Supplementary Information (SI) for Nanoscale.
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1 **SUPPLEMENTARY MATERIAL**

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3 # Preserved properties of non-pyrogenic chains of
4 # magnetosomes following storage in powder form
5 # and re-suspension under isotonic conditions.

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19 **Supplementary materials et methods:**

20 **Strains**

21 *Pseudomonas aeruginosa* (VT000266-1VL), *Bacillus Subtilis* (VT000036-1VL), *Staphylococcus*
22 *Aureus* (VT000326-1VL), *Candida Albicans* (VT000546-1VL), *Aspergillus Brasiliensis* (VT000532-
23 1VL) were obtained from Sigma Aldrich. *Clostridium Sporogenes* (0317E3) was obtained from
24 Microbiologics.

25 **Growth promotion test**

26 Under aseptic conditions, 9.9 mL of fluid thioglycolate medium (FTM) were inoculated with 100
27 µL (not more than 100 CFU) of the following microorganisms: *Staphylococcus aureus (SA),*
28 *Pseudomonas aeruginosa (PA), Clostridium sporogenes (CS)*. 9,9 mL of tryptic soy broth (TSB) were
29 inoculated with 100 µL (not more than 100 CFU) of the following microorganisms: *Bacillus subtilis*
30 *(BS), Candida albicans (CA), Aspergillus brasiliensis (AB).* All these microorganisms were incubated
31 separately at 35°C for those in FTM and 25°C in TSB. Incubation was not more than 3 days in the
32 case of bacteria and not more than 5 days in the case of fungi. FTM and TSB without micro-
33 organisms were considered as negative controls (NC).
34 The media were suitable if a clearly visible growth of the microorganisms occurs and an increase
35 in the optical density at 600 nm (OD_{600}) was determined using a spectrophotometer with 900 µL
36 of medium (Secoman, Uviline 9400).

37 **Method suitability test**

38 Under aseptic conditions, 20 mg of each lyophilized magnetosome powder were incubated in 9.9
39 mL of culture media, FTM and TSB. Then 100 µL (not more than 100 CFU) of viable micro-
40 organisms were added to the medium. The same micro-organisms described above under the
41 Growth promotion test are used and inoculated in their corresponding medium. A growth
42 promotion test was considered as a positive control of this test. Incubation was not more than 5
43 days for all the samples. At the end of incubation, 1 mL was taken from each nanoparticle sample
44 and placed against the magnet for 10 min to only recuperate the medium. The turbidity was then

45 measured at 600 nm with 900 µL of such medium using a spectrophotometer (Secoman, Uviline
46 9400).
47 If clearly visible growth of microorganisms and an increase in the OD_{600} were determined after
48 the incubation, visually comparable to that in the control tube without magnetosomes, the
49 product therefore possesses no antimicrobial activity under the conditions of the test. The test
50 for sterility may then be carried out without further modification. If clearly visible growth and an
51 increase in the OD_{600} were not obtained in the presence of the product to be tested, visually
52 comparable to that in the control vessels without product, the product possesses antimicrobial
53 activity that has not been satisfactorily eliminated under the conditions of the test.
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55 **Supplementary Figures and Tables:**

56 **Figure S1:** (a) Evolution of the biomass (OD_{565}), oxygen concentration (%) and Fed-Batch volume
57 added (mL) during the growth step of MSR-1 bacteria in a semi-automated 40 L fermenter.

58 **Figure S2:** Schematic representation of the M-CMD formulation step

59 **Figure S3:** Schematic representation of stability measurement of magnetosomes suspensions

60 **Figure S4:** Sterility assay: (a) growth promotion test for 2-4 days (D1 to D3-D5) using 6 stains. (b)
61 suitability test for 3 days using 6 stains mixed with MgC, M-uncoated and $(M\text{-}CMD)_F$.

62 **Figure S5:** Schematic representation of LIU hyperthermia treatment on PC3 cells with or without
63 $(M\text{-}CMD)_F$.

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65 **Table S1:**Composition of pre-growth medium, growth medium, and iron-rich Fed-batch medium
66 for the cultivation of MSR-1 magnetotactic bacteria. All chemicals were in pharmaceutical grade
67 purchased from Merck (Darmstadt, Germany).

