Hyperthermia Induced in Magnetic Scaffolds for Bone Tissue Engineering

Manuel Bañobre-López[1], Yolanda Piñeiro-Redondo[2], Monica Sandri[3], Anna Tampieri[3], Roberto De Santis[4], Valentin Alek Dediu[5], and Jose Rivas[1,2]

[1]International Iberian Nanotechnology Laboratory, Braga 4715-330, Portugal
[2]Department of Applied Physics, Faculty of Physics, University of Santiago de Compostela, Santiago de Compostela 15782, Spain
[3]Institute of Science and Technology for Ceramics, National Research Council, Faenza 48018, Italy
[4]Institute for Polymers, Composites and Biomaterials, National Research Council, Naples 80125, Italy
[5]Institute for Nanostructured Materials, National Research Council, Bologna 40129, Italy

The design and fabrication of advanced biocompatible and bioresorbable materials able to mimic the natural tissues present in the human body constitutes an important challenge in regenerative medicine. The size-dependent properties that materials exhibit at the nanoscale as a consequence of their higher surface-to-volume ratio have opened a wide range of opportunities for applications in almost every imaginable field. In this regard, the incorporation of magnetic nanoparticles (MNPs) into biocompatible scaffold formulations provides final materials with additional multifunctionality and reinforced mechanical properties for bone tissue engineering applications. In addition to the biological implications due to their magnetic character (i.e., magnetic stimuli that favor the cell adhesion/proliferation, guiding of growth factors loaded magnetic nanocarriers, etc.), the ability of superparamagnetic scaffolds to simultaneously show magnetic hyperthermia when a dynamic external magnetic field is applied become promising to treat critical bone defects caused by malignant bone cancer through a combined therapy consisting of on demand temperature increase and thermally activated drug delivery. In this paper, we will comment on several different approaches to construct magnetic scaffolds with hyperthermia properties for bone tissue engineering. Experimental details about the design, fabrication and physicochemical characterization of a representative set of magnetic scaffolds have been described, focusing on their hyperthermia properties. The following synthesis procedures to magnetize biocompatible scaffolds reported in this paper covers dip coating of biocompatible gelatin-based scaffolds in aqueous MNPs dispersions, iron doping of the hydroxyapatite (HA) crystal structure, and incorporation of magnetic bioresorbable HA nanoparticles into poly-ε-caprolactone-based polymeric matrices.

Index Terms—**Magnetic hyperthermia (MH), magnetic scaffolds, magnetite nanoparticles.**

I. Introduction

THE use of biocompatible scaffolds as good mechanical supports able to induce bone tissue regeneration is one of the most active research topics in biomedicine. The fabrication of 3-D templates that mimic natural biologic structures and induce their regeneration, is a challenge in regenerative medicine nowadays [1].

Nanotechnology emerges as a powerful discipline able to provide original and novel solutions in many different fields. The discovery of novel size-dependent properties in materials at the nanoscale has opened a big variety of opportunities for applications in almost every imaginable field. For example, the superparamagnetic properties found in iron oxide particles as their sizes decrease below the multidomain-to-single-domain limit, together with their assumed biocompatibility and nontoxicity, have allowed their incorporation in a big amount of material formulations for biomedical applications, providing them with powerful multifunctionality and reinforced mechanical properties. Particularly interesting is their incorporation into biocompatible scaffolds for bone tissue engineering applications [2]. The main objective for designing biocompatible and bioresorbable magnetic scaffolds is the possibility to obtain structures that can be manipulated *in situ* by applying external magnetic fields. The influence of magnetic fields in the cell stimulation has been recently reported (magneto-mechanical stimulation of cell constructs [3], mechano-sensitive ion channels [4], magnetic cell seeding, and controlled cell proliferation and differentiation) in [5] and [6]. Furthermore, the magnetic field fluxes and gradients generated in the vicinity and inside the scaffold constitute the driving force needed to control specific processes at cell level, allowing magnetic carriers to transport biomolecules and growth factors (VEGF, BMP, etc.) that eventually stimulate bone tissue regenerations and vascular remodeling [7].

In addition, especially interesting is the ability of some magnetic nanoparticles (MNPs) to transform the electromagnetic energy into heat, that allows to induce a localized temperature increase by external application of an oscillating magnetic field [magnetic hyperthermia (MH)] [8]. Incorporated into synthetic/natural scaffold formulations, MNPs could act as heat-generating sources able to thermally activate, on demand, the release of therapeutic compounds and growth factors, thus triggering cell behaviour (e.g. proliferation and differentiation) and promoting tissue regeneration. It is important to remark that the radiation used in MH is in the range of the radiofrequency of the electromagnetic spectrum, having a very good penetration length that allows it to access any tissue and organ inside the human body and being completely safe.

Manuscript received May 7, 2014; revised May 23, 2014; accepted May 23, 2014. Date of current version November 18, 2014. Corresponding author: M. Bañobre-López (e-mail: manuel.banobre@inl.int).

Color versions of one or more of the figures in this paper are available online at http://ieeexplore.ieee.org.

