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# Magnetic Particle Imaging for the Evaluation of Gastrointestinal Health by Measuring Gastrointestinal Permeability

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**Abstract**

Various diseases and immune-related issues have been associated with the gastrointestinal system's health. Gastrointestinal permeability - a measure of transport across the GI tract's cell lining from the lumen - is a functional parameter that is affected and is a viable factor in GI health assessment. Current diagnosis of GI-related disease involves the use of invasive exploratory surgery to minimally invasive colonoscopy. Magnetic particle imaging (MPI) is a non-radioactive and highly sensitive tracer imaging modality. The nanoparticle tracers used for MPI are FDA approved and can readily be translated into the clinic. In this research, we provide the first proof-of-concept using MPI to evaluate GI permeability in an *in vitro* model of the epithelial barrier lining of the GI tract.

## I. Introduction

The health of the GI tract has implications for gut-related disorders, brain health, immune health, and even the successful outcome of immunotherapy treatments [1]. Studies have shown a link between a patient's gut health and their response to immunotherapy treatments. Over the past decade, there has been an increasing discussion on the correlation between altered intestinal permeability and chronic GI disorders like Inflammatory Bowel Disease, Crohn's disease, Ulcerative Colitis, and even non-GI-related diseases like Alzheimer's and Parkinson's. Apart from food digestion and absorption, the GI tract is home to many essential symbiotic bacteria that help maintain good gut health and a protective mucus lining. The intestinal barrier lining consists of mucus layer(s), an epithelial layer, and immunological defenses. This intestinal barrier performs two key functions: (a) It must prevent bacteria and other infectious agents from permeating through and triggering an immune response (b) It must allow the passage of nutrients and absorption of fluids [2]. If this intestinal barrier is compromised, it can lead to pathological conditions - inflammation and changes in the gut flora. Currently, there is no *robust* standard test to measure GI permeability. In a standard procedure, the ratiometric sugar assay of lactulose to mannitol (L/M) concentrations is measured by collecting urine for over 6 hours. However, both sugars are diuretics and can alter GI permeability, introducing confounds and rendering the test unreliable [3].

Minimally invasive imaging techniques of endoscopy or colonoscopy are also used for GI health. These are used to image the inside of the intestine to check for ulcerations, inflammation and cancer. However, the procedure is uncomfortable, and patient participation in screening is often abysmal. X-ray imaging provides high-resolution anatomical features of the contrast-enhanced GI tract, which seldom provides information on gut func-

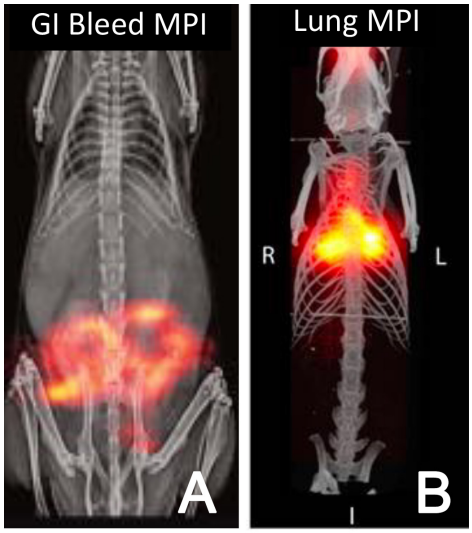


**Figure 1:** Magnetic Particle Imaging (MPI) is particularly useful in imaging and diagnosis of tissues such as the GI tract and lungs. (A) MPI is by far the most sensitive modality for detecting the GI bleed [4] and (B) MPI images of tracer distribution in the lung, also known as lung ventilation assessment [5].

