# Bacteria encapsulation in a magnetic sol–gel matrix†

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**The encapsulation of *Escherichia coli* bacteria within ferrihydrite gels favours the long-term viability of the entrapped cells while preserving the magnetic properties of the host material.**

Over the last few years, progress has been made in the use of sol–gel chemistry for cell entrapment and design of biosensors and bioreactors.[1,2] Almost all sol–gel hosts evaluated for cell encapsulation are based on silica, as it confers a non-toxic and biologically inert environment. In contrast, metal oxides can exhibit functionalities such as conductivity or magnetism, but most of them are known to be toxic to a large number of living systems. A first demonstration of the possibility to encapsulate bacteria in a non-silica sol–gel host was very recently reported, based on the development of a colloidal route to alumina gels compatible with the long-term preservation of *Escherichia coli* bacteria.[3] This result suggests that the colloidal route provides a new pathway for cell encapsulation in functional metal-oxide sol–gel matrices. Hence it becomes possible to envision 'living' materials[2a] where the biological activity of living organisms and the physical properties of the inorganic host contribute synergetically to a functional device. However, despite its use in biomedical devices, alumina does not exhibit any intrinsic properties that could influence or be modified by entrapped cells. In this communication, we demonstrate that the sol–gel colloidal approach can be extended to ferrihydrite gels, showing for the first time that living cells can be preserved within a magnetic inorganic host. This possibility not only paves the route to the design of magnetic cell-based biosensors[4] but also provides a model system to study the impact of magnetic fields on the metabolism of living cells, with implications in geomicrobiology[5] and in the medical field.[6]

Iron oxyhydroxide nanoparticles (*ca.* 5 nm in diameter) were obtained by alkalinisation of an iron(III) chloride solution by addition of a solution of sodium hydroxide at pH 7.[7] The obtained gelatinous precipitate was recovered by centrifugation. In order to remove the chloride anions and to reduce the ionic strength, the nanoparticles were thoroughly washed until no $Cl^-$ could be detected in the supernatant upon $Ag(NO_3)$ addition. A concentrated sol was obtained after re-suspension of the precipitate in deionized water. The addition of phosphate buffer (100 mM) solution with or without 10 wt% glycerol led to the fast (<30 s) formation of a brown and opaque gel (see ESI† for full experimental details). The X-ray diffraction (XRD) diagram of the freeze-dried gels indicates the formation of so-called "2-line" ferrihydrite nanoparticles, together with another poorly crystallized oxyhydroxide iron phase, either a "6-line" ferrihydrite or goethite (see ESI†).[7] The presence of glycerol does not significantly modify the XRD diagram suggesting that this additive does not influence the nanoparticle crystallinity.

The success of gel formation could be explained on the basis of zeta-potential measurements, indicating that the isoelectric point (IEP) of ferrihydrite particles was $7.2 \pm 0.1$ at 0.1 M ionic strength (see ESI†). Thus, in the conditions of cell encapsulation, nanoparticles bear no net surface charge, avoiding inter-particle electrostatic repulsion and favouring gel formation.

SEM observations indicate that the $CO_2$ supercritically dried gels, with or without added glycerol, consist of a homogeneous macroporous network of aggregated submicron particles (Fig. 1a and ESI†). TEM images suggest that these particles consist of nanoparticles, less than 10 nm in size, aggregated in a mesoporous packing arrangement (Fig. 1b and ESI†).‡ Adsorption–desorption isotherms of $N_2$ at 77 K provide further insight into the structure of the gels. For both samples a type IV isotherm characteristic of mesoporous materials is obtained (Fig. 1c),[8] indicating specific surface areas ($S_{BET}$) of ~310 $m^2g^{-1}$ and ~320 $m^2g^{-1}$ and porous volumes ($V_p$) of ~0.18 $cm^3g^{-1}$ and ~0.17 $cm^3g^{-1}$ in the absence or presence of

