Biomaterials Science

Magnetic composite biomaterials for tissue engineering

Journal:	*Biomaterials Science*
Manuscript ID:	BM-MRV-02-2014-000041
Article Type:	Minireview
Date Submitted by the Author:	05-Feb-2014
Complete List of Authors:	Gil, Sara; University of Minho, 3B's Research Group Mano, Joao F; 3Bs Research Group, Department of Polymer Engineering

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

Minireview

Magnetic composite biomaterials for tissue engineering

Sara Gil[a,b] and João F. Mano[a,c*]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Magnetic nanoparticles (MNPs) have been increasingly used in tissue engineering and regenerative medicine. These particles have been mainly employed as elements directly incorporated within cells or interacting with cell membranes; however, MNPs are starting to be combined with biomaterials to create other functionalities of the structural framework used to support cells, namely for controlling cellular responses and for enhancing drug delivery and release. This mini-review summarizes and highlights the latest developments and applications of polymeric/ceramic biomimetic scaffolds and hydrogels that contain MNPs for such purposes, also addressing future perspectives for the use of these magnetic composite biomaterials in biomedicine.

Introduction

Magnetic materials have been widely used in biomedicine. The preparation of stronger and smaller permanent magnets allowed the creation of more delicate biomedical applications like in the fields of ophthalmology (magnetically assisted cataract surgery), dentistry (temporary fixing prosthesis), cardiology and gastroenterology (guiding catheters through the body), and neurology (navigating within the brain).[1] In particular, much effort has been devoted to the synthesis of magnetic nanosized materials, due to their small size and unusual superparamagnetic properties.[2-5] Magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) are the most common iron oxides used for biomedicine due to their low toxicity, relative ease of functionalization and high magnetization at room temperature.[5-8] Such materials are easily fabricated into the shape of nanoparticles.[9] Magnetic nanoparticles (MNPs) exhibit a superparamagnetic behavior at sizes below 20 nm, demonstrating high potential for *in vivo* applications because they do not retain any remanent magnetization upon removal of a magnetic field, which prevents aggregation and enables them to redisperse rapidly after withdrawing the magnetic field.[3,5,8,10]

MNPs and magnetic lipossomes have been increasingly exploited in the field of biomedicine. They have controllable sizes (few nanometers up to tens of nanometers) which are compatible to those of viruses (20-450 nm), proteins (5-50 nm) and genes (2 nm wide by 10-100 nm long). Besides their small size, nanoparticles and liposomes can be functionalized with other materials enabling their interaction and specific binding to other biological entities, and enhancing their colloidal stability and biocompatibility. Also, through the action of a magnetic field it is possible to trace and control the localization of these nanomaterials within the human body, through minimally invasive methods.[3-5]

Tissue engineering (TE) is an interdisciplinary field, exploiting biological and engineering principles that, when combined with suitable biochemical factors, allows for the development of functional substitutes of loss or damaged tissue.[11] An emerging TE strategy, named magnetic-force based tissue engineering, employs cells that have been magnetically labeled with MNPs or magnetic cationic liposomes (MCLs) in the biofabrication of more complex tissue constructs.[8,12] For example, the cellular culture and co-culture techniques applying this principle can be used in magnetic cell patterning, magnetic cell seeding and magnetic cell levitation.[12] Such works mainly focused on the direct contact between MNPs or MCLs and cells. In another perspective, magnetic elements can be combined to biomaterials that are usually used as a structural framework for supporting cells to attach, proliferate and differentiate. Such strategy could allow for the production of hybrid structures with enhanced functionalities, including devices able to provide mechanical stimuli to cells or to deliver on demand growth factors (GFs) or other bioactive molecules. This mini-review overviews the latest development of polymeric/ceramic scaffolds and hydrogels that contain magnetic particles for such purposes. The results reported so far indicate an increased interest by the researchers on these topics, foreseeing that magnetic particles can be used as a stimulus to influence cellular activity, as well as cell proliferation and differentiation, and will bring new prospects and major improvements in the fields of drug delivery and tissue regeneration.

