Sustained release from biodegradable metallic matrix—The entrapment of drugs within iron

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Abstract
Iron and its alloys have been widely used for variety of medical implants. These are used for long term applications as cheap implants with high inertness and low corrosion rate, and also as implants with high biocompatibility (the fourth-generation type). Such degrading implants can provide a temporary scaffold while the body heals. In addition to the needed mechanical support, it is highly desirable to provide local drug therapy, providing antibacterial properties, preventing rejection of the implant, and more. So far, the combination of a degradable metallic implant which serves also as a three-dimensional matrix for drug release, remained unanswered. Here we present, we believe for the first time realization of this concept: Entrapment of drugs within a 3D degradable metal matrix—iron—from which the entrapped drugs are sustain-released. This new type of material is based on the molecular metals entrapment materials methodology, resulting in drugs@Fe. Two drugs have been successfully entrapped and released: chlorhexidine - an antiseptic drug, and rapamycin—used for avoiding transplant rejection. The delivery profiles of the composites were studied in two forms—powders and pressed discs showing two different types of drug release profiles. The release of the drugs from the powder has a first order release profile, while the pressed disk is a slower, zero-order release profile, which is highly desirable due to the constant rate of the release. Full characterization of the metallic biomaterials is provided, including XRD, SEM, TGA, elemental analysis, and surface area/porosity analysis.

1. Introduction
Metallic iron and its alloys are widely used for verity of permanent and temporary implants. Permanent implants based on iron and iron alloys have a number of advantages such as corrosion resistance, good mechanical properties and low cost compared with other materials [1]. Temporary implants are useful as well because they provide the critical initial support for healing, allowing the injured tissue to recover and to replace the implant while being bio-absorbed [2–4]. Implants made of biodegradable metals (also termed as the 4th generation [5]) are implants that are planned to corrode gradually in vivo, with non-toxic corrosion products which slowly dissolve along the healing process [2]. The duration of such degradation is usually optimized to last for 6–12 months. Among the variety of metals, two have been intensively studied for this kind of application—iron and magnesium which bio-corrode to their non-toxic oxides and hydroxides - as stents, pins, nails, screws, wires, and more [2–4]. A recent review of Birbilis et al [6], took this medical approach one step further and suggested that: ‘Metals themselves can be used for delivering pharmaceutics… however to the best of our knowledge there has been no current research into the possibility of resorbable metallic implants that can provide a route to drug delivery through the degradation of the metal itself’. Such new material might provide not only mechanical support, but also degrade while providing sustained release of the drug [6]. In general, sustained release of drugs from an implant serves for a variety of purposes such as preventing infections or implant rejection, while
releasing drugs slowly and locally in the target site, to improve their efficiency, lower their toxicity, and improve overall patient compliance [7, 8]. Here we report the successful entrapment of two relevant drugs in iron, and their subsequent release from within this metallic matrix in kinetics which are affected by corrosion. It extends our previous studies where we have shown that metals can be used as reservoir of drugs for delivery, specifically platinum as an example of an inert metal [9], and silver as an example of a bioactive metallic matrix [10]. Iron now completes the generalization of this approach, by taking the third type of metal—a corroding one. The basic entrapment mechanism of molecules within the metals is based on the reduction of the metal cations in the presence of the desired molecules, followed by an aggregation of the formed metallic nanocrystals, leading eventually to the entrapment of the drugs within the newly formed porous metal matrix [11, 12]. This molecular doping methodology has already created a wide arsenal of functional composites with new or improved properties compared to the separated components such as obtaining biocidal synergism by the entrapment of an antibacterial agent in silver [13–15], and more [11, 12].

Iron has a low reduction potential that poses a challenge for the reductive entrapment process in water, without damaging the entrapped molecules. Thus, a major part of this study has been to find the right reductive conditions that will provide the desired drug-doped iron, capable of releasing its load. The optimal procedure we developed uses sodium borohydride in water at low temperatures (equation (1)) [16], Experimental details, with which we developed successful protocols for the entrapment of two types of drugs: chlorhexidine (chd)—a broad-spectrum biocidal drug that is used also in sustained release formulations [17–19]; and rapamycin (rap)—used for treatment transplant rejection [20, 21](figure 1).