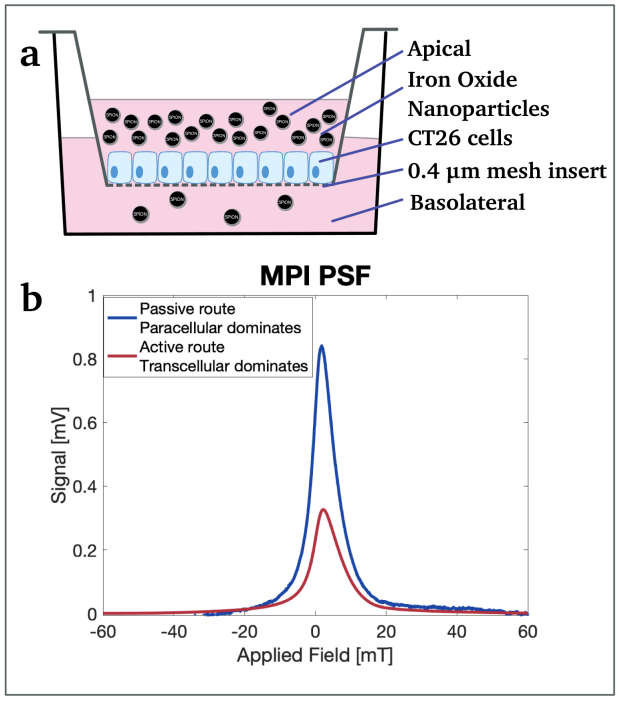


**Figure 2:** *In vitro* model of GI permeability using a transwell membrane and CT26 murine cells. (a) MPI tracers were loaded onto the apical side of the monolayer, and the tracer's permeability across the cell monolayer was evaluated by sampling the basolateral side. A sensitive in-house arbitrary waveform relaxometer was used to evaluate the amount of permeated MPI tracer. (b) The point-spread function (PSF) of MPI signal on the basolateral side with (active route, transcytosis) and without (passive route) a cell monolayer after 72 hours incubation.

tion or permeability. MPI is a tracer-based imaging modality that can image superparamagnetic iron-oxide (SPIO) tracers with excellent contrast and nanogram sensitivity [6, 7]. MPI has zero tissue attenuation and zero ionizing radiation. MPI tracers are FDA-approved and can be used directly without laboratory preparation [8, 9] and are particularly useful to image organs like the liver and lungs (Fig. 1). By measuring the clearance rate of an oral dose of MPI tracers from the gut to the liver, we can measure GI permeability and provide a means of non-invasive and in-house diagnosis of a person's gut health. The GI tract can be imaged to see its outline, and the health of the GI tract can thus be assessed. In this research work, we evaluated GI permeability using an *in vitro* model of the gut epithelium using MPI.

## II. Methods and Materials

An intestinal *in vitro* model was set up using the Corning™ Costar™ Flat Bottom Cell Culture 6-well Microplates with Transwell mesh inserts of 0.4 $\mu$m pore size (see Figure 2a). Perimag® (130 nm, Micromod Partikeltechnologie GmbH, Germany) was used to evaluate the permeability of the monolayer [10]. CT26 colon cancer cells were seeded onto the 0.4 $\mu$m mesh and incubated in cell-culture media for 24 hours to ensure single monolayer formation [11]. MPI tracers were added on the apical side of the transwell membrane as shown in Fig. 2a. The solutions in the apical and basolateral sides of the membrane were collected at the end of 72 hours, and the amount of iron that had permeated through was

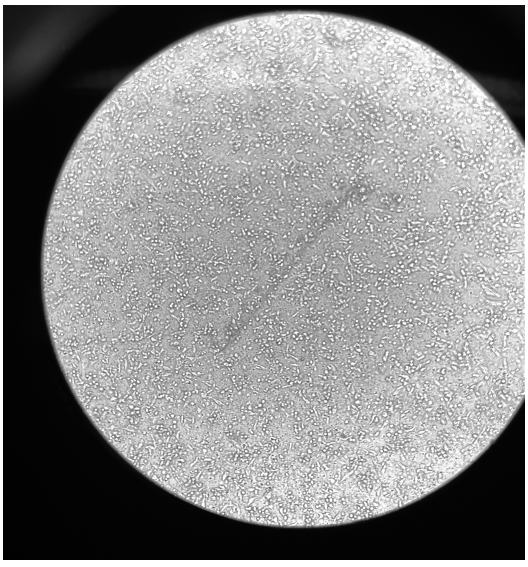

**Figure 3:** The *in vitro* transwell setup with the CT26 cell monolayer on the 0.4 $\mu$m mesh insert.

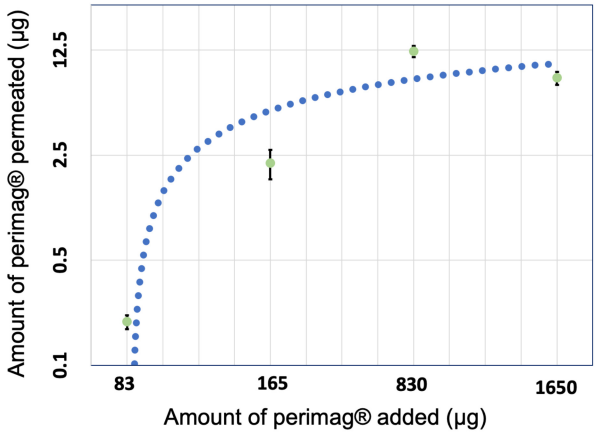


**Figure 4:** The data shows the effect of iron loading on the monolayer versus the amount of nanoparticles that permeated across. At low loadings, the amount permeated increased with the nanoparticle load. In contrast, at higher loading, the permeation was purely limited by the cell monolayer and saturated (as shown by the trendline through the data points).

evaluated. A non-monolayer setup was used as a control.