Digital Object Identifier 10.1109/TMAG.2014.2327245

In this paper, experimental details about the synthesis and hyperthermia properties of different biocompatible scaffold formulations containing magnetic iron oxide particles (magnetite) will be shown. As some examples, we will focus on the hyperthermia properties of both natural and synthetic scaffolds [i.e., gelatin-, hydroxyapatite (HA)-based scaffolds] magnetized either by dip coating in aqueous MNPs dispersions or using magnetite nanoparticles as starting material in the formulation, as well as by intrinsic metal transition doping of the HA lattice. Special emphasis will be focused on the development of an innovative biocompatible and bioresorbable superparamagnetic scaffold synthesized by doping HA with Fe ions, which is very promising for application either as active scaffold for bone angiogenesis and osteochondral regeneration or as nontoxic bioresorbable shuttle for magnetic nanocarriers [9]. Magnetic nanocomposites formed by a biodegradable poly-ε-caprolactone (PCL) matrix and superparamagnetic iron-doped HA (FeHA) nanoparticles (NPs) at different PCL/FeHA compositions have been also successfully prototyped, layer on layer, through 3-D bioplotting, which show interesting hyperthermia properties and cell proliferation [10], [11].

II. Magnetic Scaffolds

To use MH as an effective healing strategy for bone tissues damaged by the effect of diseases or severe injuries, a strong effort is being done to find biocompatible magnetic scaffolds. The candidate scaffolds have to mimic the natural bone structure and functionalities, and therefore, they have to show adequate porosity, resistance, biocompatibility, controlled degradation kinetics, and in addition being magnetically active to provide mild MH for drug release or cancer cell necrosis.

Magnetic activity is not only crucial for the MH performance, but is necessary for endowing the scaffolds with added functionalities, such as magnetic stimulation for a differentiate cell growth, magnetic docking or guiding of specifically developed magnetic therapeutic nanocarriers and contrast enhancement of certain locations for magnetic imaging techniques [12], [13]. Moreover, scaffold fixation by magnetic forces constitute an innovative solution to the old challenge in osteochondral defect surgery, which would guaranty a stable bone/scaffold interface for tissue growth.

The synthetic procedures that allow obtaining magneto-mechanically functional bioscaffolds for bone implants that will be discussed here comprise different synthesis approaches. The simplest one is by physicochemical processes based on mixing magnetic NPs with biocompatible scaffolds, whereas a more complex approach is achieved by chemically doping of the HA crystal structure with magnetic ions.

A. Poly-ε-Acrylic Acid-Coated Fe_3O_4 MNPs as Nanoheaters

The most extensively ferrofluid-based MH applications use superparamagnetic iron oxide NPs as magnetic core materials for *in vivo* setups [14], [15] due to their good magnetic response (M_{sat}bulk = 80–90 emu/g), biocompatibility, nontoxicity, and the absence of magnetic interaction without magnetic stimulation.

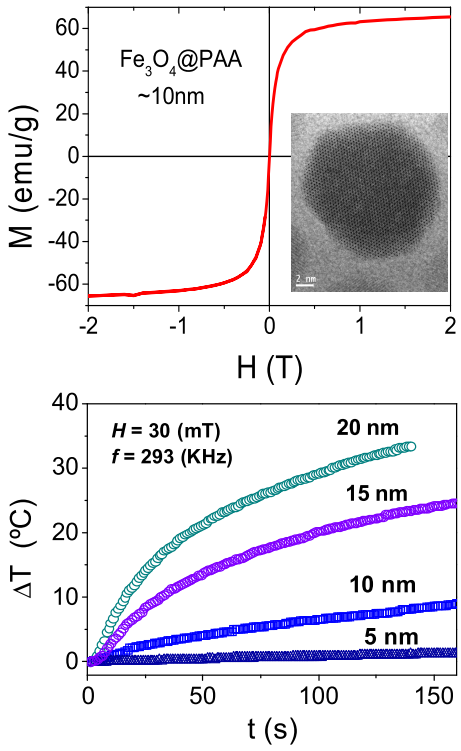

Fig. 1. Top: SPM curve of Fe_3O_4@PAA MNPs. Inset shows a TEM image of one single particle. Bottom: particle size dependence of MH of Fe_3O_4@PAA MNPs.

The final efficiency of the whole setup depends on the optimization of the instrumental parameters (externally applied magnetic field intensity and frequency) and physicochemical properties of the magnetic nanosized systems (i.e., MNPs size distribution, particle shape, and magnetic and embedding polymeric materials) [16]–[18].

The MNPs used to simultaneously magnetize biocompatible scaffolds and act as heating generating sources under an oscillating magnetic field were small Fe_3O_4 NPs of about $d \approx 10$ nm coated with polyacrylic acid (PAA). They were synthesized by coprecipitation of an aqueous stoichiometric mixture of Fe^{2+} and Fe^{3+} salts with molar ratio $Fe^{3+}/Fe^{2+} = 1.49$ in ammonium hydroxide (NH_4OH, 28%). Then, the PAA coating was added in molar excess to the particles dispersion. The rationale for using PAA is to increase the colloidal stability in aqueous solutions and leave around the particles carboxyl functional groups that can be used for further functionalized steps if necessary [19].