2. Experimental details

2.1. Chemicals
Iron sulfate heptahydrate, chlorhexidine digluconate (20% w in water), chlorhexidine (free amine), and aluminum chloride, were purchased from Sigma Aldrich. Rapamycin was purchased from Fermetek LTD and sodium borohydride from Stem Chemicals.

2.2. Syntheses

2.2.1. Synthesis of chlorhexidine@Fe
The reaction was carried out in a flask equipped with five needles for inlets of Ar, the reducing solution and the drug, and for outlets of Ar and H2 (which forms from the hydrolysis of NaBH4). The Ar is needed to avoid air oxidation of the forming iron. 5.0 g (0.018 mol) of iron sulphate heptahydrate and 0.1 ml of AlCl3 solution (prepared from 10 mg of AlCl3 dissolved in 1.0 ml double-distilled water (DDW); the function of the AlCl3 is the acceleration of the aggregation) were added to 10 ml DDW. The solution was stirred at 30 °C and 300 RPM for five minutes. Meanwhile, 0.4 g of sodium borohydride was dissolved in 20 ml DDW (lithium borohydride was found to react too fast, but potassium borohydride could be used as well). Optimization of the reduction/entrapment process resulted in the following slow, step-wise procedure: At first, the stirring rate was decreased to 50 RPM, and the 20 ml of the reducing solution was added gradually during 60 min with a peristaltic pump at a rate of 20 ml/hour. Then, the stirring rate was increased to 300 RPM for 10 min, because it was found to increase the aggregation. This sequence of addition of the reducing agent was repeated four times with a total of 1.6 g (0.043 mol) of sodium borohydride which is an excess of 0.007 mol over the Fe $^{+2}$ (equation (1)).
FeSO_4(aq) + 2NaBH_4(aq) + 6H_2O → Fe(s) + 7H_2(g) + 2B(OH)_3(aq) + Na_2SO_4(aq) (1)

At the beginning of the third addition, 0.5 ml of chd digluconate diluted with 9.5 ml DDW was added gradually for 30 min with the peristaltic pump, in parallel with the addition of the reduction agent. At the end of the fourth batch of addition of the reducing solution, stirring at 300 RPM was carried out for 30 min. The whole entrapment process takes ~5.5 h. The product was concentrated at the bottom with a magnet, and the supernatant solution was decanted, followed by washing with three portions of 20 ml of DDW. The composite was immediately vacuum-dried with an oil pump at 150 mbar over-night, after which the vacuum was slowly released with argon flow. The product, chd@Fe was weighed (1.2 gr) and immediately sealed in order to avoid oxidation. The somewhat higher-than-theoretical (1.0) yield is mainly due to some residual entrapped water and minute oxide formation (see TGA below). The product was tested either as powder or as compressed discs 13 mm in diameter (thickness: 0.5 mm) prepared from 200 mg powder by using infrared pellet press at 10,000 PSI for 5 min.

2.2.2. Synthesis of rapamycin@Fe
The entrapment of rap was carried out similarly with two changes: (1) The iron sulphate heptahydrate was dissolved in 15 ml DDW (instead of 10 ml), and (2) unlike the chd solution (which was 10 ml DDW)—the rap solution was made by dissolving 30 mg of rap in 5 ml ethanol and its addition took 15 min. The yield: 1.2 gr, as above.

2.3. Entrapment quantification measurements
10 mg of either chd@Fe or 25 mg of rap@Fe powders were extracted with 5 ml ethanol, which is a good solvent for both chd and rap (see table S1, supplementary material (SM) for details. Available online at stacks.iop.org/MRX/7/065404/mmedia). The suspensions were put in an incubator shaker at 100 RPM, 30 °C for five hours, and the content of the supernatant solution quantified by UV–vis, using the characteristic peaks of 260 nm for chd and 278 nm for rap.

2.4. Sustained release measurements
10 mg of either chd@Fe or 25 mg of rap@Fe in their powder or pressed forms were immersed in 5 ml phosphate-buffered saline (PBS, pH 7.4), and shaken with an incubator shaker at 100 RPM, 30 °C. PBS is a known solution that mimics in–vivo conditions and is not too strong for rapid extractions of the drugs. 2.5 ml of the suspension was taken for measurements and then immediately returned to the vail. (From the absorbance of the drugs the scattering base at 325 nm was subtracted).

