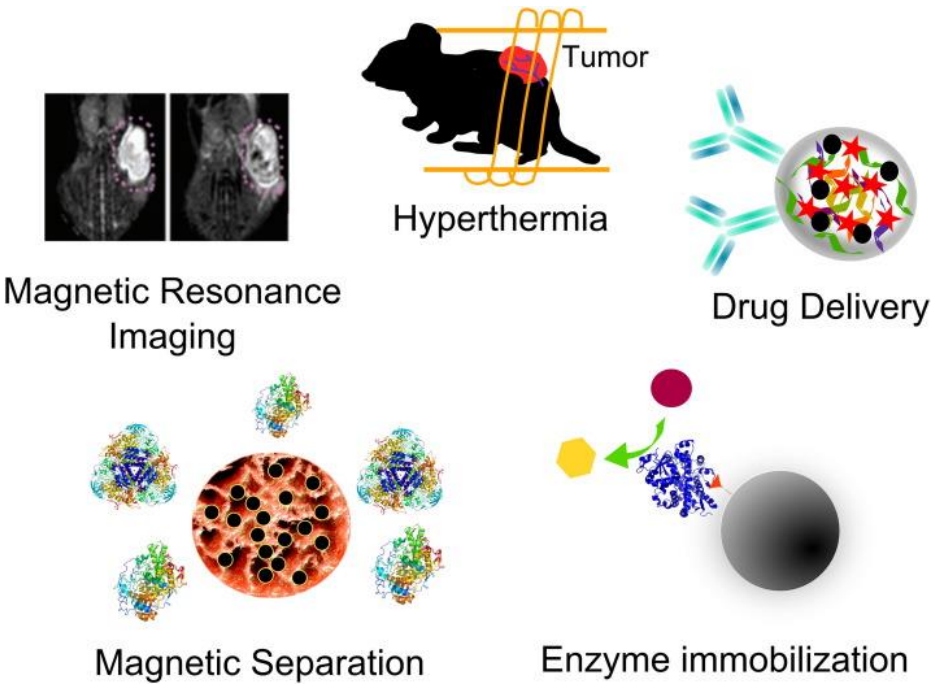

Figure 4. Schematic representation of biomedical and biotechnological applications of IONPs. Reprinted from Biotechnology Advances, Volume 33, Issue 6, Part 2, 1 November 2015, Katerina Hola, Zdenka Markova, Giorgio Zoppellaro, Jiri Tucek, Radek Zboril, Tailored functionalization of iron oxide nanoparticles for MRI, drug delivery, magnetic separation and immobilization of biosubstances, Pages 1162-1176, Copyright (2010), with permission from Elsevier (33).

4.2.1 Drug Delivery

One of the major drawbacks with the use of chemotherapeutic drugs is the frequency and severity of side effects observed due to their systemic application. IONPs are of interest for use in the targeted delivery of these, and other, drugs (51–53). Drug delivery is a pharmaceutical approach to transporting drugs to a desired location in the body, often reducing the required dose. In general, magnetic IONPs used for drug delivery consist of a core-shell structure with the IONPs as the core, coated in a biocompatible component (36). The magnetic properties allow the pharmaceutically functionalised nanoparticles to be delivered to the site of interest by application of a magnetic field to a specific region of the body.

A further application in cancer therapies is in thermoablation where local overheating of the cancer cells can be achieved by hyperthermia (54). Hyperthermia can be achieved due to the ability of magnetic nanoparticles to adsorb alternating current (AC) energy and convert it to heat (33). By heating to a temperature between 41˚C and 46˚C thermal stress can cause the cancer cells to undergo apoptosis, a programmed cell death.