The MPI tracer signal and point-spread function from both the apical and the basolateral side were measured using our in-house arbitrary waveform relaxometer (AWR) with gradiometer shimming operating at 20 mT amplitude and 20kHz frequency with a bias field sweep from -60 to 60 mT. The signal obtained was reconstructed using the x-space reconstruction algorithm, and the peak signal of the PSF was used to determine the amount of iron oxide that permeated through [12, 13].

## III. Results and Discussion

Fig. 2b shows the PSF from the data obtained under two experimental conditions: with and without the cell monolayer. There are two mechanisms through which nanoparticles cross the GI epithelial barrier. In the passive or *paracellular* route, nanoparticles cross through the tight junctions between the epithelial cells barrier due to a mere concentration gradient across the barrier. While in the active or *transcellular* route, the nanoparticles pass through the cells of the barrier in an active phagocytic manner. We determined for our *in vitro* model of GI permeability that the setup with cell monolayer mimicked the active or the *transcellular* route of nanoparticle transport using our MPI system. While the nanoparticle transport via the passive route is a gross overestimate given the fact that the tight junctions are relatively smaller (0.8-1.3 nm) than the 0.4 $\mu$m that was currently tested out [14]. Next, we evaluated the dose dependence and its effect on permeability. Figure 4 shows the effect of varying amounts of iron from 83-1650 $\mu$g, and the amount permeated measured using our arbitrary waveform relaxometer (AWR). The tracer permeating through the transmembrane seems to saturate at a higher dose (> 165 $\mu$g, based on the trendline). While at the lower doses, the permeation depended on the amount loaded. This preliminary study informs us on planning the translational work for evaluating gut health in murine models subject to hyperpermeable GI disorders.

## IV. Conclusion

Our initial proof-of-concept *in vitro* study suggests that nanoparticle tracers used in MPI can permeate across the gut epithelium through the transcellular pathway. However, we need to ascertain the finding further using a blocking agent that prevents transcellular permeation. Nevertheless, using MPI tracers that are FDA-approved, a GI permeability study can be carried out in the clinic via oral administration of MPI tracers. Estimating the MPI tracer permeation across the GI epithelium to the liver can be one of the first non-invasive methods to estimate GI permeability and assess Gastrointestinal health.

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## Author's statement

Conflict of interest: Dr. Steven Conolly is a co-founder and holds stock of a startup company that manufactures and sells preclinical MPI scanners. The authors declare no other conflict of interest.

## References

[1] V. Gopalakrishnan, C. N. Spencer, L. Nezi, A. Reuben, M. C. Andrews, T. V. Karpinets, P. A. Prieto, D. Vicente, K. Hoffman, S. C. Wei, A. P. Cogdill, L. Zhao, C. W. Hudgens, D. S. Hutchinson, T. Manzo, M. P. de Macedo, T. Cotechini, T. Kumar, W. S. Chen, S. M. Reddy, R. S. Sloane, J. Galloway-Pena, H. Jiang, P. L. Chen, E. J. Shpall, K. Rezvani, A. M. Alousi, R. F. Chemaly, S. Shelburne, L. M. Vence, P. C. Okhuysen, V. B. Jensen, A. G. Swennes, F. McAllister, E. M. R. Sanchez, Y. Zhang, E. L. Chatelier, L. Zitvogel, N. Pons, J. L. Austin-Breneman, L. E. Haydu, E. M. Burton, J. M. Gardner, E. Sirmans, J. Hu, A. J. Lazar, T. Tsujikawa, A. Diab, H. Tawbi, I. C. Glitza, W. J. Hwu, S. P. Patel, S. E. Woodman, R. N. Amaria, M. A. Davies, J. E. Gershenwald, P. Hwu, J. E. Lee, J. Zhang, L. M. Coussens, Z. A. Cooper, P. A. Futreal, C. R. Daniel, N. J. Ajami, J. F. Petrosino, M. T. Tetzlaff, P. Sharma, J. P. Allison, R. R. Jenq, and J. A. Wargo.