Structural characterization by X-ray powder diffraction (XRD) showed a single phase of magnetite with the absence of any impurity (data not shown). Fig. 1 (upper) shows the magnetization curve as a function of the magnetic field up to 2 T at room temperature, from which a clear superparamagnetic behavior with nor remanence or coercive fields is evidenced. On other hand, the saturation magnetization was as high as 60 emu/g, which represents actually a very high value for such small NPs. Inset of Fig. 1 is a transmission electron microscope (TEM) image of one single NP, showing that they have a very high structural quality with the absence

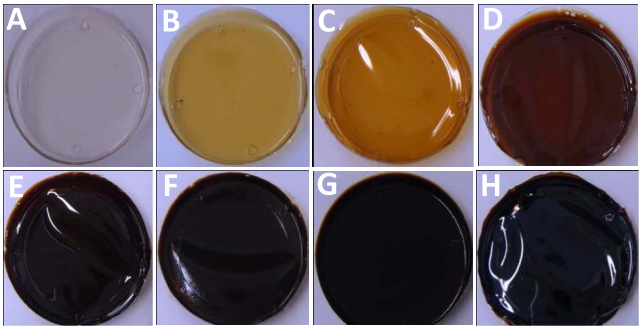

Fig. 2. Gelatin-based magnetic scaffolds with increasing loading of Fe_3O_4@PAA MNPs from A to H.

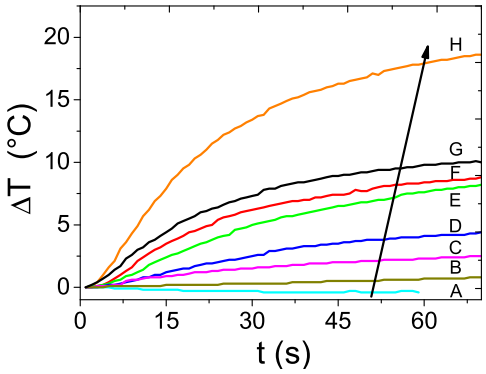

Fig. 3. MH curves for gelatin-based scaffolds containing increasing MNPs loadings from A to H, as indicated by the arrow.

of crystalline multidomains. Fig. 1, lower, shows the hyperthermia properties of these Fe_3O_4@PAA NPs as a function of the particle size when they are under an oscillating field of frequency of 293 kHz and magnitude of 30 mT. Their heating power is clearly appreciated, showing a temperature increase of almost 10 °C for the 10 nm particles in less than 3 min. The bigger the particle size, the higher the temperature increase, being of more than 30 °C for the bigger particles. These results suggest that these particles are very promising candidate to be used as small nanoheaters able to provide final scaffold materials with mild hyperthermia properties useful for multifunctional healing therapies in bone regenerative medicine.

B. Gelatin-Based Magnetic Scaffolds

Magnetically doped biohybrid composites, such as Fe_3O_4 NPs doped gelatin and HA, combine the magnetic/hyperthermia activity of magnetite with some natural biologically inspired processes as gelatin fibers assembly or HA porosity. These materials are only a set of those possible combinations that can provide improvement in bioactivity, angiogenesis, or mechanical properties to bone mineral components. In this way, benefiting from biological properties of natural materials like gelatin and HA, we synthesized magnetic scaffolds using these materials as matrix and incorporated into them the MNPs described above. In the case of HA matrices, the magnetic functionalization was carried out by dip coating, by which a preformed natural template was immersed into solutions containing different concentration of colloidal Fe_3O_4@PAA NPs. The incorporation of these MNPs into the formulation takes place by physicochemical adsorption to the HA particle surface. As a representative example of these biological miming materials, Fig. 2 shows final gelatin-based scaffolds morphologies with increasing MNPs loadings. In this case, the magnetic functionalization of the gelatin matrices was performed by physical mixing of different amounts of magnetite NPs (in aqueous dispersion) and gelatin, which led to stable and homogeneous solutions. These solutions were dropped into circular templates and stayed after gelation. The final materials are compositionally completely homogeneous. The magnetic loading in the final scaffolds can be tuned by using different MNPs concentration dispersions; the higher the MNPs concentration, the darker the color.

Fig. 3 shows the MH curves obtained for these samples under an oscillating magnetic field of frequency of 293 kHz and magnitude of 30 mT. All the samples show a temperature increase that is dependent on their MNP content (no heating is observed in sample A, which is a blank without MNPs). Their thermal response is additionally in total concordance with the magnetic results, in which the saturation magnetization obtained from the M versus H curves increases with the amount of MNPs in the samples from A to H (data not shown), as expected from the saturation magnetization dependence of the Néel relaxation time. A temperature increase about 20 °C is observed in only 1 min for the gelatin-based scaffold with the highest MNPs concentration. Therefore, magnetic-doped gelatin bioagents show a very good performance that make them suitable for remote magnetic control biomedical applications, promising for bone tissue engineering.