4.2.2 MRI contrast agent

Magnetic resonance imaging (MRI) is a non-invasive diagnostic technique which is often the preferred imaging technique due to its high spatial resolution (~100nm), long effective imaging window, the absence of exposure to ionising radiation and rapid *in vivo* image acquisition (55) MRI contrast agents contain paramagnetic or superparamagnetic metal ions which have a positive effect on the MRI signal properties of surrounding tissue (56). Superparamagnetic IONPs (SPIONs) are

capable of substantially altering the spin-spin relaxation of water molecules (T2 relaxation) near the magnetic nanoparticles which can enhance the negative contract of the image (56). The biocompatibility of IONPs makes them the preferred contrast agents over other metal oxides.

4.2.3 Antibacterial Agent

Metal oxide nanoparticles have been shown to interact with the cell membrane of bacteria by electrostatic interaction, inducing toxic oxidative stress on the bacteria by free radical formation; the reactive oxygen species (ROS) (57,58). Iron oxide nanoparticles have been shown to impart antimicrobial properties against both Gram-negative and Gram-positive bacteria. Ismail *et al.* synthesised maghemite nanoparticles of diameter 50 – 110nm by pulsed laser ablation and an agar well diffusion assay was used to assess the antibacterial activity (59). Inhibition zones were present for all Gram-negative (*Pseudomonas aeruginosa, Escherichia coli and Serratia marcescens)* and Gram-positive (*Staphylococcus aureus*) bacteria tested.

5. Nanoparticle-nanofibre composite fabrication

There are currently a number of different techniques employed to obtain iron oxide nanoparticle-nanofibre composites. These can be grouped into three categories; (1)pre-synthesised IONPs, (2) Post-treatment synthesised IONPs and (3) *in-situ* synthesised IONPs. Nanoparticles (NPs) are more commonly synthesised before electrospinning or precursors are electrospun and the consequent nanofibres are treated to synthesise the nanoparticles within the nanofibres. More recently, *in-situ* synthesis techniques have emerged which allow for NPs to be synthesised during the electrospinning process or in the solution to be electrospun with no pre-processing of NPs.

The electrospinning process is generally unchanged with all particle synthesis techniques. If particles are pre-synthesised a co-electrospinning technique is generally employed where the nanoparticles are dispersed in a polymer solution before electrospinning. The other technique which can be used is coaxial electrospinning as discussed in section 3.

5.1 Pre-synthesised nanoparticles

Electrospinning NPs which have already been synthesised is the most basic and therefore most commonly used technique for the fabrication of nanoparticle-nanofibre composites. However, this process can often be multi-stage and time consuming requiring particles to be pre-synthesised and subsequently functionalised to reduce the effects of particle agglomeration and allow homogenous distribution throughout the nanofibres.

The particle synthesis techniques employed are as outlined in section 3.1, with an additional functionalisation stage if necessary. Wang *et al.* used a co-precipitation technique with the reduction of iron(III) chloride hexadydrate and iron(II) chloride tetrahydrate using ammonium hydroxide in the presence of a graft copolymer to arrest the growth of NPs and prevent aggregation (60). IONPs were then added to solutions of PEO and PVA before electrospinning using a needle electrospinnning set-up. Amarjargal *et al.* presented an alternative technique where magnetite nanoparticles (MNPs) were prepared using a modified precipitation method followed by a dyrothermal treatment (61). Polyurethane (PU) nanofibres were electrospun and superparamagnetic iron oxide nanoparticles (SPIONS) were assembled on the nanofibres using a facile polyol immersion technique. The nanofibrous mats were immersed in a colloidal solution containing the SPIONs under vigorous shaking at 60˚C. Ahn *et al.* acquired IONPs, separated out the SPIONs and dispersed them in a Poly(ethylene terephthatlate) (PET) solution before electrospinning using a needle electrospinning set-up (62). Ting Tan *et al.* prepared MNPs in an aqueous solution in the presence of a surface-active agent to supress aggregation (63). MNPs were exposed to air for a period of time to allow them to oxidise to maghemite before dispersion in poly(hydroxyethyl methacrylate) (PHEMA) and poly-l-lactide (PLLA) solutions for needle electrospinning. EDX analysis confirmed the presence of iron in the fibres and magnetization curves show the composites to display magnetic properties characteristic of IONPs. Tsioptsias *et al.* dispersed commercially available iron(III) oxide NPs in a cellulose acetate solution (64). The solutions were then electrospun using a needle electrospinning set-up. Sung *et al.* fabricated core/sheath magnetic