Cellular behavior

Stem cell behavior is highly influenced by the physical properties of the scaffold and the chemical/biochemical landscape over its surface. However, other external factors may affect cellular behavior, such as mechanical stimulation. Many studies have shown that cells and tissue growth increase in response to mechanical stresses generated by the mobility of the surface matrix or by fluid flow.[13-15] This has been the basis of the development of bioreactors.[16]

Mechanotransduction is a well known pathway by which cells convert physical stimuli into biochemical activity. For many TE

and regenerative medicine applications, mechanical cues provide important stimuli to the cells that promote the production of functional tissue matrix. For example, the differentiation of mesenchymal stem cells into bone, cartilage, muscle and connective tissue is particularly conditioned by mechanical cues.[17,18] However, applying the correct stress profiles to cells growing in a 3D scaffold within a bioreactor or within a patient's body has proven difficult.

To provide mechanical stimuli similar to those experienced *in vivo* by cells, the *in vivo* environment must be mimicked inside the bioreactor. Currently, the available bioreactors do not allow the application of spatially varying stresses in three dimensions, in order to form complex tissue structures. Direct magnetic actuation can provide the application of controlled forces in order to precisely regulate cellular function. In this context, MNPs can be attached to specific ion channels present on cellular membrane, acting as a stress generator.[19] Cells can thus be mechanically conditioned by magnetic remote actuation. Cartmell and co-workers demonstrated that mechanical stimulation of primary human osteoblast cells by adhered magnetic particles promoted the regeneration of bone matrix when under the influence of a magnetic field.[20]

However, a possibility that has seldom been considered is the application of MNPs in tissue regeneration by incorporating them into scaffolds. These superparamagnetic scaffolds can be "activated" through the application of an external magnetic field. The field acts on the nanoparticles along the gradient vector, producing compressive or tensile forces that are sensed by the cells in the scaffold. The forces necessary to activate the mechanosensitive channels via cell membrane deformation are really small (in the order of picoNewtons).[21] Therefore we believe that superparamagnetic scaffolds can provide the necessary cues for stimulating stem cell differentiation.

X. B. Zeng and co-workers investigated the behavior of rat osteoblast and mice preosteoblast cells on a series of MNP-hydroxyapatite (HA) magnetic scaffolds with different MNPs content (from 0 to 2 wt%).[22] The results demonstrated the positive influence of MNP-HA scaffolds on cell adhesion, proliferation and differentiation when compared to non magnetic HA scaffolds, suggesting enhanced cell behavior due to the incorporation of MNPs.[14,22] Furthermore, these results were significantly intensified when the MNP-HA scaffolds were under the influence of an external static magnetic field, suggesting a likely synergistic effect between the magnetic scaffolds and the exterior magnetic field.[22-24] Likewise, a positive correlation between MNP content and cell proliferation was observed.

With sizes of 20 nm or less, MNPs become superparamagnetic and behave like common materials in the absence of an external magnetic field. However, at the nanoscale level, each MNP in the scaffold acts like a single magnetic domain, providing micromotions on the interface between cells and the scaffold that might affect the ion channels on the cell membrane, and trigger the mechanotransduction pathway.[25] Nevertheless, as explained earlier, once MNPs are exposed to a magnetic field, they are rapidly magnetized providing enhanced therapeutic effect.[26]

Although the majority of groups attribute the direct effect of MNPs on cell activity only to magnetism, we cannot rule out the possibility raised by Y. Sapir and co-workers, which states that in addition to the scaffold magnetic properties, the integration of MNPs into the scaffolds also changes the surface roughness of the scaffold pore walls and scaffold stiffness.[27] In fact, scaffold elasticity properties are known to influence cell behavior.[28-30] Adhesive forces are formed when a cell binds to a certain substrate. These forces are generated by the cellular cytoskeleton, allowing the cell to spread. Substrate stiffness is a parameter that allows the control of cell behavior and the extent of cell spreading.[31,32]

Sapir et al. observed an enhanced effect in metabolic cell activity in the MNPs-scaffold when exposed to a magnetic field. They did not report an increase in proliferation, but rather an induction of other cellular processes such as cell organization.[27] However, to clarify if this is solely due to the magnetic component of the scaffolds, it would be interesting to perform a more systematic study, where a scaffold impregnated with non-magnetic particles, but with the same elastic/storage modulus as a MNPs impregnated one, would act as a control.