 

Gut microbiome modulates response to anti–pd-1 immunotherapy in melanoma patients. *Science*, 359(6371):97–103, 2018, doi:10.1126/science.aan4236.

[2] C. V. Almario, M. L. Ballal, W. D. Chey, C. Nordstrom, D. Khanna, and B. M. R. Spiegel. Burden of gastrointestinal symptoms in the united states: Results of a nationally representative survey of over 71,000 americans. *Am J Gastroenterol*, 113(11):1701–1710, 2018, doi:10.1038/s41395-018-0256-8.

[3] A. S. Rao, M. Camilleri, D. J. Eckert, I. Busciglio, D. D. Burton, M. Ryks, B. S. Wong, J. Lamsam, R. Singh, and A. R. Zinsmeister. Urine sugars for in vivo gut permeability: Validation and comparisons in irritable bowel syndrome-diarrhea and controls. *Am J Physiol Gastrointest Liver Physiol*, 301(5):G919–28, 2011, doi:10.1152/ajpgi.00168.2011.

[4] E. Y. Yu, P. Chandrasekharan, ...., and S. M. Conolly. Magnetic Particle Imaging for Highly Sensitive, Quantitative, and Safe in Vivo Gut Bleed Detection in a Murine Model. *ACS Nano*, 11(12):12067–12076, 2017.

[5] Z. W. Tay, P. Chandrasekharan, X. Y. Zhou, E. Yu, B. Zheng, and S. Conolly. In vivo tracking and quantification of inhaled aerosol using magnetic particle imaging towards inhaled therapeutic monitoring. *Theranostics*, 8(13):3676–3687, 2018, doi:10.7150/thno.26608.

[6] B. Gleich and J. Weizenecker. Tomographic imaging using the nonlinear response of magnetic particles. *Nature*, 435(7046):1214–1217, 2005, doi:10.1038/nature03808.

[7] P. Chandrasekharan, Z. W. Tay, ...., and S. Conolly, Chapter 15 - magnetic particle imaging for vascular, cellular and molecular imaging, in *Molecular Imaging (Second Edition)*, B. D. Ross and S. S. Gambhir, Eds. Academic Press, 2021, 265–282, ISBN: 978-0-12-816386-3. doi:https://doi.org/10.1016/B978-0-12-816386-3.00015-6.

[8] B. Zheng, T. Vazin, P. W. Goodwill, A. Conway, A. Verma, E. U. Saritas, D. Schaffer, and S. M. Conolly. Magnetic Particle Imaging tracks the long-term fate of in vivo neural cell implants with high image contrast. *Sci Rep*, 5:14055, 2015.

[9] X. Y. Zhou, K. E. Jeffris, E. Y. Yu, B. Zheng, P. W. Goodwill, P. Nahid, and S. M. Conolly. First in vivo magnetic particle imaging of lung perfusion in rats. *Phys Med Biol*, 62(9):3510–3522, 2017.

[10] P. Chandrasekharan, Z. W. Tay, ..., and S. Conolly. A perspective on a rapid and radiation-free tracer imaging modality, magnetic particle imaging, with promise for clinical translation. *Br J Radiol*, 91(1091):20180326, 2018, doi:10.1259/bjr.20180326.

[11] A. Fedi, C. Vitale, G. Ponschin, S. Ayehunie, M. Fato, and S. Scaglione. In vitro models replicating the human intestinal epithelium for absorption and metabolism studies: A systematic review. *Journal of Controlled Release*, 335:247–268, 2021, doi:https://doi.org/10.1016/j.jconrel.2021.05.028.

[12] Z. W. Tay, P. W. Goodwill, D. W. Hensley, L. A. Taylor, B. Zheng, and S. M. Conolly. A High-Throughput, Arbitrary-Waveform, MPI Spectrometer and Relaxometer for Comprehensive Magnetic Particle Optimization and Characterization. *Sci Rep*, 6:34180, 2016.

[13] P. W. Goodwill and S. M. Conolly. The x-space formulation of the magnetic particle imaging process: 1-d signal, resolution, bandwidth, snr, sar, and magnetostimulation. *IEEE Trans Med Imaging*, 29(11):1851–9, 2010, doi:10.1109/tmi.2010.2052284.

[14] J. Linnankoski, J. Mäkelä, ..., and M. Yliperttula. Paracellular porosity and pore size of the human intestinal epithelium in tissue and cell culture models. *Journal of Pharmaceutical Sciences*, 99(4):2166–2175, 2010, doi:https://doi.org/10.1002/jps.21961.

 