2.5. Instrumentation
The slow reduction and entrapment process was carried out with a peristaltic FH 100 M multichannel pump system, to which silicon tubes, inside diameter of 0.8 mm, were connected to the pump. Thermogravimetric analysis (TGA) was carried out with a Mettler-Toledo TGA/SDTA 851e, from 50 to 650 °C, at a heating rate of 10 °C per minute under nitrogen atmosphere. Density measurements were carried out with a Micromeritics AccuPyc 1340 instrument using helium as the displacing gas. UV–vis absorbance spectroscopy was carried out with Hewlett-Packard 8452A diode-array UV–vis spectrophotometer. Scanning electron microscope (SEM) was carried out on a Sirion (FEI) high resolution (HR) SEM instrument. X-ray powder diffraction measurements were performed on a D8 Advance diffractometer (Bruker AXS, Karlsruhe, Germany) with a secondary Graphite monochromator, 2° Sollers slits and 0.2 mm receiving slit. The powder samples were placed on low background quartz sample holders. X-ray diffraction (XRD) patterns from 5° to 85° 20 were recorded at room temperature using CuKα radiation (λ = 0.15418 nm) with the following measurement conditions: tube voltage of 40 kV, tube current of 40 mA, step scan mode with a step size of 0.02° 20 and a counting time of 1 s per step. The iron crystallite size was determined from the experimental XRD data using Scherrer equation. The instrumental broadening was determined using LaB6 powder (NIST SRM 660). Surface area analyses were determined by adsorption–desorption N2-BET isotherms analysis obtained from Micromeritics ASAP-2000 physisorption instrument. Elemental analysis of (C, N, H,) was determined by using the Thermo Flash 2000 CHN-O elemental Analyzer. Cl was determined by decomposition of the organic compound followed by potentiometric titration using a 835 Titrodir Metrohm Titroprocessor and by Ion chromatography analysis using a Dionex IC system (more details can be found on the following website [22]).
3. Results and discussion

3.1. Developing the drugs entrapment protocols

The entrapment process, as mentioned above, is based on the reduction of the metal cation in the presence of the drug molecules. The initial conditions resemble the synthesis of metal nanoparticles, which in our application translates to the drug molecules stabilizing the formed metal nanoparticles by adsorption on the newly formed surfaces. This prevents massive aggregation and no precipitation, which, if fact, we need for the formation of the drug@Fe composite. Furthermore, another related problem emerged from the need to use sodium borohydride for the iron cations, because this strong reducing agent is also known to be used for stable nanoparticles synthesis \[16\]. In addition, sodium borohydride also releases hydrogen gas during the reduction, which further interfere with the formation of the entrapping conditions \[23\]. Therefore, in order to promote the needed aggregation, the physical and chemical conditions were adjusted towards that goal. The parameters that were used in order to promote precipitation were using low temperature, high metal cation concentration, adding three-valent metal cation (from AlCl3 in order to enhance the aggregation), minimizing the amount of the reducing agent, slowing the reduction rate, optimizing the stirring rate, and developing a special multi-step protocol for adding the reducing agent and the drug—all of these led to the procedures described in the Experimental Details.

3.2. Materials characterization

The resulting material is a typical black iron powder (figure 2(a)), which also nicely responds to an external magnet (figure 2(b)). The powder is of hierarchical structure and is composed of granulated particles in the hundreds of microns scale (figure 2(c)), which, in turn, are built of a porous microns scale iron network, the building blocks of which are nanometric iron nanocrystallites, as determined from the XRD analysis (detailed next).

Indeed, it was found that the densities — 4.7 g cm\(^{-3}\) for the composites and 5.0 g cm\(^{-3}\) for pure iron prepared by the same method—are 40% lower compared to the literature \[24\]. The nitrogen adsorption-desorption isotherms—figure 3(a) and figure S1, SM—are typical of open meso- and macroporous materials. The BET (Brunauer–Emmett–Teller) adsorption equation fits perfectly these isotherms (figure 3(b) and figure S1, SM; \(R^2\) of fit better than 0.998 in all cases), which is usually an indication of the chemical homogeneity of the
material, and the calculated surface areas along with other surface and porosity parameters, are collected in table 1. It is interesting to note the difference in the C-BET constant; it also shows the sensitivity of the architecture of these materials to variations in the preparation procedures: ethanol was added in the synthesis of rap@Fe only, influencing the pore morphology [25].