It can be concluded that magnetic scaffolds clearly have an influence in cellular aspects such as adhesion, proliferation and differentiation. The MNPs-impregnated scaffolds demonstrate an enhanced effect on cell behavior, promoting the remote activation of the mechanotransduction pathway which in turn triggers the biochemical one. Magnetic scaffolds present an excellent alternative and improvement in bioreactor and scaffold design, as they can provide mechanical cues that can be enhanced upon the application of a remotely generated external magnetic field. From the research done so far, few are the works that clearly study the mechanism by which cell behavior is influenced. Some authors attribute such differences to scaffold magnetic properties, which are synergistically enhanced when under the action of a magnetic field, but others also state that changes on surface topography and scaffold stiffness are parameters that also need to be considered. In the future it would be important to increase the systematization of the studies related with the fundamental understanding of the effect of the presence of MNPs in biomaterials (with and without application of an external magnetic field), isolating for example stiffness/topography components.

Tissue engineering applications

Drug Delivery

For the complete biological and histomorphological maturation of tissues, synthetic systems, able to control the delivery of bioactive systems, are particularly promising as devices for enhancing tissue regeneration. Therefore scaffolds capable of mimicking the molecular regulatory characteristics, combined to an adequate three-dimensional architecture are necessary for guiding functional angiogenesis, controlling stem cell proliferation and differentiation, and for tissue repairing.[33,34]

Through the introduction of magnetic nanoparticles into the scaffold, unique properties are imparted to the resulting material. In particular, nanocomposites sensitive to magnetic field exhibit the specific property of being responsive to remote actuation, thus allowing high control of the release of therapeutic agents by the influence of external magnetic field.

Controlled drug delivery is mainly aimed at the sustained delivery of therapeutic substances over a prolonged period of

time. Nevertheless, pulsatile drug delivery is also very attractive.[35] Through an adequate scaffold design and time/intensity control of the external field one could, in principle, achieve zero-order or more complex (e.g. pulsatile) delivery profiles, capable of mimicking the physiological needs of bioactive agents, and thus leading to optimum drug delivery.[36]

The use of magnetic scaffolds responsive to "on demand" magnetic field allows to overcome the limitations faced by conventional scaffolds. These are often pre-loaded with GFs or other therapeutic molecules, resulting in devices with limited control of the release profile.[37,38] Also, systems with a constant release rate, very popular in the pharmaceutic field, may not be adequate in TE strategies. The body's need for a drug during a regenerative process is not always constant,[38] thereby it is believed that magnetic scaffolds could provide a controlled delivery that could be compatible with the endogenous production and availability of GFs, hormones and other bioactive molecules.

Such principles were already validated in the field of drug release systems, especially using composite hydrogels. For example, R. Langer and co-workers designed a magnetic subcutaneous implant based on ethylene-vinyl acetate copolymer (EVAc) hydrogel able to liberate insulin at higher rates, upon demand.[39] When the device was implanted on diabetic mice the glucose level was kept at a near constant value. However, when a magnetic field was applied the blood glucose level clearly decreased. The movements induced by the field on the magnet inside the implanted hydrogel exerted pressure on the implant's matrix, causing the squeezing of the drug out of the pores. Therefore the control of the delivery of insulin could be achieved by varying parameters such as frequency, strength and duration of the external magnetic field.