C. Magnetic Scaffolds by Magnetic Doping of HA

A more complex approach to synthesize biocompatible magnetic scaffolds is achieved by chemically doping of the scaffold crystal nanostructure with magnetic ions. We have produced magnetic HA NPs by doping specific sites on its crystal lattice with magnetic Fe^{2+}/Fe^{3+} ions, keeping its original biocompatible structure and forming intrinsic iron oxide regions responsible for the magnetic and hyperthermia properties. Superparamagnetic FeHA NPs have been synthesized following a neutralization method in which both Fe species are simultaneously introduced under controlled synthesis conditions. Following this synthesis method, during the stage of HA formation, the crystallographic position Ca(1) and Ca(2) of the apatite lattice are selectively substituted by iron species, Fe^{3+} and Fe^{2+}, respectively, leading to the formation of local iron oxide domains that provide the mainly HA lattice with intrinsic superparamagnetism behavior [9], [20]. Experimental details about the method followed for the synthesis of these FeHA NPs have been described in [9]. FeHA nanopowder morphology investigation by TEM showed calcium phosphate particles with needle-like morphology, rather heterogeneous in size, 5–20 nm in width and up to 50–80 nm in length [Fig. 4(a)]. It also confirmed that the quasi-amorphous calcium phosphate

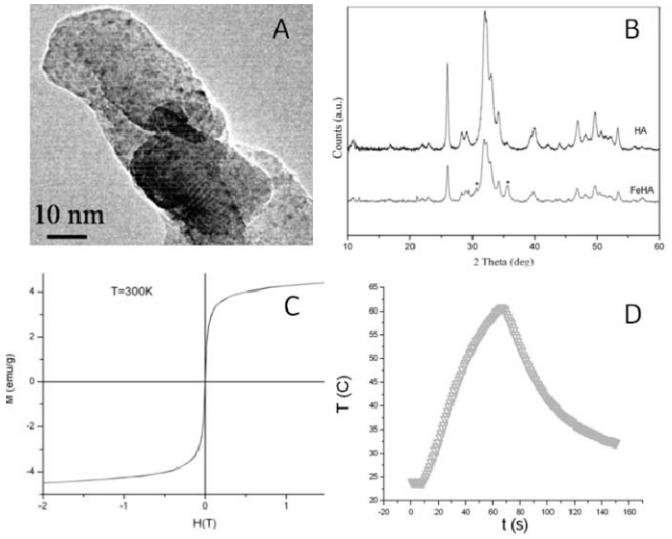

Fig. 4. FeHA NPs characterization. (a) TEM micrographs showing FeHA MNPs with needle-like morphology. (b) XRD analysis profiles obtained from FeHA and HA nanopowders synthesized at the same temperature and conditions. FeHA spectra underline a lower crystallinity and a little amount of magnetite (*) (∼2 vol.%) as secondary phase. (c) Magnetization curve obtained from the analysis of FeHA MNPs, which displays a typical superparamagnetic behavior. (d) Temperature increase with time under an oscillating magnetic field of frequency of 293 kHz and magnitude of 30 mT. The maximum of the curve represents the switch OFF of the magnetic field, from which the material starts cooling down.

matrix contained iron uniformly distributed, although a very low concentration of dark spots (5–10 nm in size) corresponding to inclusions of iron rich phases was also observed. Further XRD studies reveal that the synthesis method employed leads to a low crystalline apatite with a crystallinity extent much lower than that of the nonsubstituted HA prepared at the same temperature and with a very low content of magnetite (<2 vol.%) as secondary phase [Fig. 4(b)]. Moreover, by Rietveld analysis, an increase of the *a*-axis from 9.4218(5) to 9.4557(1) and a decrease of the *c*-axis from 6.8813(3) to 6.8785(1) was detected as expected in the case of Ca substitution with ion species having a lower radius, confirming that the Fe^{2+}/Fe^{3+} ions are not situated in cell interstitial positions but in Ca-substituting crystallographic sites. The superparamagnetic behavior of the FeHA nanopowders is evidenced by the magnetization curve as a function of the applied magnetic field, typical of single-domain MNPs [Fig. 4(c)]. Contrary to what would be expected on the basis of the amount and aggregate size of magnetite present in the powder as secondary phase and detected by XRD, the saturation magnetization value of the FeHA nanopowder was higher (4.0–4.2 emu/g) confirming the additional intrinsic magnetic property of the powder due to the substitution of iron ions in the HA lattice. Regarding the hyperthermia properties of the FeHA powders, the temperature versus time curve reported in the Fig. 4(d) exhibits a significant temperature increase of about 40 °C in 60 s, which once implanted in large bone defects or damaged areas in the human body make them very promising to induce thermal stimuli that can be used either for killing tumor cells or to favor the local release of previously adsorbed growth factors that promote cell activity and vascularization. So far, previous preliminary *in vitro* and *in vivo* study performed on the FeHA NPs showed that those novel superparamagnetic MNPs enhance cell proliferation and exhibit a good biocompatibility level after implantation into a critical size lesion of the rabbit condyle [21], demonstrating that might be suitable for biomedical applications.

In conclusion, the magnetic properties of the new FeHA crystallographic phase open the door of regenerative medicine to a conceptually new family of biomimetic materials able to be biologically manipulated or activated *in situ* by means of an external magnetic field. In addition, the wider field of theranostics may benefit from solutions represented by these completely biocompatible and biodegradable magnetic nanocarriers.

