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Intraluminal magnetisation of bowel by ferromagnetic particles for retraction and manipulation by magnetic probes

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Abstract

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Keywords (3-10) Characterisation, magnetic particles, physical properties, magnetic bowel retraction, minimal access surgery (MAS)

1. Introduction

Retraction of bowel during minimal access surgery (MAS) remains problematic as the moist low friction serosal surface is difficult to grasp with laparoscopic graspers. One study documented the low percentage (62 %) of successful grasping actions and indicated the need for improvement in laparoscopic grasper design [1].

We have been investigating tissue magnetization by magnetic nano- and micro-particles for MAS applications and have previously reported two tissue ferromagnetisation approaches for tissue retraction: *i)* surface magnetization by applying a small volume of glue-based magnetic media to the mucosal/ serosal surface [2], and *ii)* by interstitial injection of phosphate-buffered saline (PBS) ferrofluids [3]. In these experiments injected ferromagnetisation was shown to be superior to surface magnetisation by surface magnetic pellets, as the latter tended to peel off the tissue during retraction by magnetic probes. However, the restricted sub-mucosal space limited our injected media volume thus retraction force in previous interstitial injection method.

In the present study, we report a novel method of magnetisation of a bowel segment by intra-luminal injection of magnetic ferrofluids using a custom-designed intra-abdominal suction-injection probe. We compare the behaviour of stainless steel microparticles (SS-µPs) and iron oxide nanoparticles (IO-nPs). The latter family of nPs was chosen due to their well-established clinical use for applications including MRI contrast agents [4-8]. Detailed characterisation of magnetic particles used for making the magnetic ferrofluids for intraluminal bowel magnetisation and the retraction forces obtained in ex-vivo experiments using porcine bowel segments are reported and the comparison of four micro- and nano-particles allowed the identification of the rules to engineer the next generation of particles.

2. Materials and methods

2.1. Magnetic particles

Stainless steel microparticles and iron oxide nanoparticles were used as model magnetic particles. Two types of stainless steel microparticles (SS-µPs) were investigated: stainless steel type 410 microparticles (SS410-µPs, from Goodfellow) and stainless steel type 430 microparticles (SS430-µPs, from Alfa). Iron oxide nanoparticles were made in-house by forced hydrolysis (IO_{iH}-nPs) and compared with commercial iron oxide powder (IO_{Alfa}-nPs, from Alfa). These types of materials were previously used in reported studies on medical/surgical applications [9-11].

Preparation of in-house IO_{iH}-nPs: aqueous $FeCl_3$ and $FeCl_2$ solutions were prepared separately in degassed DI-H_2O (Milipore). The reaction environment was conditioned with a continuous and sustained flow of nitrogen and with a mechanical stirrer. Co-precipitation of the iron ions was completed at room temperature and by dropwise injection of a NH_4OH solution at a rate of 60 mL/h. The iron oxide formed a black precipitate, which was collected at the bottom of the container and washed with degassed DI-H_2O several times to remove free amine. The NH_3-free nanoparticles were then dried in vacuum before use.

All chemical reagents, unless otherwise stated, were purchased from Sigma, used without further purification and degassed before use only if specified: Iron(III) chloride ($FeCl_3$, reagent grade >97 %), Iron(II) chloride tetrahydrate ($FeCl_2.4H_2O$, puriss. p.a. ≥ 99.0 %), ammonium hydroxide aqueous solution (NH_4OH, 28-30 %).

2.2. Characterisation of magnetic particles

The properties of the four types of particles were characterised with the following experimental techniques.

SQUID: A 5.0 Tesla Superconducting Quantum Interference Device (SQUID) magnetometer from Quantum Design (MPMS XL^{TM}) was used to quantify the particles

magnetic properties. They were dispersed in a polymeric matrix to prevent interaction between nearby particles. The resulting sample was loaded into a low magnetic background gelatin capsule. Hysteresis measurements were completed at 300 K. The magnetization of the gelatine capsules and the polymeric matrix was subsequently subtracted.