68 **Table S2:** Composition of mineral elixir.

69 **Table S3:** Composition of vitamin mixture.

70 **Table S4:** Number of colony forming units (CFU) of culture media incubating different types of
71 magnetosomes for 14 days, determined by inoculating 100 µL of each medium on an agar plate
72 and incubating it for 3 days. Negative controls were the culture media without nanoparticles.

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Ingredient	Pre-growth medium (g/L)	Growth medium (g/L)	Fed-batch medium (g/L)
Sodium lactate	2.600	1.300	100.0
Ammonium chloride (NH_4Cl)	0.400	0.223	4.800
Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$)	0.100	0.027	2.400
Dipotassium phosphate (K_2HPO_4)	0.500	0.067	6.000
Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$)	0.000	0.000	2.000
Mineral Elixir[(a)]	0.500 mL	0.080 mL	7.000 mL
Vitamin mixture[(b)]	0.100 mL	0.067 mL	1.000 mL

74 [(a)] see the composition in Table S2. [(b)] See the composition in Table S3

75 **Table S1**

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Ingredient	Mineral Elixir (g/L)
Iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$)	1
Calcium chloride ($CaCl_2$)	30

85 **Table**
86 **S2**
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Ingredient	**Vitamin mixture (1000X) (g/L)**
Biotin	0.002
Calcium pentothenate	0.400
Folic acid	0.002
Inositol	2.000
Nicotinic acid	0.400
p-Aminobenzoic acid	0.200
Pyridoxine HCl	0.400
Riboflavin	0.200
Thiamine HCl	0.400

103 **Table S3**

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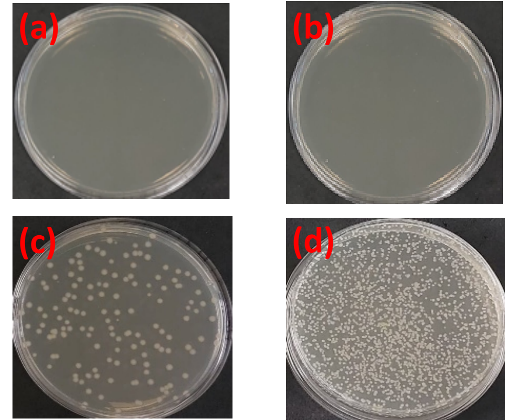
	Number of CFU per 100 µL	
	TSB 25°C	**FTM 35°C**
Negative controls	0 **(a)**	0 **(b)**
MgC	146 ± 21 **(c)**	> 300 CFU **(d)**
M-uncoated	0	0
(M-CMD)$_F$	0	0