XRD measurements—figure 3(c)—indicate that the matrices (and their pure Fe blanks) are mainly metallic iron (98%), with some (~2%) sodium sulphate residues. Analysis of the peak shapes by the Rietveld refinement mode of the ‘double-phase model’ [27], suggests bimodal nanocrystallite sizes: The main phase, 74% and 78% of the chd and rap composites, has nanocrystallites of average size of 1 nm, while the second phase — 24 and 20%, has nanocrystallites of average size of 9 nm. Morphologies of aggregated materials are very sensitive to particle size and shape, because these dictate the details of the chemical interactions between one particle and the other, as have been shown, for instance, by Chowdhury et.al [28]. The two nanocrystals types have lead perhaps to the two morphologies seen in figures 2(d), (f) of beads and strings. In figures 2(e), (f) one can notice the entrapped drug as darker nanoparticles of different sizes and shapes entrapped within the metal aggregates.

TGA of chd@Fe (figure 3(d)) shows the weight decline caused by the decomposition of chd, with the well characterized peak at 234 °C that has been reported in our previous publication [15]. The monotonic decline of 3.2%, with another characterized peak at 100 °C, is apparently due to entrapped water residues. Similar features are seen for the TGA of rap@Fe—figure S2, SM. Elemental analysis was carried out as well, proving the presence of the organic component in both composites with the detection of carbon, hydrogen, nitrogen and chloride—see table S2, SM.

3.3. Drug delivery measurements
The initial total load was determined by extraction with a strong solvent, ethanol. The entrapment weight percentages for chd and rap are 1.6% and 1.1% (supported by the %C elemental analysis which provided 1.6 and 1.3%). This extraction serves also for another important check: As the reduction is carried out with a strong reducing agent, it was important to determine that the drug molecules are not affected by it or by the other components of the entrapment process. Figure S3, SM, confirms that this is indeed the case: UV–vis spectra before entrapment and after extraction are practically the same. Both drug-composites showed successful release from the powder and the disk forms into PBS. The release profiles are shown on two-time scales: 24 h (figures 5(a)–(b)) and 20 days (figures 5(c)–(d)). During the first 24 h, the release kinetic from the powders was found to be first order, according to equation (2):

![Figure 3. (a) Nitrogen adsorption-desorption isotherms and (b) the fit to the BET adsorption equation for chd@Fe. See figure S1 for the other isotherms including pure Fe, and their fits. c) XRD measurements of chd@Fe (black), Fe made under similar conditions (red), rap@Fe (blue) and Fe made under similar conditions (purple). Note that the Y-axis refers to chd@Fe (black); the others are shifted. Red and blue vertical lines on the bottom: metallic iron and sodium sulphate lit. data, respectively [26]. (d) TGA analysis: The weight loss as a function of temperature (black) and the first derivative (red) of chd@Fe. See figure S2, SM for rap@Fe.](image-url)
| Material | Density (g/cm³) | Surface area (m²/g) | Mesopores volume (cm³/g) | Pore size (nm) | Micropores volume (cm³/g) | C constant |
|----------|----------------|---------------------|--------------------------|----------------|----------------------------|------------|
| chd@Fe   | 4.7            | 2.6                 | 0.0014                   | 4.7            | 0.00045                    | 240        |
| Fe       | 5.0            | 2.7                 | 0.0013                   | 4.1            | 0.00043                    | 210        |
| rap@Fe   | 4.7            | 2.9                 | 0.0031                   | 4.2            | 0                          | 40         |
| Fe       | 4.7            | 2.0                 | 0.0017                   | 3.6            | 0                          | 20         |

Table 1. The densities, surface areas, pore volumes and sizes and C constants of drugs@Fe and Fe.
\[
m(t) = m_b + m_s \left(1 - \exp \left(-\frac{t}{\tau_s}\right)\right)
\]  

where \(m_b\) represents a very small initial burst of the drug molecules, located on the exposed surface area of the metallic matrix, and \(m_s\) represents the population that is released slower with the first order, representing the major population that has been entrapped with a time constant — \(\tau_s\). All parameters are summarized at table 2.