nanofibres using a coaxial electrospinning set-up (65). The core was provided by a magneto-rheological fluid that contained a blend of ferrofluid and mineral oil. The sheath was formed by Poly (ethylene terephthalate) (PET) in trifluoroacetic acid (TFA) and Pellethane in a co-solvent (7:3 N-N-dimethylformamide (DMF): dichloromethane (DCM)). The morphology was studied using SEM and TEM and magnetic properties analysed using magnetic curves with hysteresis loops typical of a superparamagnetic material. Lai *et al.* prepared MNPs using a high temperature solution phase reaction before dispersion in a Poly(lactic-co-glycolic acid) (PLGA) solution (66). Nanoparticle-nanofibre composites were prepared using electrospinning. FTIR was then used to demonstrate peaks representative of MNPs, DLS showed an average particle dimeter of 8.47 ± 2.12 nm and XRD confirmed the phase of the MNPs to be magnetite. The magnetic loops on magnetization curves illustrated the typical superparamagnetic behaviour of the MNPs. Meng *et al.* pre-synthesised MNPs using a modified emulsion technique and then dispersed the MNPs in a solution of Poly(DL-lactide)/Dimethylacetamide before electrospinning (67).

5.2 Post-treatment

Post-treatment techniques involve the inclusion of a precursor in the electrospun nanofibres which undergo post-electrospinning processing technique to form IONPs within the nanofibres. Xiao *et al.* prepared nanofibres from PVA/PAA before immersion in an aqueous solution of ferrous tichloride to allow ferric cations to complex with free carboxyl groups on PAA through ion exchange (68). Sodium borohydride was then added to the mats to reduce the ferric ions to IONPs. Another example of post-treatment nanocomposite fabrication is the work of Barakat who prepared nanofibres from a solution of PVA and Iron (II) acetate (FeAc) (69). The nanofibre mats were dried for 24 hours before undergoing calcination at 700˚C for 5 hours in an argon atmosphere to form IONPs. XRD confirmed there was no hematite present and FTIR confirmed there was no magnetite present, showing that the IONPs present were maghemite.

5.3 *In-situ* synthesis

In recent years, research has focussed on the development of *in-situ* synthesis techniques combined with electrospinning with fewer steps, more simplicity and lower production costs. This is an area that has been investigated in more depth for other metal nanoparticles. For example, Wang *et al.* presented a method to prepare silver nanoparticles dispersed in polyacrylonitrile (PAN) nanofibres combining a reduction reaction with electrospinning (70). Jin *et al.* presented a one-step technique to prepare silver nanoparticles in Poly(vinylpyrrolidone) PVP nanoparticles (71). In their study silver nitrate ($AgNO_3$) was reduced in a PVP/DMF solution with DMF as the reducing agent. Solutions were then electrospin resulting in PVP nanofibres containing silver nanoparticles.

Nataraj *et al.* presented a three-stage *in-situ* synthesis technique where the chemical reagents ($FeSO_4$ and $FeCl_3$) were added to a PAN solution and electrospun (72). The electrospun mat was then immersed in KOH for 4 hours, stabilised at 280˚C in air for 2 hours and carbonized at 800˚C in nitrogen atmosphere. XPS was used to identify the phase of the IONPs, which were identified as Fe_2O_3. Faridi-Majidi *et al.* present a one-stage in-situ synthesis technique for the electrospinning of PEO nanofibres containing IONPs (73). In their technique, $FeCl_3$ and $FeSO_4$ were added to the PEO/distilled water electrospinning solution. Electrospinning was carried out in an ammonia atmosphere to reduce to iron compounds to IONPs. SEM and TEM confirmed the nanofibrous morphology and presence of nanoparticles. XRD was used to identify the phase of iron oxide nanoparticles as maghemite.