XRD: Wide-angle powder X-ray diffraction (XRD) was used to assess the particle crystallinity and grain size. The data were collected on a Stoe STADI/P powder diffractometer operating in transmission mode and with a small angle position sensitive detector. Incident radiation was generated using a $Fe_{K\alpha 1}$ source (λ=1.936 Å). The strongest peak was fitted with Lorentzian-shaped peaks using STOEwinXpow and KaleidaGraph software packages to determine the diffraction peak positions and widths. The crystalline grain size, D_{XRD}, of the particles was calculated according to Scherrer's formula[12]:

$$D_{XRD} = \frac{0.9\lambda}{B\cos\theta} \quad (1)$$

where D_{XRD} is the "average" dimension of the crystallites, λ is the wavelength of the X-ray source (for Fe source is equal to 0.193604 nm), B is the full width at half maximum of the peak intensity, θ is the glancing angle.

Electron microscopy: Their sizes and morphology determined by electron microscopy (Transmission (TEM) and Scanning (SEM) electron microscope). TEM images were recorded using a Gatan CCD camera on a JEOL JEM-2011 microscope operating at 200 kV. SEM images were recorded using a Hitachi S-4800 microscope.

ICP-OES: The chemical composition of all particles was analysed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). A Perkin Elmer Optima 5300 DV was used as Inductively Coupled Plasma Optical Emission Spectrometer. The particles were mixed with hydrochloric acid (Trace SELECT®, ≥37 %) and nitric acid (Trace SELECT®, ≥69.0 %) in a 3:1 acid volume ratio and left overnight at room temperature. After

full dissolution of the particles, the solution was diluted with deionised water to prepare inductively coupled plasma-atomic emission spectrometry measurements.

2.3. Preparation of magnetic ferrofluids for intra-luminal bowel injection

Magnetic particles were dispersed in phosphate buffered saline (PBS) together with high viscosity fluids such as glycerol (80 % v/v) in order to improve their suspension and dispersion [3]. Concentrations of magnetic particles ranging from 0.1 to 1 g/mL were used for the magnetic bowel retraction studies. In a typical preparation protocol, for one ferrofluid formulation containing 0.25 g/mL fluid, 1.0 g of magnetic particles were added into a Sure/SealTM bottle which already contained 4 mL glycerol/PBS (80 % v/v) fluid. The magnetic particles were suspended in the liquid by vigorous hand and mechanical shaking of the sealed bottle prior to using the ferrofluid for intraluminal bowel injection.

A 10 mm diameter intra-abdominal suction-injection probe was designed and made-in-house. The probe applies initial suction through small suction holes at its distal end before its long needle (19-gauge) is inserted, via a central channel of the probe, into the lumen of the bowel for injection of ferrofluid in large volume. More detailed illustration of our proposed transperitoneal injection can be found from our recently published glue-based magnetic fluid for bowel magnetisation and retraction [13]. This design facilitates intraluminal injection without the need for use of additional graspers.

Harvested porcine colons were injected with the ferrofluids, after which the injection device was removed and the magnet probe was brought into contact with the bowel at the injection site. In these experiments, the magnetic attraction force was measured using a tensiometer [2] (Model 5564, Instron Ltd, Buckinghamshire, UK). The magnetic probe was made from neodymium iron boron (NdFeB) disc magnets with a remanence of 1.20 T (grade N35, Eclipse Magnetics Ltd, Sheffield, UK), and diameters of both 5 mm and 10 mm (30 mm

in length). The magnetic field at the surface of the magnets was measured with a magnetometer (Model #DCM 2320, AlphaLab, Inc., Salt Lake City, UT) and found to be equal to 0.46 T and 0.53 T for the 5mm and 10mm probes, respectively.

3. Results

3.1. Characterisation of magnetic particles

Four types of magnetic particles were investigated, Table 1 lists the physical properties as obtained by SEM and XRD, SQUID at 300 K, and ICP-OES.

Table 1

Characterization of the magnetic particles: size as obtained by SEM and XRD characterizations, magnetization at saturation (M_s) and coercivity (H_c) measured with a SQUID magnetometer at 300 K, Iron and chromium percentage as deduced from ICP-OES measurements, average maximum of the magnetic retraction forces F_{max} of a magnetised bowel segments in 20 ex-vivo experiments using the 10 mm magnet probe (injected magnetic media at concentration 0.25 g/mL: intraluminally injected 4 mL). Normalised retractions forces with the surface of the magnet and the magnetization at saturation.