D. Polycaprolactone-Based Magnetic Scaffolds

In general terms, the physical and chemical synthetic procedures that allow obtaining magneto-mechanically functional bioscaffolds for bone tissue engineering (some examples described above) cover a wide variety of well-known approaches, such as calcinations at high temperatures, ball milling to produce powders, suspensions in solvents or foaming agents, casting into molds, sintering procedures at high temperatures (in cases up to 1200 °C), exposure to controlled atmospheres, reduction or oxidation of metallic salts in the presence of HA nucleation reactions or simultaneous addition of metallic salts during neutralization processes. The main challenge in this step is to avoid the degradation of the magnetic phase during the synthesis. However, although ceramic materials can act as substitutes of natural bone and possess osteoconductive properties, they are brittle, stiff, and do not present a good mechanical performance [22]–[24]. Therefore, a more suitable alternative comprises the physical mixture of the magnetic scaffold with biopolymers as polylactic acid, polyglycolic acid, poly(lactic-*co*-glycolic acid), or PCL, which have been widely studied in [22] and [23]. These polymer matrix provides flexibility to the whole ensemble and the final macroscopic shape is obtained by rapid prototyping through extrusion procedures that are optimized to ensure the adequate nanocomposite matrix structure, mechanic strength, and a morphology with an adequate pore distribution required to favor cell growth.

In this sense, PCL, a bioresorbable polymer, is becoming the reference material in tissue engineering due to its numerous advantages: easy to manipulate, exceptional blend compatibility, rheological and viscoleastic properties, tailorable degradation kinetics and mechanic properties, facile and inexpensive production routes, easiness of shaping, and pores distribution manufacture [25].

On the other hand, magnetic nanocomposites represent a further biomaterial advancement introduced to trigger biological events occurring during tissue growth simply by activating, on demand, an external magnetic field. This feature has been recently incorporated into bone scaffolds [10], [26]–[28]. Similarly to bone cements and modified cements [10], [11], the incorporation of MNPs into a scaffold material can also

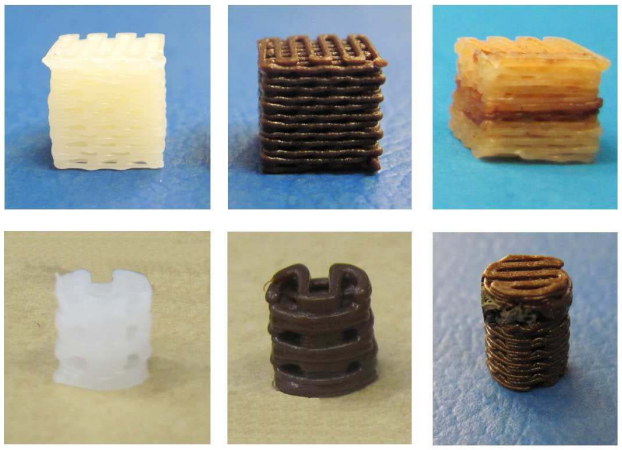

Fig. 5. PCL and nanocomposite scaffolds for bone tissue engineering. Top line: cubic scaffolds made of PCL, PCL/FeHA 80/20, and gradient PCL/FeHA. Bottom line: cylindrical scaffolds made of PCL, PCL/FeHA 80/20, and gradient PCL/FeHA.

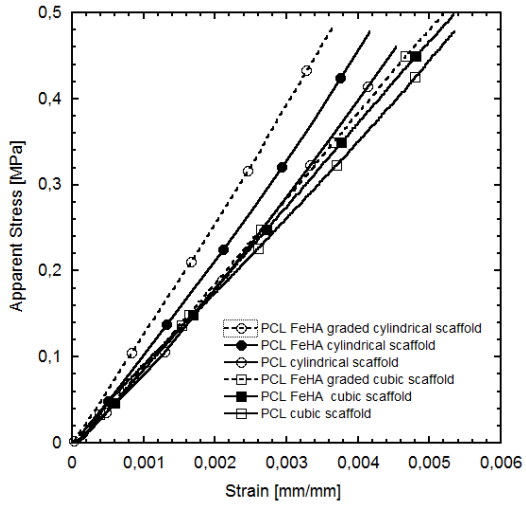

Fig. 6. Stress–strain curves at low-strain levels of cubic scaffolds made of PCL, PCL/FeHA 80/20, graded PCL/FeHA and of cylindrical scaffolds made of PCL, PCL/FeHA 80/20, graded PCL/FeHA.

be an interesting approach for treating bone cancer through an hyperthermia effect.

With the aim of exploring new materials that combine many of the above described biomagnetic functionalities, we have provided PCL matrices with magnetic and hyperthermia properties by embedding into the formulation Fe_3O_4 or FeHA NPs, leading to final scaffolds with enhanced mechanical properties and biological compatibility for tissue engineering applications.

Magnetic PCL/FeHA nanocomposite pellets were prepared to feed the syringe of the 3-D bioplotting equipment. PCL pellets (weight-average molecular weight 65 000) were dissolved in tetrahydrofuran (THF) through stirring at room temperature. The FeHA NPs described in the previous section were used as magnetic loading of the PCL matrix. They were added together with ethanol to the PCL/THF solution during stirring, and a PCL/filler weight ratio (wt/wt) of 80/20 was used. The obtained nanocomposite pellets consisting of PCL loaded with FeHA fillers were processed through 3-D bioplotting equipment to manufacture 3-D cubic (length of 8 mm) cylindrical scaffolds (6 mm in diameter, 8 mm in height) with a 0°/0°/90°/90° lay-down pattern. Pellets were placed in the stainless steel syringe and heated to a temperature of 110 °C–130 °C using a heated cartridge unit placed on the mobile arm of a 3-D bioplotter dispensing machine. The materials were extruded through a nozzle with an inner diameter of 400 and 580 μm for the cubic and cylindrical scaffolds, respectively. A continuous filament was deposited at a speed of about 30 mm/min using nitrogen pressure of 8.5–8.9 bar applied to the syringe of the bioplotter through a cap. Fig. 5 shows the PCL and the magnetic nanocomposite scaffolds (cubic and cylindrical) that were obtained. Furthermore, magnetic cubic and cylindrical prototyped scaffolds were also processed to provide a gradient in the FeHA distribution into the scaffold. In particular, for the PCL/FeHA graded cylindrical scaffold, a layer consisting of a preformed porous FeHA disc (having a diameter 6 mm and a height of 1.5 mm) was incorporated into the cylindrical 3-D bioplotted architecture (Fig. 5).