V.M. Paoli and co-workers studied the effect of an oscillating magnetic field on the morphology and release profile of dextran-Rhodamine's (Dex-R) from magnetic collagen hydrogels containing nanoparticles and microparticles. Regarding drug release profiles, it was observed that the release rate followed an exponential profile for both formulations. However the amount of drug released is almost doubled upon application of the magnetic field.[40]

The change in the release profile in the system described is limited to the control of the magnification of the release when the field is turned on. However, we consider that the combination of different magnetic stimuli with the design and composition of hydrogel structures could bring new perspectives to the drug delivery field, and help to obtain release profiles other than first order release. For example, S. Y. Chen and co-workers fabricated a magnetic hydrogel by mixing poly(vinyl alcohol) with Fe_3O_4 MNPs through freeze-thawing cycles, and studied the drug release profile when under a pulsed magnetic field.[41] When the field is "switched on" MNPs aggregate together instantly, producing a bulk magnetic moment and causing a rapid reduction of the hydrogel's porosity. In this state, the rate of drug release is at a lower level and the hydrogel possesses a "closed" configuration. However, when the magnetic field is "switched off", the hydrogel returns to its original geometrical conformation (swelling rate increases) resulting in a burst-like release profile, that turns back to a normal diffusion mode shortly after the burst.

On the other hand, other groups reported different results, stating that the application of an external magnetic field causes a rapid burst in the drug release.[36] Again, considering the design of the scaffolds, they could also be advantageous for the stabilization of drugs or GFs if those were attached to MNPs that are impregnated in the scaffold. This way they would always be available for cells encapsulated within the scaffold as opposed to the dispersed molecules.[42,43]

The studies performed so far have thus shown that hydrogels containing magnetic elements may exhibit distinct, and even opposite, drug release behaviors upon the action of external magnetic fields. In the future, we could even envisage more sophisticated devices. For example, the combination of magnetic nanoparticles with responsive polymers,[44] could open new prospects for externally mediated treatments *in vivo*, not only including drug release, but also hyperthermia and combinations thereof.[45]

In tissue engineering there is a constant need for a spatially controlled delivery of cells and/or specific GFs to foment rapid and well organized cell scaffold colonization. However, there is a limitation towards the amount of biological material that can be incorporated in a scaffold before implantation. In this context, magnetic responsive scaffolds can be also envisaged as reloading systems for long term biochemical stimuli. Such scaffolds could function as a fixed station, capable to attract and fix, for example, nano/micro magnetic particles containing the required therapeutic molecules via magnetic driving. These particles could be administrated on demand and delivery the cargo in site of the implanted scaffold.

Although magnetic responsive composites have been developed for the delivery of therapeutics to treat different diseases, they were not yet optimized to be used specifically for regenerative purposes. We believe that there is an immense potential of both fundamental and applied research in this field that should clearly need the cooperation of multidisciplinary teams.

Tissue regeneration

Strategies used nowadays for tissue regeneration do not often promote a successful growth of tissue nor the complete integration of scaffolds on the tissue. Successful regeneration largely depends on interface interactions between cells and scaffolds. Therefore, scaffolds should not act as static elements. They should be "activated" during cell colonization, re-structuring their architecture according to their different mechanical and anatomical characteristics and to the different maturation phases of the tissue. As previously discussed, one of the most important stimuli that promote cellular differentiation into bone, cartilage, muscle and connective tissue is the mechanical one, and magnetic stimulation emerges as a possible means of achieving this stimulation *in vivo*.

The application from static or alternating magnetic fields into clinical studies has already been reported by some groups, and has been proved as beneficial in regeneration, integration, and ingrowth of tissues into ceramics.[46,47] Also, as commented before the incorporation of magnetic nanoparticles into scaffolds used in tissue engineering has already been validated as having a beneficial role on cellular behavior in *in vitro* studies. Therefore,

we consider that the incorporation of MNPs into scaffolds acting synergistically with magnetic field *in vivo* would improve cellular proliferation, and differentiation, and promote an enhancement in tissue integration with the scaffold, a crucial step towards the clinical applications of the composites. Besides, another factor that should be taken into consideration is the fixation of scaffold in the defect, that could, in principle, be improved by the help of an external magnetic field. An efficient mechanical fixation would prevent macro and microscopic movements at the interface between the scaffold and body tissue, thus enhancing integration of the new formed tissue.