	SS410-µPs	SS430-µPs	IO_{iH}-nPs	IO_{Alfa}-nPs
Size (diameter)	up to 50 µm ^{SEM}	up to 40 µm ^{SEM}	~ 13 nm ^{XRD}	~ 25 nm ^{XRD}
M_s (emu/g)	168.0	110.0	11.0	2.0
H_c (kOe)	0.01	0.02	0.25	0.06
Fe (w %) Cr (w %)	86.6 ± 6.8 12.6 ± 1.0	82.2 ± 6.7 16.9 ± 1.4	na	na
F_{max} *(N)*	5.9 ± 1.0	5.8 ± 0.6	1.3 ± 0.4	2.0 ± 0.1
σ (kPa)	75.67 ± 12.5	74.0 ± 7.0	16.69 ± 4.3	24.97 ± 1.4
F_{max}/M_s *(Ng/emu)*	0.035 ± 0.006	0.053 ± 0.005	0.12 ± 0.03	0.98 ± 0.10

Fig. 1a presents the morphology as observed by electron microscopy for iron oxide nanoparticles. XRD patterns, Fig. 1b, are characteristic of Fe_3O_4 and were used to extract the

crystalline grain size. Magnetic properties were quantified at room temperature in terms of magnetization as function of applied magnetic field and the resulting curves are presented in Fig. 1c. The morphologies and magnetisation curves for the commercial stainless steel microparticles were reported earlier elsewhere [13]. As expected, the curves are symmetric and the magnetization at saturation, Ms, can be deduced from the plateau, when the magnetization does not increase any more with the magnetic field.