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108 **(a) (b) (c) (d)** indicating the photos of agar plates inoculated with 100 µL of TSB, FTM, TSB with MgC and
109 FTM with MgC, respectively. For the culture media of M-uncoated, and (M-CMD)$_F$, no colonies were
110 observed in their agar plates as exhibited in those of negative controls
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112 **Table S4**
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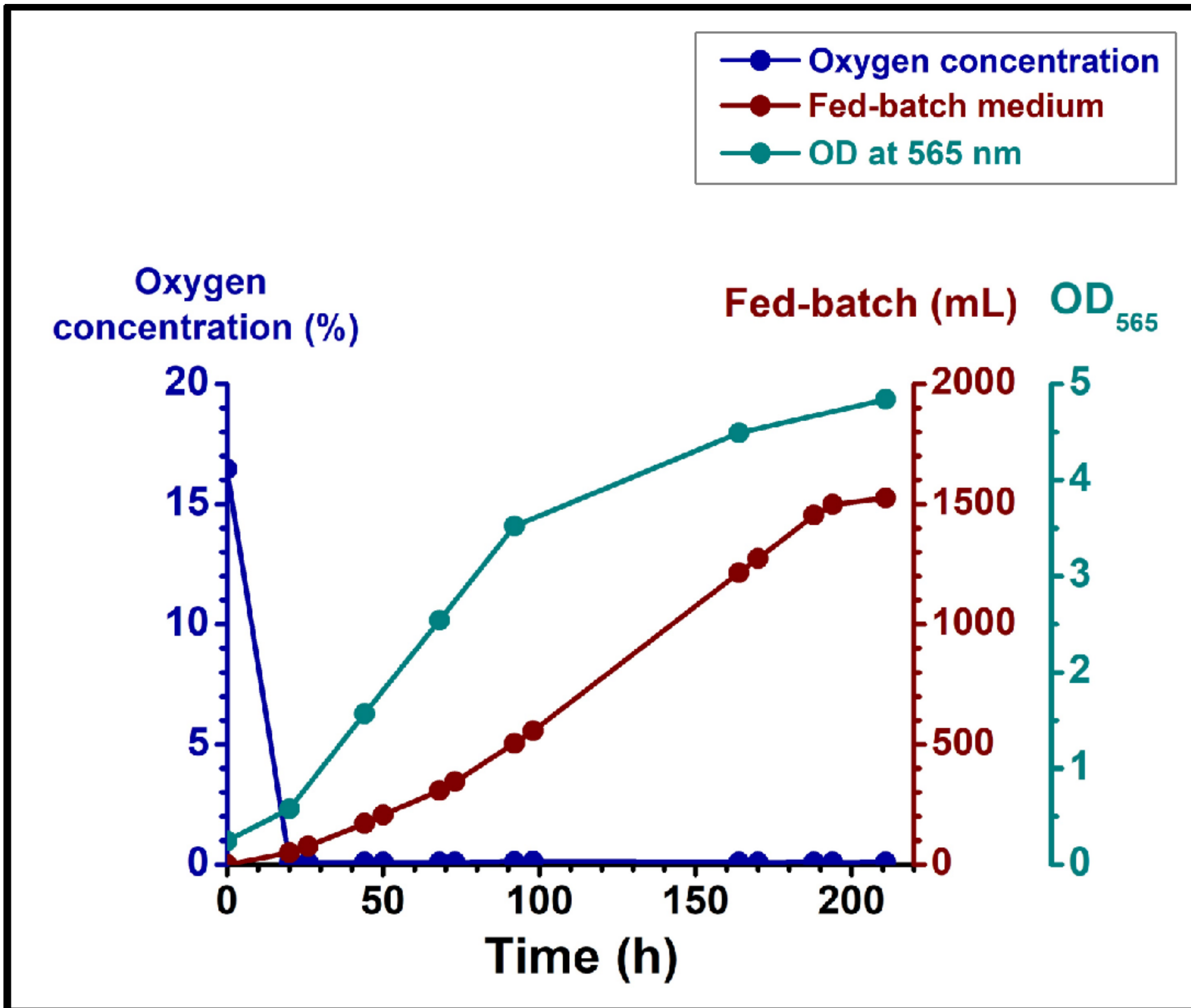