The apparent elastic modulus of PCL and of superparamagnetic-based scaffolds was measured through compression tests carried out at a rate of 1 mm/min up to a stress limit of 0.5 MPa. Fig. 6 reports the stress–strain data up to a stress of 0.5 MPa for scaffolds architectures shown in Fig. 5. A statistical significant difference ($p < 0.05$) was found between cubic and cylindrical scaffolds. For both the cylindrical and cubic scaffolds higher apparent modulus were observed for the PCL/FeHA graded structures, being of 133 (standard deviation 12) and 98 (standard deviation 10) MPa, respectively. The rapid prototyping approach was confirmed to be a powerful technique to manufacture PCL and nanocomposite-based scaffolds [26], [29]–[31]. Mechanical results clearly shows that the effect of superparamagnetic NPs provoke an increase of the stiffness of these scaffolds, and this effect is consistent with [10], [27], and [28]. The apparent elastic moduli measured for the cubic scaffolds are in the range of those reported in [26], and both the cubic and cylindrical 3-D scaffolds show an apparent elastic modulus that is in the range of the apparent modulus of trabecular bone [32]. The higher values of the apparent elastic modulus measured for the cylindrical scaffolds may be ascribed to the architecture of the scaffold and to the thickness of the plotted fiber. As scaffolds are compressed, the stress mainly transfers along the regions where the plotted fibers cross each other [33], however, for the cylindrical scaffolds, the compressive stress also transfers along the regions of the round boundary. Interestingly, these round boundary regions occupy about 20% of the whole circular base (Fig. 5).

In addition to the mechanical properties, engineered 3-D superparamagnetic architectures have the potential to attract, on demand, magnetic functionalized aggregates and cells, thus providing a tool for guiding the regeneration process. On the other hand, if a dynamic external magnetic source is applied to superparamagnetic scaffolds, a hyperthermia effect can be observed in [10]. This temperature increase can offer other options to further take advantage from superparamagnetic scaffolds; there is a great potential to use PCL/FeHA scaffolds

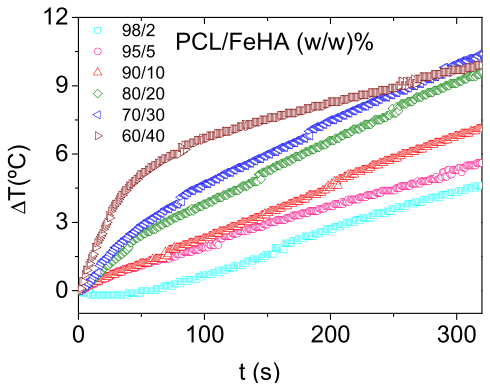

Fig. 7. Hyperthermia curves of magnetic PCL-based scaffolds at several PCL/concentrations and with cubic structure. The frequency and magnetic field of the generated oscillating magnetic field were 293 kHz and 30 mT, respectively.

in the case of critical bone defects caused by malignant bone cancer through a combined therapy consisting of on demand temperature increase and drug delivery. In particular, it has already been suggested to incorporate superparamagnetic NPs into acrylic-based bone cement to treat bone tumors through the hyperthermia effect [34]. As an example of the heating properties of these materials, Fig. 7 shows the hyperthermia curves of magnetic PCL/FeHA scaffolds with cubic structure at different PCL/FeHA concentrations. The scaffolds containing a low amount of superparamagnetic FeHA NPs showed hyperthermia responses quite moderate, whereas the most promising temperature increases corresponded to the highest magnetic loadings, i.e., PCL/FeHA 80/20 (shown in the Fig. 5) exhibited a temperature increase as high as 9 °C in 5 min of exposure to an externally applied oscillating field.

The possibility to manufacture 3-D graded superparamagnetic scaffolds could allow tuning the hyperthermia effect along the scaffold. Regions of the scaffolds containing higher amount of superparamagnetic HA will undergo a higher temperature increase. Therefore, if the critical bone defect is caused by malignant bone cancer, by increasing the amount of NPs in the regions of the scaffolds interfacing with the hosting bone tissue, it would be possible to locally increase temperature of these scaffolds to treat the cancer tissue, without affecting the regeneration process inside the scaffold. Therefore, the optimal composition will be the one that allow us to achieve a significant temperature increase while keeping the mechanical performance as high as possible.