M. Marcacci and co-workers demonstrated for the first time how collagen magnetic scaffolds, fixed *in vivo* with external magnets, could induce controlled regeneration in a well 3D pattern.[48] Under the effect of the static magnetic field the scaffolds become "activated" and oriented according to the field, thus allowing an oriented ECM deposition, which mimics the site specific collagen/apatite orientation.[49]

In vivo study for tissue formation and enhancement mediated by magnetic or superparamagnetic responsive composites has been rarely reported, although pioneer studies have demonstrated valuable improvements in tissue regeneration.[24,50,51] In addition to magnetic biomaterials properties, the synergistic effect of an external magnetic field results in site specific oriented tissue architecture that shortens tissue remodeling, by accelerating the balance between mature tissue formation and scaffold degradation. Moreover, this methodology allows to reduce the strength of the magnetic field applied to the tissues, since weak magnetic force stimulation has significant effect on the scaffold, and consequently on tissue formation, homogeneity, and stability of the scaffold when implanted in the injured site.

Therefore, TE using magnetic composites holds great promise, since it benefits in optimizing the control of timing, delivery of GFs, magnetic strength and scaffold fixation in tissue formation allowing a control of the processes governing interface regeneration and homeostasis.

Conclusions and future perspectives

Nowadays, magnetic iron oxides, especially in the form of MNPs, are being used in TE applications. In particular, MNPs are being incorporated into scaffolds, providing functional three dimensional (3D) engineered systems which can respond to exterior magnetic field stimuli.

Such magnetic responsive composites can provide other functionalities to implantable devices, either by directly influencing the interaction of the scaffold with the cells (affecting adhesion, proliferation and differentiation) or to be used as smart drug delivery and tissue regeneration systems. Scheme 1 resumes some effects that can be explored by such devices in the context of TE, explored in this mini-review. Regarding cell interaction it was possible to conclude that MNPs stimulate cell adhesion, proliferation, and even differentiation, being this effect amplified in the presence of an external magnetic field. Magnetic composites can thus provide local mechanical stimuli to cells, enhancing the regeneration potential of implantable devices.

The magnetic properties of polymeric matrices, especially hydrogels, also allow to finely tune and accurately control drug's release profile in a spatiotemporal context. More work will be necessary in order to improve the drug release profile (including multiple drug release specifically designed to stimulate the regenerative process) using magnetic scaffolds under the presence of a magnetic field.

Furthermore, it is believed that magnetic composites could be an useful tool for controlling the delivery and availability of GFs for tissue regeneration, being more cost effective when conjugated with magnetic nanoparticles than when freely available. Moreover, remote actuation could allow for scaffold reloading with these molecules, thus benefitting tissue regeneration. Also, magnetic scaffolds can be fixed in the body by the action of an external magnetic field, overcoming issues related to scaffold micro- and macro-movements in injured sites.

Besides the applications discussed in this review, magnetic composites could offer other possibilities that could be explored in the field of TE. For example, Utkan Demirci and co-workers developed a technique that enables 3D microgel assembly, by mimicking the repeating cellular functional units that compose tissues.[52] The technology presented herein offers an alternative to known approaches, which face cell seeding limitations and microenvironment control.

Also we envision for the future other kinds of applications, such as: magnetic responsive surfaces that could control better the cellular behavior; implantable constructs with shape controlled by magnetic fields; magnetic beads for stem cell expansion;[53] and magnetic hydrogel composites as remoted activated microfluidic devices.[54]

Notes

[a] *3B's Research Group - Biomaterials, Biodegradables and Biomimetics; ICVS/3B's – PT Government Associate Laboratory. AvePark, Zona Industrial da Gandra, S. Cláudio do Barco, 4806-909 Taipas, Guimarães, Portugal.*

[b] *Fax: +351253510909; Tel: +351-253510912; E-mail: sara.gil@dep.uminho.pt*

[c] *Fax: +351253510909; Tel: +351-253510904; E-mail: jmano@dep.uminho.pt;*

[*] *Corresponding author.*

Acnowledgements

The present work was supported by the Portuguese Foundation for Science and Technology (FCT) through the Suprarelax project (PTDC/FIS/115048/2009).