In a recent study we have developed a novel one-stage *in-situ* synthesis technique to fabricate PEO and PVP nanofibres containing magnetite MNPs (28). We have also demonstrated an ability to scale up the process from laboratory to industrial scale using a commercially available free-surface electrospinning set-up. In our technique a 2:1 molar ratio of ferric and ferrous chloride are added to a PEO solution in deionised water containing sodium borohydride, used to reduce the ions to nanoparticles. The reaction is allowed to progress before being electrospun (Figure 4). Nanofibre mats were crosslinked using UV irradiation, EDX was used to

confirm the presence of iron, DLS showed the average nanoparticle diameter to range from 8nm (PVP) to 26nm (PEO), XRD confirmed the phase of the nanoparticles to be magnetite and NMR showed a shortening in both T1 and T2 relaxation times confirming the nanoparticles could provide a suitable relaxation channel.

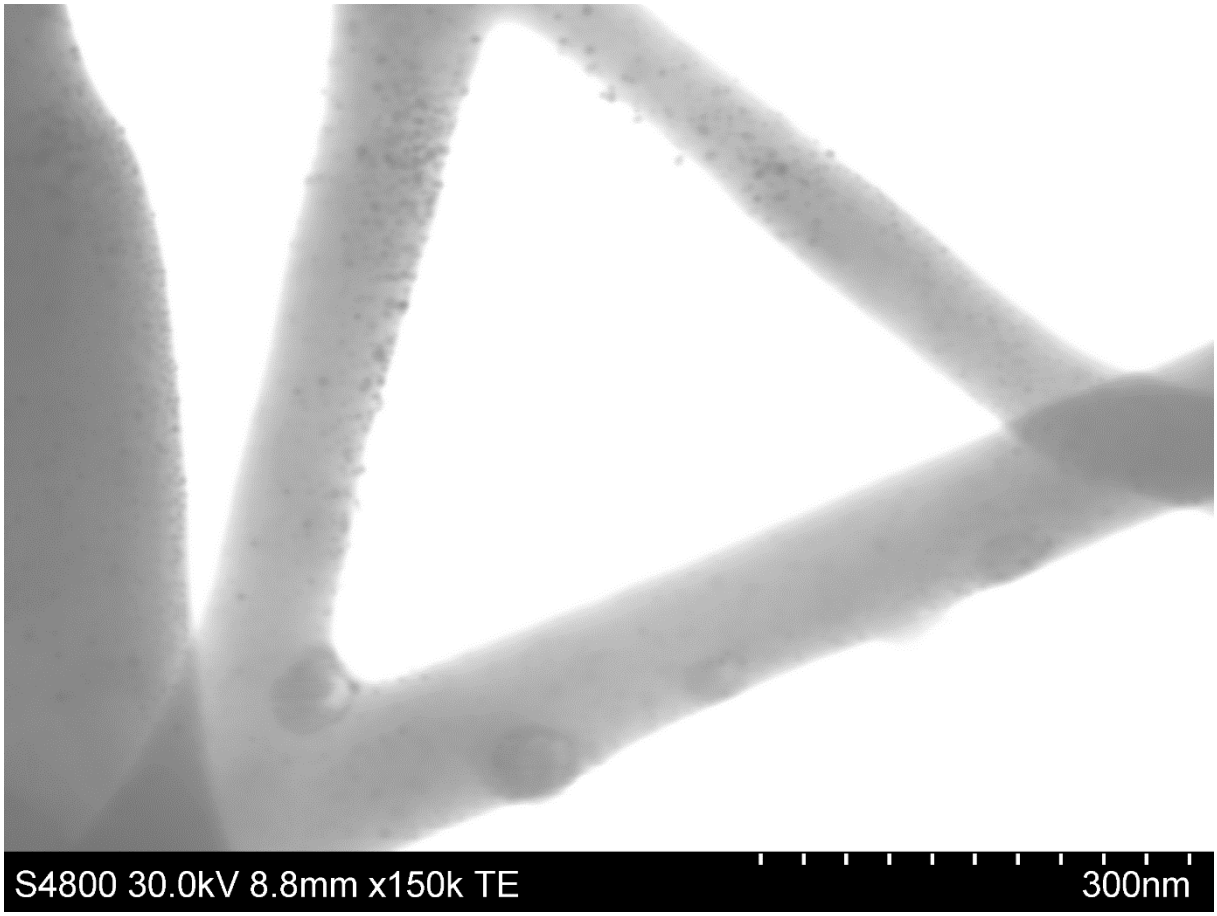

Figure 5. High magnification Scanning Transmission Electron Microscopy image of PEO nanofibre containing MNPs. Reprinted from Materials Science and Engineering: C, Volume 70, Part 1, 1 January 2017, Pages 512-519, Luke Burke, Chris J. Mortimer, Daniel J. Curtis, Aled R. Lewis, Rhodri Williams, Karl Hawkins, Thierry G.G. Maffeis, Chris J. Wright, In-situ synthesis of magnetic iron-oxide nanoparticle-nanofibre composites using electrospinning, Copyright (2017), with permission from Elsevier (28).

6. Applications of iron oxide nanoparticle-nanofibre composites

6.1 Tissue Engineering

The incorporation of soluble factors and control of surface chemistry of tissue engineering scaffolds to provide biochemical cues have been well documented (74–76). Magnetic scaffolds have been investigated for the regeneration and repair of damaged or diseased tissue (50). The incorporation of MNPs into scaffolds is also believed to increase the rate of both bone cell growth and differentiation. This is due to the tissue's ability to recognise the mechano-electrical conversion that can lead to increased cellular proliferation and expression levels of a number of genes related to bone differentiation (48,49).