III. Conclusion

Bone tissue engineering is recognized as a promising solution to regenerate injured bone tissue, overcoming the drawbacks that an autologous bone grafts harvested from healthy bone districts present, such as the amount of tissue to be supplied and donor site morbidity. Scaffolds for bone tissue engineering have to provide a mechanical function similar to that of the natural tissue to properly withstand loads and to transfer the stress to the hosting tissue. The use of polymer-based composite materials to manufacture scaffolds for bone tissue engineering represents a valuable approach since this strategy allows the design of the material most suitable for the specific purpose simply by modulating the ratio between the matrix and the reinforcement and the distribution and/or orientation of the reinforcement into the polymeric matrix. Moreover, these polymeric composite scaffolds can be programmed to undergo a tailored degradation process to gradually transfer the mechanical function to the newly forming tissue, and ideally, completely replaced by newly formed bone. Magnetic scaffolds can offer new interesting functionalities, they can be externally activated by applying a magnetic field and so generate remotely magnetic stimuli that can be used simultaneously to guide growth factors-loaded nanocarriers to injured bone areas and to induce MH to locally kill surrounded tumor cells or to trigger thermal activated processes, such as thermally-induced drug delivery from the nanocarriers. In particular, in this paper, we have described different experimental approaches to design and fabricate magnetic scaffolds showing promising hyperthermia properties, in addition to their biocompatible character and enhanced mechanical properties. These experimental procedures include dip-coating magnetization of natural biological compounds miming gelatin-based scaffolds, chemical doping of specific sites of the crystal lattice of HA NPs and magnetic nanocomposites formed by a biodegradable PCL matrix and superparamagnetic FeHA NPs at different PCL/FeHA compositions, successfully prototyped, layer on layer, through 3-D bioplotting.

In particular, in this paper, we have focused on the promising hyperthermia properties of a set of representative magnetic scaffolds developed by different synthesis methods, in addition to their biocompatible character and enhanced mechanical properties.

Acknowledgment

This work was supported by the European Community's under the FP7-Cooperation Programme through the MAGISTER project Magnetic Scaffolds for *in vivo* Tissue Engineering Large Collaborative Project FP7–21468.

References

[1] R. Langer and J. P. Vacanti, "Tissue engineering," *Science*, vol. 260, no. 5110, pp. 920–926, 1993.
[2] N. Bock *et al.*, "A novel route in bone tissue engineering: Magnetic biomimetic scaffolds," *Acta Biomater.*, vol. 6, no. 3, pp. 786–796, 2010.
[3] A. E. Markaki and T. W. Clyne, "Magneto-mechanical stimulation of bone growth in a bonded array of ferromagnetic fibres," *Biomaterials*, vol. 25, no. 19, pp. 4805–4815, 2004.
[4] S. Hughes, S. McBain, J. Dobson, and A. J. El Haj, "Selective activation of mechanosensitive ion channels using magnetic particles," *J. Roy. Soc. Inter.*, vol. 5, no. 25, pp. 855–863, 2008.
[5] J. M. Kanczler *et al.*, "Controlled differentiation of human bone marrow stromal cells using magnetic nanoparticle technology," *Tissue Eng. A*, vol. 16, no. 10, pp. 3241–3250, 2010.
[6] J. J. Mack *et al.*, "Achieving nutrient pumping and strain stimulus by magnetic actuation of tubular scaffolds," *Smart Mater. Struct.*, vol. 18, no. 10, p. 104025, 2009.
[7] A. Tautzenberger, A. Kovtun, and A. Ignatius, "Nanoparticles and their potential for application in bone," *Int. J. Nanobiomed.*, vol. 7, pp. 4545–4557, Aug. 2012.
[8] J. Rivas, M. Bañobre-López, Y. Piñeiro-Redondo, B. Rivas, and M. A. López-Quintela, "Magnetic particles and clusters through a cross-disciplinary approach," *J. Magn. Magn. Mater.*, vol. 324, no. 21, pp. 3499–3502, Oct. 2012.

[9] A. Tampieri *et al.*, "Intrinsic magnetism and hyperthermia in bioactive Fe-doped hydroxyapatite," *Acta Biomater.*, vol. 8, no. 2, pp. 843–851, 2012.

[10] M. Bañobre-López *et al.*, "Poly(caprolactone) based magnetic scaffolds for bone tissue engineering," *J. Appl. Phys.*, vol. 109, no. 7, pp. 07B313-1–07B313-3, 2011.

[11] A. Gloria *et al.*, "Magnetic poly(ε-caprolactone)/iron-doped hydroxyapatite nanocomposite substrates for advanced bone tissue engineering," *J. Roy. Soc. Inter.*, vol. 10, no. 80, p. 20120833, 2013.

[12] J. Meng *et al.*, "Super-paramagnetic responsive nanofibrous scaffolds under static magnetic field enhance osteogenesis for bone repair *in vivo*," *Sci. Rep.*, vol. 3, no. 2655, pp. 1–7, 2013.

[13] A. Russo *et al.*, "A new approach to scaffold fixation by magnetic forces: Application to large osteochondral defects," *Med. Eng. Phys.*, vol. 34, no. 9, pp. 1287–1293, Nov. 2012.

[14] R. B. Campbell, "Battling tumors with magnetic nanotherapeutics and hyperthermia: Turning up the heat," *Nanomedicine*, vol. 2, no. 5, pp. 649–652, Oct. 2007.

[15] A. K. Gupta, R. R. Naregalkar, V. D. Vaidya, and M. Gupta, "Recent advances on surface engineering of magnetic iron oxide nanoparticles and their biomedical applications," *Nanomedicine*, vol. 2, no. 1, pp. 23–39, Feb. 2007.