120 **Fig S1**

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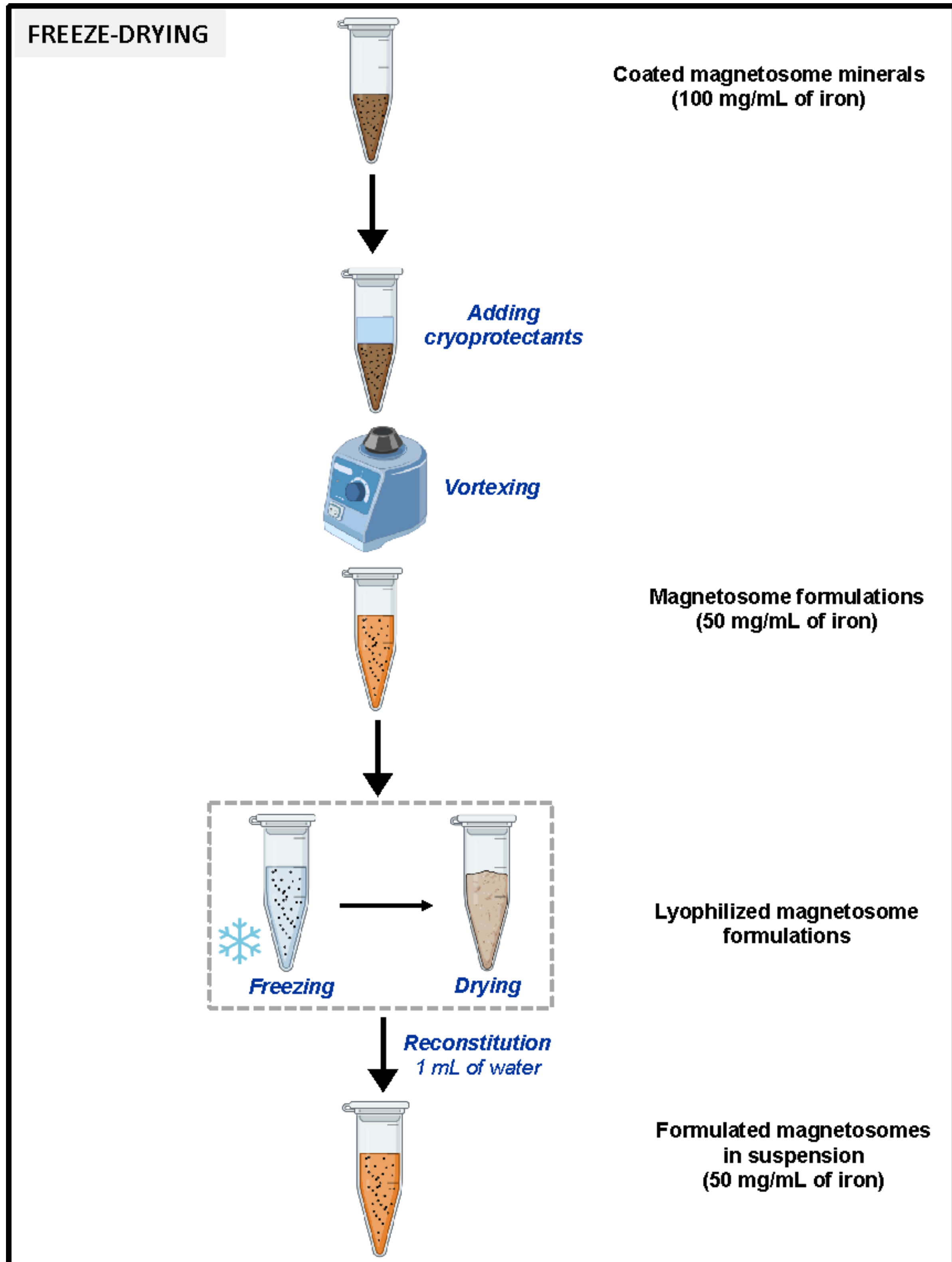

122 **Fig S2**

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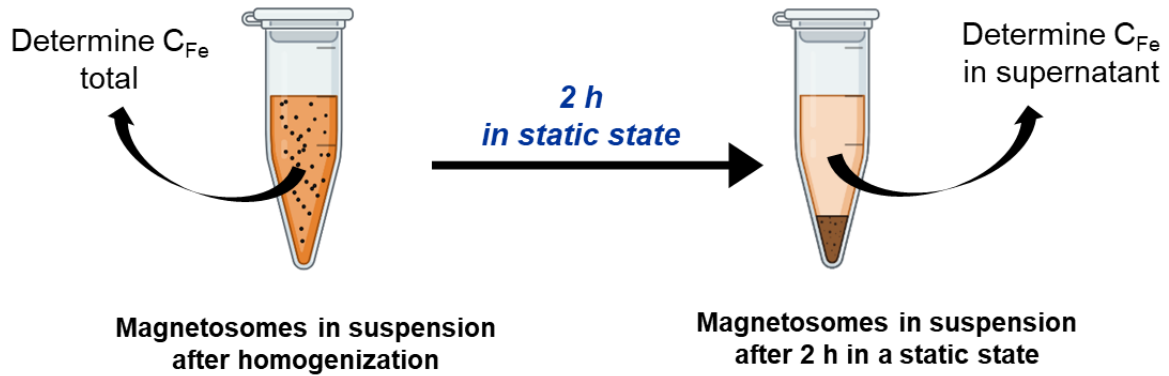
Measurement of colloidal stability



$$Stability\ (\%) = \left(\frac{C_{Fe}\ in\ supernantant}{C_{Fe}\ total}\right) \times 100\%$$