As expected, the release kinetics from the disk (see inset of figure 4(a)) is slower compared to the powder. Another significant change is that the process changed from 1st order to a zero-order, as clearly seen in figures 4(a), (b). It is important to note that zero order release profile is desirable due to the constant dose that is released from the matrix, which maintains the plasma concentration steady [29]. In order to compare between the powders and the discs, a good approximation for the powders is to linearize the initial release from the powders. It is seen — table — that the release rates of the drugs from table 3 the discs are slower by a magnitude of order.

Figures 4(c), (d) show a phenomenon that which is unique for the iron, and which we did not see neither for the release from Pt [9], nor for the release from silver [10], namely that for iron the release from the powders passes through a maximum: After 24 h and during the following days — the release percentage of the drugs continuously decreased to 70% for chd and 30% for rap. It is suggested that this behavior is caused by a gradual

| Parameter | chd@Fe | rap@Fe |
|-----------|--------|--------|
| \(M_b\) (%) | 2      | 1      |
| \(M_s\) (%) | 98     | 100    |
| \(\tau_s\) (hrs) | 2.3    | 3.4    |
| \(R^2\) | 0.997  | 0.995  |

Table 2. The kinetic parameters of the release of chd and rap from the powder form.

Figure 4. Drug release kinetic profiles of chd (a) and rap (b) during the first 24 h from the powders (black dots) and from the disks (red dots). Inset picture: the disk form of chd@Fe. Drug release kinetic profiles during 20 days: (c) — chd and (d) — rap. ('Maximum release' on the y-axes refers to release to PBS).
oxidation of the iron forming the oxide which is a good adsorbent. This oxidation can be clearly seen visually (the slight reddish hue compared with figure 2(a) is visible) and by XRD analysis of the matrix after the release (figure 5). Oxidation of the disc is much slower because of its compactness, and therefore this decrease is not seen for this format. It could also be that the residual iron phosphate (indicated in the XRD) helps in preventing the penetration of oxygen [30]. The release profiles from the discs also show that there is a population of drug molecules that is so tightly entrapped, that it remains unreleased, compared to powder in PBS: 35% of chd and 20% of rap are released from the discs.

4. Conclusion and outlook

The use of iron, a bioabsorbable metal with low toxicity, for sustained release application has been demonstrated in this study. The release kinetics was found to be based mainly on the solubility equilibrium between the drug that is entrapped and the solution (PBS medium; rap is very hydrophobic with low solubility in water, and chd has low solubility in media rich in chloride and phosphate anions), on the adsorption equilibrium between the drug molecules and the surface of the matrix, on the porosity of the matrix, and by the oxidation of the iron matrix in the powder form. The drug is entrapped mostly within the pores created by the iron aggregated particles, and this morphology enables the drug molecules to be extracted to the solution, which, as seen in previous reports [9, 10], leads to 1st order release kinetics. For the discs the release rates change, as we have seen, to zero order. Zero order rate is known to occur when the matrix itself—such as tight porosity—is the barrier for the release [31]. A sub-population of the entrapped drug molecules is not accessible to the surrounding solvent and remains entrapped. That population is expected to be release upon the in-vivo very slow biological degradation, that is, implants can act as reservoir while the implant is decomposed and bio-absorbed, as suggested by Birbilis et al [6]. Both forms can be used for various applications: The disk as a direct implant and the powder in the form of coating or filler, or as a suspension for direct injection. The two selected drugs have been chosen due to their specific need after implantation—preventing infection and implant rejection. Orthopedic implants and drug delivery are both two technologies worth billions of dollars market [32, 33]. Here we have presented the combination of these two technologies as a proof of concept with a potential to entrap other needed drugs, either within iron or within other low reduction potential metals.

| Composite name | Initial release (%/hrs.) |
|----------------|--------------------------|
| chd@Fe powder  | 32                       |
| chd@Fe disk    | 3.1                      |
| rap@Fe powder  | 24                       |
| rap@Fe disk    | 1.2                      |

Table 3. The initial release rates of the powder and disk forms.

Figure 5. Left: XRD measurements of chd@Fe (black) and of rap@Fe (red) at the end of the sustained release experiment (both spectra are upshifted from the base line). Red vertical lines: metallic iron, blue lines: magnetite, green lines: hematite, purple lines: sodium iron phosphate hydrate. lit. data [26]. Right: rap@Fe powder at the end of the sustained release experiment. Compare with figure 2(a).
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