References

1 G.T. Gillies, R.C. Ritter, W.C. Broaddus, M.S. Grady, M.A. Howard, R.G. McNeill, *Rev. Sci. Instrum.*, 1994, **65**, 533-562;

2 Y.W. Jun, J.W. Seo, J. Cheon, *Acc. Chem. Res.*, 2008, **41**, 179-189;

3 S.F. Medeiros, A.M. Santos, H. Fessi, A. Elaissari, *Int. J. Pharm.*, 2011, **403**, 139-161;

4 Q.A. Pankhurst, J. Conolly, S.K. Jones and J. Dobson, *J. Phys. D: Appl. Phys.*, 2003, **36**, 167-181;

5 J.M. De Teresa, C. Marquina, D. Serrate, R.F. Pacheco, L. Morellon, P.A. Algarabel, M.R. Ibarra, *Int. J. of Nanotechnology*, 2005, **2**, 3-22;

6 N. Tran, T.J. Webster, *Wiley Interdisicp. Rev. Nanomed. Nanobiotechnol.*, 2009, **1**, 336-351;

7 C. Cunha, S. Panseri, D. Lannazo, A. Piperno, A. Pistone, M. Fazio, A. Russo, M. Marcacci, S. Galvagno, *Nanotechnology*, 2012, **23**, 465102;

8 J.L. Corchero, A. Villaverde, *Trends Biotechnol.*, 2009, **27**, 468-476;

9 C. Yang, J. Wu, Y. Hou, *Chem. Commun. (Camb)*, 2011, **47**, 5130-5141;

10 S. Gil, E. Castro, J.F. Mano, *Matter. Lett.,* 2013, **100**, 266-270;

11 R. Langer, J.P. Vacanti, *Science*, 1993, **260**, 920-926;

12 E. Castro, J.F. Mano, *J. Tissue Eng. Regen. M.,* 2013, **9**, 1129-1136;

13 S. Nomura, T. Takano-Yamamoto, *Matrix Biol.,* 2000, **19**, 91-96;

14 K. Lai, W. Jiang, J.Z. Tang, Y. Wu, B. He, G. Wang and Z. Gou, *R. Soc. Chem. Adv.,* 2012, **2**, 13007-13017;

15 G. Bao, S. Suresh, *Nat. Mater.,* 2003, **2**, 715-725;

16 L.E. Freed and G. Vunjak-Novakovic, in *Principles of Tissue Engineering*, ed. R.P. Lanza, R. Langer, J. Vacanti, Elsevier, San Diego, 2nd edn., 2000, ch. 13, pp. 143-156;

17 M. Wright, P. Jobanputra, C. Bavington, D.M. Salter, G. Nuki, *Clin. Sci. (Lond)*, 1996, **90**, 61-71;

18 Q.A. Pankhurst, N.T.K. Thanh, S.K. Jones, J. Dobson, *J. Phys. D: Appl. Phys.*, 2009, **42**;

19 J. Dobson, S.H. Cartmell, A. Keramane, A.J.E. Haj, *IEEE Trans. Nanobioscience*, 2006, **5**, 173-177;

20 S.H. Cartmell, J. Dobson, S.B. Verschueren, A.J.E. Haj, *IEEE Trans. Nanobioscience*, 2002, **1**, 92-97:

21 L.M Walker, A. Holm, L. Cooling, L. Maxwell, A. Oberg, T. Sundgvist, A.J.E. Haj, *FEBS Lett.*, 1999, **459**, 39-42;

22 X. B. Zeng, H. Hu, L.Q. Xie, F. Lan, W. Jiang, Y. Wu, Z.W. Gu, *Int. J. Nanomedicine*, 2012, **7**, 3365-3378;

23 J.T. Kannarkat, J. Battogtokh, J. Philip, O.C. Wilson and P.M. Mehl, *J. Appl. Phys.*, 2010, **107**, 307-373;

24 J. Meng, B. Xiao, Y. Zhang, J. Liu, H. Xue, J. Lei, H. Kong, Y. Huang, Z. Jin, N. Gu, H. Xu, *Sci. Rep.*, 2013, **3**, 2655-2662;