A number of different techniques have been used to fabricate magnetic scaffolds, however this review only focuses on those using electrospinning. Other examples can be found here (77–83). Bock *et al.* prepared magnetic scaffolds from hydroxyapatite/collagen dipped in a dispersion of magnetite nanoparticles (82). Their studies indicate the ability of the scaffolds to support adhesion and proliferation of human bone marrow stem cells *in vitro*. Lai *et al.* fabricated superparamagnetic nano-composite scaffolds for promoting bone cell proliferation and defect reparation (66). MNPs were prepared and electrospun into PLGA nanofibres of average diameter 400-600nm. Rosc17/2.8 (osteosarcoma cell lines) and MC3T3-E1 (osteoblast precursor cell lines) were used for their studies. They found that PLGA scaffolds containing MNPs promoted faster and better cell attachment when compared with the PLGA control. It was also found that in the presence of MNPs cells proliferated significantly faster than in the PLGA control. Meng *et al.* reported that the presence of an electrospun nanofibrous material containing MNPs inserted within a bone fracture site in a rabbit model increased osteocalcin expression by osteoblasts and improved healing rates over 100 days (67). In their work, IONPs were synthesised using a modified emulsion technique along with hydroxyapatite nanoparticles and electrospun into PLA nanofibre scaffolds. This study used the MNPs as an intrinsic component of the scaffold to produce huge amounts of miniature magnetic force under an external magnetic field allowing continual stimulation of osteoblast cell proliferation and secretion of ECM. Their studies showed that under an external magnetic field the scaffolds

induced earlier and higher amounts of osteocalcin positive cells *in-situ,* which led to an earlier and faster bone formation in the defect. This was evidenced by the faster achievement of cortical bone and medullar cavity continuity along with pathological observations when compared to those without the presence of a magnetic field. These results are a strong indication that continuous weak magnetic force stimulation has a significant effect on bone regeneration and repair, which they achieved by applying an external magnetic force to super-paramagnetic responsive scaffolds. Furthermore, the stimulation using the magnetic field resulted in a faster degradation rate of the scaffold, which is another important factor determining bone repair.