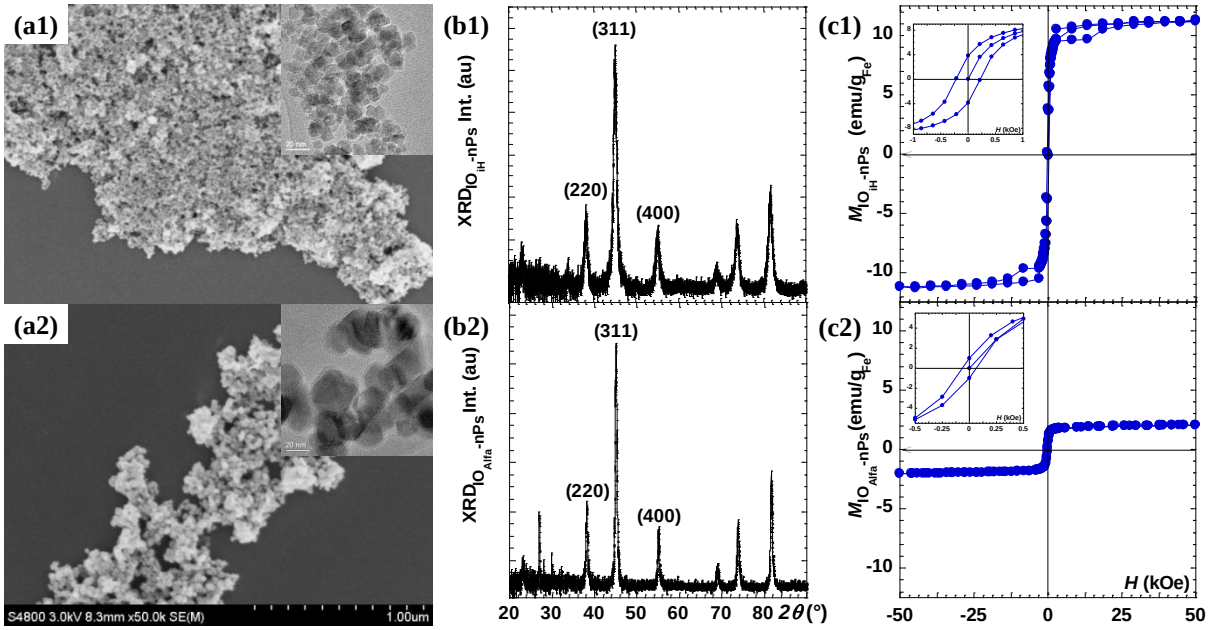

Fig. 1: For IOiH-nPs (1) and IOAlfa-nPs (2): Scanning Electron Microscopy images (a), XRD spectra of the Fe_3O_4 nPs (b) and hysteresis curves completed at room temperature (c). Insets present TEM images of IO-nPS with a 20 nm scale bar (a) and zoomed magnetisation response for low magnetic fields (c).

The magnetization curves of both µPs fell to zero magnetisation when the magnetic field equalled zero, indicating that there was no significant coercivity (H_c) and no observable remanence (Table 1). The coercivity refers to the magnetic field which needs to be applied to reduce the magnetization of a material down to zero after the magnetization of the sample has been driven to saturation. This implies that SS-µPs even though too large to form stable solution would not face further difficulty in dispersion in the fluid due to magnetic

interactions since the particles experience no mutual magnetic attraction until placed in a magnetic field. In contrast, the smaller size nPs would face magnetic interactions at room temperature so that a degree aggregation/ clumping occurs, as observed on the SEM and TEM images in Fig. 1ab.

3.2 Ferrofluid injection and bowel retraction by magnet probe

Table 1 also summarises the magnetic retraction forces of a magnetised bowel segments in 20 ex-vivo experiments using the 10 mm magnet probes with a 4 mL ferrofluid injection (Fig. 2a-b), that is, the number of measurements per type of ferrofluid and probe size was 20 with standard deviations shown in Table 1 and Fig. 2c. Ferrofluids based on both SS-MPs could generate maximal force near 6 N while IO-nPs-based media could only provide about 1 to 2 N retraction force.

In our previous study [3], we observed that magnetic attraction force increased proportionally with the injected volume of a solution of constant particle concentration. However, the maximal possible injected volume for interstitial (sub-mucosal) injection is limited to 0.2 mL and this limits the retraction force possible with this form of tissue magnetisation. This problem is avoided with intraluminal injection into the bowel lumen. Although one can inject as many fluids as possible into the bowel lumen, a practical threshold could be estimated. For example, as shown in Fig. 2c, there was no or just minor increase in its magnetic retraction force beyond 4 mL injection. Specifically, magnet probes with 10 mm and 5 mm diameters were used. With 10 mm probe, magnetic retraction force increased with increase of injected magnetic volume of ferrofluid and particle content/mass from 0.25 g (1 mL) up to 1 g (4 mL). There was almost no retraction force increase beyond 1 g of injected particles, indicative of saturation at this mass/content. The smaller magnet (5 mm) required even less particles to reach its saturation force. As magnetic attraction force between the magnet probe and injected particle decreases exponentially with their distance, any further

injection of magnetic ferrofluid simply pushes the magnetic particles further away from the magnet and thus contributes significantly less to the attraction force.

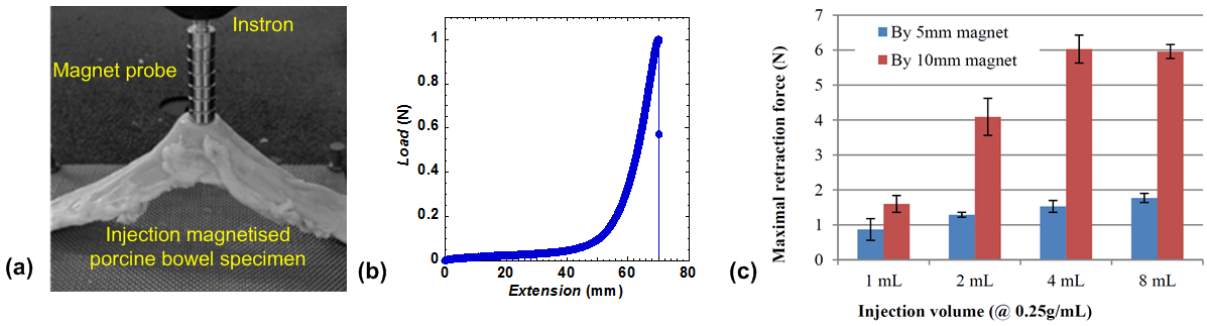

Fig. 2: Ex-vivo porcine bowel retraction and force measurement: (a) Photograph of a magnetised bowel segment retracted during Instron force measurement. (b) Extension-load curve demonstrating a maximum force before slipping occurs (normalised to unity for illustration purpose). (c) Injection volume versus magnetic retraction force using two-sized magnet probes.