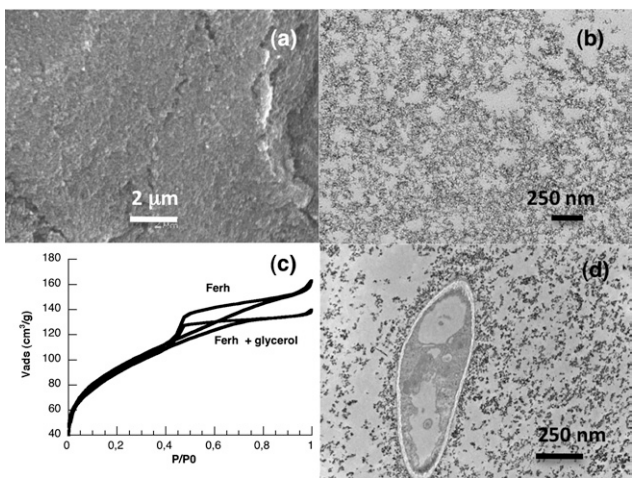


**Fig. 1** SEM (a) and TEM (b) images of ferrihydrite gels; (c) $N_2$-sorption isotherms at 77 K of ferrihydrite gels (Ferh) with or without glycerol; (d) TEM image of *E. coli* bacteria entrapped in a ferrihydrite gel.

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† Electronic supplementary information (ESI) available: Detailed experimental procedures; XRD diagrams of ferrihydrite gels; zeta-potential measurements; TEM and SEM of gels without glycerol. See DOI: 10.1039/b820433k

glycerol, respectively. Noticeably, the introduction of *E. coli* suspension in the buffer solution, with or without glycerol (WCS or WCS-gly), did not modify the kinetics of gel formation nor its structure. However, an accumulation of the inorganic colloids around the cell surface can be clearly observed (Fig. 1d). This is in agreement with previous studies showing the strong affinity of acidic and hydrophilic surfaces, including *E. coli* external membranes, for ferrihydrite and other iron oxide nanoparticles.[9,10]

The study of the short-term viability of *E. coli* bacteria encapsulated in ferrihydrite matrices was carried out one day after encapsulation, in order to study the impact of gel formation on cell survival. After gel re-suspension,[11] the plate-count technique indicates a viability rate of 30% (±6%) that does not depend on the presence of glycerol. To understand such a decrease in viability, a study of bacteria growth in the presence of ferrihydrite nanoparticles (0.5–5 $gL^{-1}$) was performed in a minimum medium containing glucose (MM) (Fig. 2). The growth curve in the absence of nanoparticles shows an initial lag time (*ca.* 1 h) followed by a rapid development phase, the 'log phase', during the first 7 hours, and by a plateau corresponding to the stationary phase where nutrients become limited. The presence of ferrihydrite colloids at 2.5 $gL^{-1}$ and 5 $gL^{-1}$ leads to a longer lag time, suggesting that bacteria are adapting to their environment. A slight increase in the slope of the curve corresponding to the log phase is also observed at these concentrations, reflecting an enhancement of bacteria growth. Interestingly, the optical density measured at the plateau is larger in the presence of ferrihydrite colloids ($OD_{600} \approx 3$) than in their absence ($OD_{600} \approx 2$). Hence, the presence of ferrihydrite particles seems to foster bacteria growth. The culture medium used here is devoid of iron cations whereas it is known that this element is necessary for growth of practically all bacteria.[12] Thus, the addition of ferrihydrite seems to compensate this deficiency. Finally, these data suggest that the loss of bacteria viability after encapsulation is not due to detrimental interactions between the cells and the colloidal species.

To dissociate short-term from long-term effects of encapsulation, the colony forming units (cfu) count after one day of encapsulation was then taken as a 100% reference for further evaluation of bacterial viability. Fig. 3 presents the evolution of free and encapsulated bacteria viability with time. In all cases, the cfu count decreases over one month but to a very different extent depending on the ageing conditions. In the mid-term, *i.e.* after 15 days, bacteria viabilities recorded in the inorganic matrix (94% (±2%) and 88% (±4%), in the presence and absence of glycerol respectively) are much larger than those recorded for bacteria aged in suspension (55% (±2%) and 60%