[16] J. P. Fortin, C. Wilhelm, J. Servais, C. Ménager, J.-C. Bacri, and F. Gazeau, "Size-sorted anionic iron oxide nanomagnets as colloidal mediators for magnetic hyperthermia," *J. Amer. Chem. Soc.*, vol. 129, no. 9, pp. 2628–2635, 2007.

[17] M. Gonzales-Weimuller, M. Zeisberger, and K. M. Krishnan, "Size-dependent heating rates of iron oxide nanoparticles for magnetic fluid hyperthermia," *J. Magn. Magn. Mater.*, vol. 321, no. 13, pp. 1947–1950, Jul. 2009.

[18] A. S. Eggeman A. M. Sara, D. Farrell, and Q. A. Pankhurst, "Size and concentration effects on high frequency hysteresis of iron oxide nanoparticles," *IEEE Trans. Magn.*, vol. 43, no. 6, pp. 2451–2453, Jun. 2007.

[19] Y. Piñeiro-Redondo, M. Bañobre-López, I. Pardiñas-Blanco, G. Goya, M. A. López-Quintela, and J. Rivas, "The influence of colloidal parameters on the specific power absorption of PAA-coated magnetite nanoparticles," *Nanosc. Res. Lett.*, vol. 6, p. 383, May 2011.

[20] M. Jiang, J. Terra, A. M. Rossi, M. A. Morales, E. B. Saitovitch, and D. E. Ellis, "Fe^{2+}/Fe^{3+} substitution in hydroxyapatite: Theory and experiment," *Phys. Rev. B*, vol. 66, no. 22, p. 224107, 2002.

[21] S. Panseri *et al.*, "Intrinsically superparamagnetic Fe-hydroxyapatite nanoparticles positively influence osteoblast-like cell behaviour," *J. Nanobiotechnol.*, vol. 10, no. 1, pp. 1–10, 2012.

[22] A. Gloria, R. De Santis, and L. Ambrosio, "Polymer-based composite scaffolds for tissue engineering," *J. Appl. Biomater. Biomech.*, vol. 8, no. 2, pp. 57–67, 2010.

[23] A. Gloria, T. Russo, R. De Santis, and L. Ambrosio, "3D fiber deposition technique to make multifunctional and tailor-made scaffolds for tissue engineering applications," *J. Appl. Biomater. Biomech.*, vol. 7, no. 3, pp. 141–152, 2009.

[24] R. Z. LeGeros, "Properties of osteoconductive biomaterials: Calcium phosphates," *Clin. Orthopaedics Rel. Res.*, vol. 395, pp. 81–98, Feb. 2002.

[25] M. A. Woodruff and D. W. Hutmacher, "The return of a forgotten polymer—Polycaprolactone in the 21st century," *Prog. Polym. Sci.*, vol. 35, no. 10, pp. 1217-1256, Oct. 2010.

[26] A. Gloria *et al.*, "Three-dimensional poly (ε-caprolactone) bioactive scaffolds with controlled structural and surface properties," *Biomacromolecules*, vol. 13, no. 11, pp. 3510–3521, 2012.

[27] R. De Santis *et al.*, "A basic approach toward the development of nanocomposite magnetic scaffolds for advanced bone tissue engineering," *J. Appl. Polym. Sci.*, vol. 122, no. 6, pp. 3599–3605, 2011.

[28] R. De Santis *et al.*, "A route toward the development of 3D magnetic scaffolds with tailored mechanical and morphological properties for hard tissue regeneration: Preliminary study," *Virtual Phys. Prototyping*, vol. 6, no. 4, pp. 189–195, Nov. 2011.

[29] D. W. Hutmacher and S. Cool, "Concepts of scaffold-based tissue engineering—The rationale to use solid free-form fabrication techniques," *J. Cellular Molecular Med.*, vol. 11, no. 4, pp. 654–669, Jul./Aug. 2007.

[30] A. Gloria, T. Russo, R. De Santis, and L. Ambrosio, "3D fiber deposition technique to make multifunctional and tailor-made scaffolds for tissue engineering applications," *J. Appl. Biomater. Biomech.*, vol. 7, no. 3, pp. 141–152, 2009.

[31] M. Domingos *et al.*, "The first systematic analysis of 3D rapid prototyped poly(ε-caprolactone) scaffolds manufactured through biocell printing: The effect of pore size and geometry on compressive mechanical behaviour and *in vitro* hMSC viability," *Biofabrication*, vol. 5, no. 4, p. 045004, 2013.

[32] R. De Santis, L. Ambrosio, F. Mollica, P. Netti, and L. Nicolais, "Mechanical properties of human mineralized connective tissues," in *Modeling of Biological Materials*. Boston, MA, USA: Birkhäuser, 2007, pp. 211–261.

[33] R. De Santis *et al.*, "Advanced composites for hard-tissue engineering based on PCL/organic–inorganic hybrid fillers: From the design of 2D substrates to 3D rapid prototyped scaffolds," *Polym. Compos.*, vol. 34, no, 9, pp. 1413–1417, Sep. 2013.

[34] M. Kawashita, K. Kawamura, and Z. Li, "PMMA-based bone cements containing magnetite particles for the hyperthermia of cancer," *Acta Biomater.*, vol. 6, no. 8, pp. 3187–3192, Aug. 2010.