25 S. Hughes, S., A.J El Haj, J. Dobson, *Med. Eng. Phys.*, 2005, **27**, 754-762;

26 A.K. Gupta, M. Gupta, *Biomaterials*, 2005, **26**, 3995-4021;

27 Y. Sapir, S. Cohen, G. Friedman, B. Plovak, *Biomaterials*, 2012, **33**, 4100-4109;

28 B. Bhana, R.K. Iver, W.L. Chen, R. Zhao, K.L. Sider, M. Likhitpanichkul, C.A. Simmons and M. Radisic, *Biotechnol. Bioeng.*, 2010, **105**, 1148-1160;

29 A.L. Sieminski, R.P. Hebbel, K.J. Gooch, *Exp. Cell Res.*, 2004, **297**, 574-584;

30 A.M. Ross, Z. Jiang, M. Bastmeyer, J. Lahann, *Small*, 2012, **8**, 336-355;

31 T. Yeung, P.C. Georges, L.A. Flanagan, B. Marq, M. Ortiz, M. Funaki, N. Zahir, W. Ming, V. Weaver, P.A. Janmey, *Cell Motil. Cytoskeleton*, 2005, **60**, 24-34;

32 N.M Alves, I. Pashkuleva, R.L. Reis, J.F. Mano, *Small*, 2010, **6**, 2208-2220;

33 W.M. Saltzman, W.L. Olbricht, *Nat. Rev. Drug Discov.*, 2002, **1**, 177-186;

34 M. Biondi, F. Ungaro, F. Quaglia, P.A. Netti, *Adv. Drug Deliv. Rev.*, 2008, **60**, 229-242;

35 T. Bussemer, I. Otto, R. Bodmeier, *Crit. Rev. Ther. Drug Carrier Syst.*, 2001, **18**, 433-458;

36 N.S. Satarkar, J.Z. Hilt, *J. Control. Release*, 2008, **130**, 246-251;

37 V.E. Santos, M.E. Gomes, J.F. Mano, R.L. Reis, *Tissue Eng. PT B-Rev.*, 2013, **19**, 308-326;

38 V.E. Santos, M.E. Gomes, J.F. Mano, R.L. Reis, *Tissue Eng. PT B-Rev.*, 2013, **19**, 327-352;

39 J. Kost, J. Wolfrum, R. Langer, *J. Biomed. Res.*, 1987, **21**, 1367-1373;

40 V.M. De Paoli, S.H. Lacerda, L. Spinu, B. Ingberg, Z. Rosenweig, N. Rosenweig, *Langmuir*, 2006, **22**, 5894-5899;

41 T.Y. Liu, S.H. Hu, T.Y. Liu, D.M. Liu, S.Y. Chen, *Langmuir*, 2006, **22**, 5974-5978;

42 O. Ziv-Polat, H. Skaat, A. Shahar, S. Margel, *Int. J. Nanomedicine*, 2012, **7**, 1259-1254;

43 H. Skaat, O. Ziv-Polat, A. Shahar, D. Last, Y. Mardor, S. Margel, *Adv. Healthc. Mater.*, 2012, **1**, 168-171;

44 J.F. Mano, *Adv. Eng. Mater.*, 2008, **10**, 515-527;

45 S.B. Campbell, M. Patenaude and T. Hoare, *Biomacromolecules*, 2013, **14**, 644-653;

46 K.L. Grace, W.J. Revell, M. Brookes, *Orthopedics*, 1998, **21**, 297-302;

47 P.A. Glazer, M.R. Heilmann, J.C. Lotz, D.S Bradford, *Spine*, 1997, **22**, 2351-2356;

48 S. Panseri, A. Russo, M. Sartori, G. Giavaresi, M. Sandri, M. Fini, M.C. Maltarello, T. Shelvakova, A. Ortolani, A. Visani, V. Dediu, A. Tampieri, M. Marcacci, *Bone*, 2013, **56**, 432-439;

49 S. Bakbak, R. Kayacan, O. Akkuş, *J. Biomech.*, 2011, **44,** 11;

50 S. Panseri, A. Russo, G. Giavaresi, M. Sartori, F. Veronesi, M. Fini, D.M. Salter, A. Ortolani, A. Strazzari, A. Visani, C. Diogini, N. Bock, M. Sandri, A. Tampieri, M. Marcacci, *J. Biomed. Mater. Res. A*, 2012, **100**, 2278-2286;