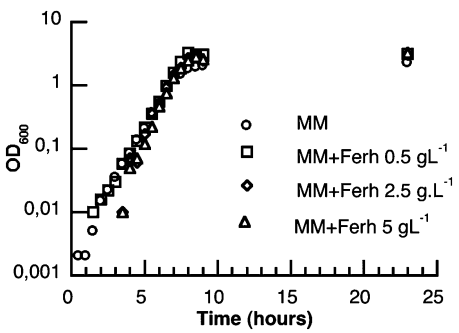


**Fig. 2** Bacteria growth in a minimum media (MM) in the presence of ferrihydrite (Ferh) nanoparticles.

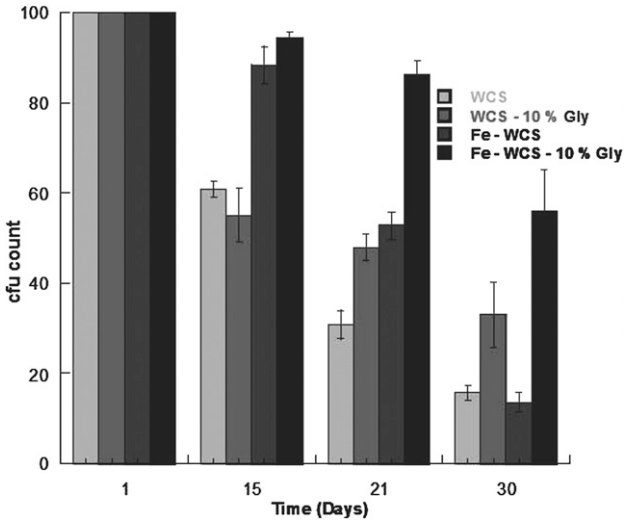


**Fig. 3** Evolution of colony formation units (cfu) count of bacteria suspension (WCS) and bacteria entrapped in ferrihydrite gel (Fe-WCS) aged 15, 21 and 30 days with or without added glycerol (cfu count after 1 day taken as 100% reference).

(±6%) respectively). This seems to be in good agreement with the data obtained from growth curves (Fig. 2), showing good adaptation of bacteria to their new environment. We must also emphasize that over this period glycerol appears to have a minor effect on bacteria viability.

In the longer term, the highest viability rates are obtained for bacteria encapsulated in the ferrihydrite gel containing glycerol (55% (±6%) after 30 days relative to 1 day cfu count). The influence of glycerol appears clearly as viability rates in the pure inorganic matrix decrease to ~15%, being therefore lower than for the cell suspension containing this additive (~35%) and comparable to suspensions without glycerol (~15%).

It is worth comparing these data with previous reports on *E. coli* bacteria encapsulation in silica and alumina matrices.[3,11] Considering the short-term, post-encapsulation viability, ferrihydrite matrices have a significant impact on cell survival as only *ca.* 30% of the initial population withstand the gel formation stress, even in the presence of glycerol. This result is similar to that observed for alumina and is much lower than that obtained for silica (*ca.* 80%). Noticeably, ferrihydrite and boehmite gels have in common that they are formed quasi-instantaneously (*i.e.* <30 s) whereas silica gels are formed within 2–3 minutes. Thus, it can be suggested that the observed loss in viability is due to the fast gel formation reaction that does not allow suitable adaptation of the bacteria to their new environment. In the long term, the presence of glycerol was found to have a beneficial effect for the three matrices. However, whereas glycerol-containing silica and alumina hosts showed similar viability after one month (*ca.* 45% relative to 1 day), bacteria encapsulated in ferrihydrite gels demonstrate a significantly higher survival rate (*ca.* 55% relative to 1 day). Two main factors may explain this difference in cell stabilization. The first one is related to the nature of the gel surface, which is neutral for the ferrihydrite matrices whereas boehmite and silica surfaces are positively and negatively charged, respectively, under the encapsulation conditions. Hence, whereas electrostatic interactions between cell membrane components and host networks can be expected for the two latter materials, much weaker interactions are


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expected for the present matrix. The second one is related to the fact that the presence of iron species is beneficial for bacteria metabolism and should therefore improve the cell survival within the gel.

As these results indicate that ferrihydrite matrices are suitable hosts for bacteria encapsulation, we turned our attention to their magnetic properties, as studied using a SQUID magnetometer. As shown in Fig. 4, the freeze-dried glycerol-containing gel exhibits the magnetic behaviour of superparamagnetic particles with a blocking temperature of *ca.* 15 K, a coercitive field $H_c$ of 3 kOe at 2 K and no hysteresis curve at 300K. The presence of bacteria does not modify the superparamagnetic behaviour but leads to a decrease of the $H_c$ value to 1 kOe. These results are in good agreement with literature data,[13] suggesting that such a decrease in $H_c$ value is related to the local aggregation of ferrihydrite colloids in the gel upon cell addition. Coming back to the TEM image of encapsulated cells (Fig. 1d), it may be suggested that this effect corresponds to the observed aggregation of colloids around the bacteria surface.