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125 **Fig S3**

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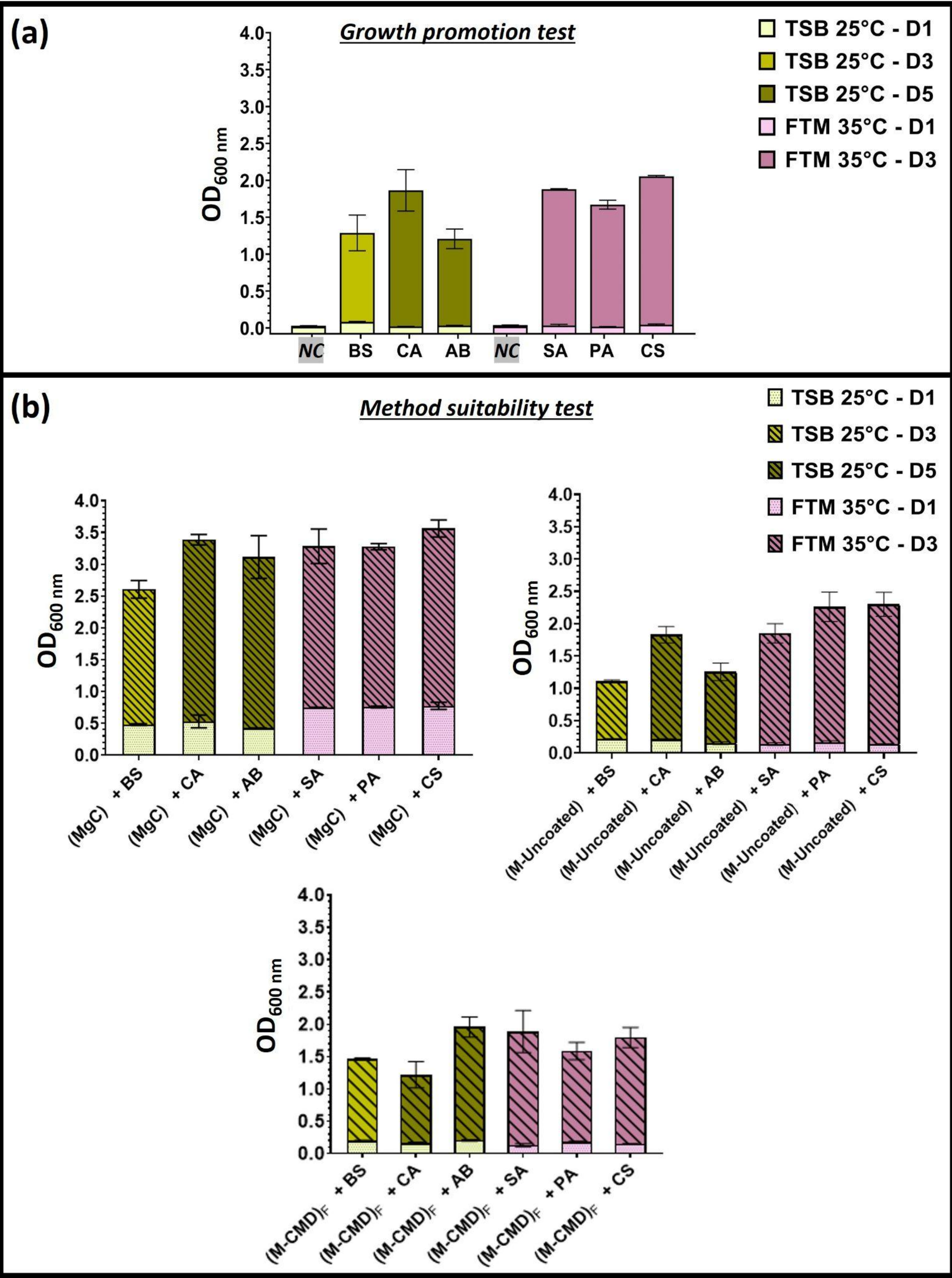