51 S. Panseri, C. Cunha, T. D'Alessandro, M. Sandri, A. Russo, G.Giavaresi, M. Marcacci, C.T. Hung, A. Tampieri, *PLoS ONE,* **7**, 38710-38718;

52 F. Xu, C.M. Wu, V. Rengarajan, T. D. Finley, H.O. Keles, Y. Sung, B. Li, U.A. Gurkan, U. Demirci, *Adv. Mater.*, 2011, **23**, 4254-4260;

53 W. Song, M.B. Oliveira, P. Sher, S. Gil, J.F. Mano, *Biomed. Mater.*, 2013, **8**, 045008;

54 N.S. Satarkar, W. Zhang, R.E. Eitel, J.Z. Hilt, *Lab Chip*, 2009, **9**, 1773-1779;

Figure 1:

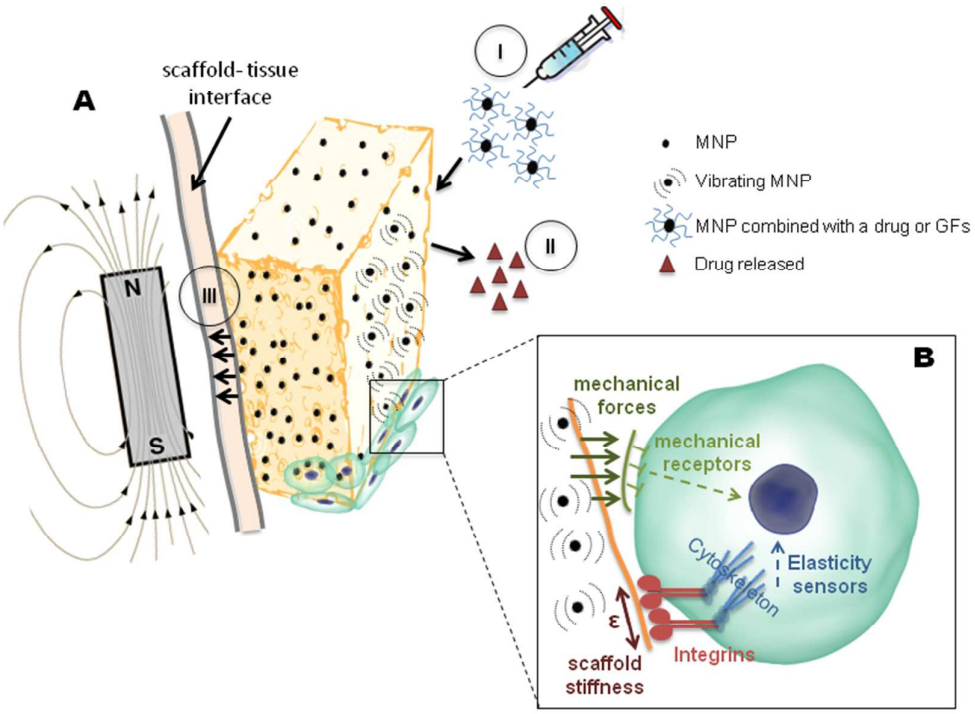

Fig. 1 A) Representative Scheme showing some possible benefits and capabilities of using magnetic nanocomposite biomaterials in tissue engineering. I - Magnetic nanoparticles, carrying drugs or growth factors, injected in the vicinity of the scaffold and attracted to it upon application of a magnetic field. II - Drug release upon vibration of magnetic nanoparticles. Different release profiles can be obtained according to the different magnetic stimulus they are exposed to. III - Magnetic scaffold fixation. B) In the magnified image it can be seen how the magnetic stimulation can influence cellular behaviors. On one hand, at the nanoscale level, each magnetic nanoparticle in the scaffold acts like a single magnetic domain, providing micromotions on the interface between cells and the scaffold that might affect the ion channels on the cell membrane, and trigger the mechanotransduction pathway. On the other hand, the incorporation of magnetic nanoparticles increases scaffold stiffness, which is a property known to influence cell adhesion.