These data indicate that the previously proposed colloidal approach allowing the design of non-silica sol–gel hosts for bacteria encapsulation[4] may be extended to a large variety of metal oxide gels. They also emphasize that key parameters responsible for the preservation of cell viability include colloid surface charge, that impacts on both gel formation conditions and cell/particle interactions, gelation time and also the effect of precursor metal ion on cell metabolism. Indeed, the performance of ferrihydrite (and alumina[3]) hosts (*ca.* 15% after one month) is significantly less than that of silica (*ca.* 40% after the same delay), due to an important stress occurring during encapsulation. However, this represents a decrease of less than one order of magnitude, *i.e.* more than $10^7$ cells per mL are still biologically active, in the absence of any nutrients. Moreover, these materials constitute the first example so far of magnetic solid hosts preserving cell life over several weeks, although further efforts are now needed to obtain encapsulation matrices exhibiting a remanent magnetization close to room temperature, using for instance larger iron oxide/hydroxide particles. In this case, it would become possible to study the effect of magnetization on the physiological state of cells, with specific interest for the impact of magnetic fields on environment and human health.[14]

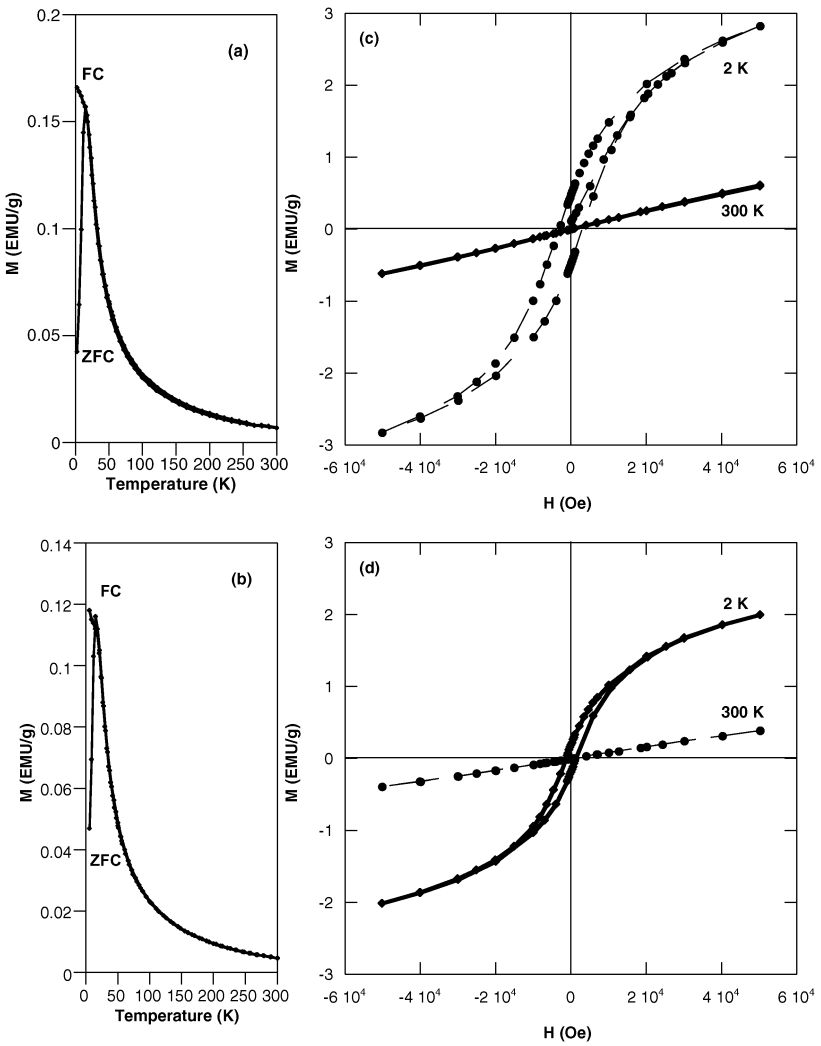


**Fig. 4** Field cooled (FC)/zero field cooled (ZFC) susceptibility curves ($H = 500$ Oe) and hysteresis loops at 2K and 300 K for ferrihydrite gels in the absence (a,c) or presence (b,d) of *E. coli* bacteria.

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## Notes and references

‡ It was not possible to obtain suitable TEM images at higher magnifications due to the crystallization of the particles upon dehydration induced by the electron beam.

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