4. Discussion

The suction-injection probe first tented the target bowel segment and generated large lumen space in order to facilitate intraluminal injection of ferrofluid into lumen. This avoids fluid to be injected into sub-mucosa or through the bowel into abdominal space. The injected fluid was temporarily maintained in the intended lumen section of the intestine due to its high viscosity (i.e., glycerol suspension), and the magnet probe was then located at the injection section immediately after withdrawal of the injection probe. Although a very small volume of fluid, e.g. 4 mL, is needed to interact with the magnet probe for generating sufficient force, in practical a larger volume (e.g. >10 mL) could be readily injected into the intended lumen section. Although the fluid would disperse throughout the lumen, in our ex-vivo experimental setup, we found there was always sufficient ferrofluid remaining to interact with the magnet probe.

The average pull force that surgeons use to provide enough tension to the bowel is known to be 2.5 N while the maximal force is just below 5 N.[14] The test results presented in Table 1 and Fig. 2 indicate that SS-μPs based bowel magnetization would be capable of providing sufficient retraction force for bowel manipulation. The pressure generated between the magnetic fluid and the probe was up to 76 kPa in the present study which would cause less trauma to the target tissue. For example, the pressure generated from this device is significantly lower than that generated by forceps or graspers (210 to 650 kPa [15]), also used for laparoscopic bowel retraction. Much higher trans-mural pressures were used in order to cause tissue ischemia in compressive magnetic anastomosis, for example in gastrointestinal (600 kPa) and bilioenteric (1 MPa) anastomosis [16]. In our application, the probe is removed once the operation is completed, thus the fluid is eliminated in the stools after surgery. A retractable permanent magnet housed inside the probe distal segment could provide an adjustable magnetic attraction force for safe tissue manipulations. For example, in one custom-design, an 8 mm-diameter and 20 mm-long magnet housed within a 10 mm tubular probe produced a maximal retraction force of 3.5 N when the probe was in contact with the magnetised bowel. The force could be reduced to 0.3 N by controlled movement of the magnet, thereby enabling controlled release of the magnetised target bowel. Details about this custom-design magnet probe will be published elsewhere.

5. Conclusion

The physical properties of magnetic particles were investigated and the relative performances were compared. Injectable high viscosity magnetic ferrofluids were developed for magnetic bowel retraction and were characterised in an ex-vivo porcine bowel model. The present approach utilizes the magnetic interactions to retract internally magnetised bowels towards an external (intra-abdominal) magnetic probe for safe and effective bowel

manipulations. The results of the current experiments indicate that with the technology described, stainless steel micro-particles provide sufficient magnetic attraction force for bowel manipulation. With at least one order of magnitude smaller magnetisation at saturation, IO-nPs fell short by only 20 % to provide of sufficient magnetic attraction force. On-going research is in progress to address the identified challenges and ideal nPs would be engineered to be either super-paramagnetic or coated with a layer providing both solubility and repulsive interactions between the nanoparticles to improve the dispersion in the selected solvent, collection from the jar, and injection into the bowel. To engineer the next generation of particles, these are more important parameters than the magnetisation at saturation of a specific magnetic material. With this in mind, the library of magnetic particles suitable to retract internally magnetised bowels towards an external (intra-abdominal) magnetic probe for safe and effective bowel manipulations should drastically expand. Finally, in-vivo animal studies should next be completed to insure efficacy and safety of ferrofluids.

Conflict of interest

The authors report no conflict of interest.

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Ethical approval

Ethical approval has not been required for this work.

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