127 **Fig S4**

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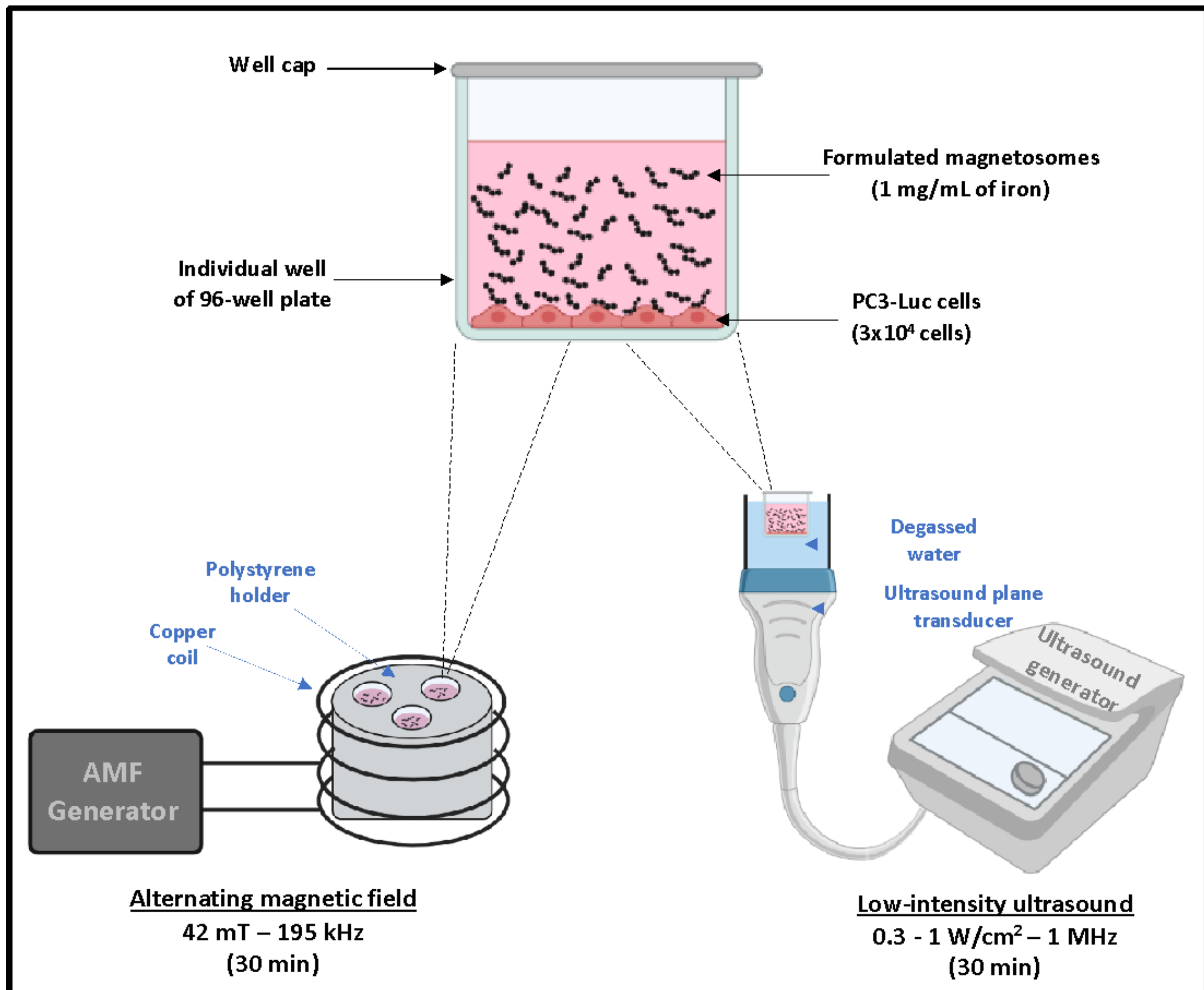

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131 **Fig S5**

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