Several different iron oxide nanoparticle-nanofibre composites have been presented for tissue engineering applications to date. The mechanism of action varies but in all instances the magnetic properties of the iron oxide nanoparticles are utilized. Generally, the magnetic properties of the scaffold are used to attract magnetic drug carriers, carrying growth factors which can promote adhesion and cell growth. Another application, as presented by Meng *et al.* is the use of the magnetic nanoparticles to provide continual stimulation to the scaffold to support cell adhesion and proliferation (67). This requires the use of an external magnetic field to provide the continuous stimulation.

7. Conclusion

There is a vast amount of research into potential scaffolds for tissue engineering. The applications of IONPs are widely reported and as such the fabrication of magnetic scaffolds for tissue engineering is growing rapidly, particularly in the area of bone tissue engineering. Electrospinning is one of the preferred methods of scaffold fabrication with many advantages including its ease of use and the capability to produce nanostructured scaffolds which can mimic the ECM. There are a number of different methods available for the synthesis of IONPs, some of which have been discussed in this review. There are also studies reported in the literature of the fabrication of nanoparticle-nanofibre composite scaffolds formed

using electrospinning. These methodologies can generally be categorised by pre-synthesised nanoparticles, post-processed nanoparticles and nanoparticles synthesised *in-situ*. Pre-synthesised and post-processed nanoparticles are more commonly reported in the literature with methodologies well established. It is *in-situ* synthesis techniques which have emerged more recently that show most promise, reducing the number of steps required and offering simple procedures for the production of magnetic scaffolds.

8. References

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Figure legends

Figure 1. A Schematic diagram of electrospinning apparatus in (a) a vertical set up and (b) a horizontal set up. Reprinted from Biotechnology Advances, Volume 28, Issue 3, Nandana Bhardwaj, Subhas C. Kundu, Electrospinning: A fascinating fiber fabrication technique, Pages 325 - 347, Copyright (2010), with permission from Elsevier [11].

Figure 2. A schematic diagram showing a free-surface electrospinning set-up. A polymer solution/melt is held in a bath and a spinning electrode connected to a high voltage power supply is utilized to form multiple jets. Nanofibers are electrospun upwards and collected on a grounded collector plate.. Reprinted from Materials Science and Engineering: C, Volume 70, Part 1, 1 January 2017, Pages 512-519, Luke Burke, Chris J. Mortimer, Daniel J. Curtis, Aled R. Lewis, Rhodri Williams, Karl Hawkins, Thierry G.G. Maffeis, Chris J. Wright, In-situ synthesis of magnetic iron-oxide nanoparticle-nanofibre composites using electrospinning, Copyright (2017), with permission from Elsevier (28).

Figure 3. Crystal structure and crystallographic data of hematite, magnetite and maghemite (the black ball is Fe2+, the green ball is Fe3+ and the red ball is O2−). Reprinted with permission from [32].

Figure 4. Schematic representation of biomedical and biotechnological applications of IONPs. Reprinted from Biotechnology Advances, Volume 33, Issue 6, Part 2, 1 November 2015, Katerina Hola, Zdenka Markova, Giorgio Zoppellaro, Jiri Tucek, Radek Zboril, Tailored functionalization of iron oxide nanoparticles for MRI, drug delivery, magnetic separation and immobilization of biosubstances, Pages 1162-1176, Copyright (2010), with permission from Elsevier [29].

Figure 5. High magnification Scanning Transmission Electron Microscopy image of PEO nanofibre containing MNPs. Reprinted from Materials Science and Engineering: C, Volume 70, Part 1, 1 January 2017, Pages 512-519, Luke Burke, Chris J. Mortimer, Daniel J. Curtis, Aled R. Lewis, Rhodri Williams, Karl Hawkins, Thierry G.G. Maffeis, Chris J. Wright, In-situ synthesis of magnetic iron-oxide nanoparticle-nanofibre composites using electrospinning, Copyright (2017), with permission from Elsevier (28).