Combined 19F MRI and CT imaging for the visualization of delayed release of compounds using pH-sensitive polymers coated capsules in vitro and in a hamster animal model

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Introduction: In vivo imaging of controlled release in the gastrointestinal tract is of major importance to assess its efficacy. Antibodies against *Clostridium difficile* represent an innovative strategy to combat one of the most common causes of nosocomial infections. Moreover, delayed controlled release of these antibodies at the site of inflammation in the small intestine will lead to a more efficient use of the antibodies and possibly reduced systemic side effects. We have monitored the release of contrast agents for in vivo ^{19}F MRI and CT from capsules coated with pH-sensitive polymers. For ^{19}F MRI we made use of signal that is only generated when the capsule starts to leak and 2-fluoro-2-deoxy-D-glucose (^{19}FDG) is dissolved. Barium sulphate ($BaSO_4$) was used as contrast agent for CT in order to monitor capsule integrity and the exact anatomical location of release of its content, utilizing the high radio opacity of barium.

Methods and Materials: Gelatine size 9 capsules (Torpac, Fairfield, USA) were coated with Eudragit L100 (Evonik Industries, Essen, Germany) in triethylcitrate in isopropanol. For in vitro studies, phantoms were prepared by filling solution at different pHs (pH = 1, 3, 5 and 7) with one intact ^{19}FDG or $BaSO_4$ loaded capsule into microcentrigufe tubes and then moved to a pre-prepared plastic container made of 2% agarose. Syrian Golden hamsters were given capsules loaded with both ^{19}FDG and $BaSO_4$ via oral gavage for in vivo studies. MRI was acquired using a 9.4 Tesla Bruker Biospec small animal MR scanner (Bruker Biospin, Ettlingen, Germany) with a home-built surface coil, tuneable-matcheable to the ^{19}F and ^{1}H resonances. Both anatomic and ^{19}F image were acquired using a RARE sequence with parameters: TE = 15.9 ms; TR = 6 s (^{1}H)/1 s (^{19}F); eight echoes/excitation; FOV = 6 cm*8 cm, 300*400 (^{1}H)/50*50 (^{19}F) matrix with 0.5 mm (^{1}H)/2.5 mm (^{19}F) slice thickness, NA = 2 (^{1}H)/ 500(^{19}F), scan time = 33 minutes. CT images were acquired on a dedicated small animal scanner (SkyScan 1076, Bruker microCT, Kontich, Belgium) with parameters: 50 kVp X-ray source voltage combined with a 0.5 mm aluminum filter, 180 µA source current, 450 ms exposure time per projection, acquiring 2 projections (for averaging) per position with 0.7° increments over a total angle of 180° and FOV covering the stomach and bowels, resulting in a scanning time of 12 minutes yielding reconstructed 3D datasets with 35 µm³ isotropic voxel size.

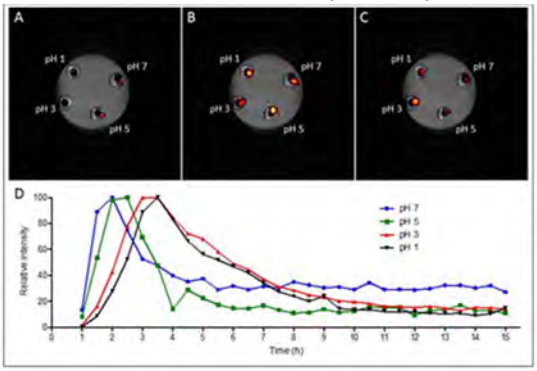

Fig.1. In vitro MRI monitoring of ^{19}FDG signal over 15 hours continuously. A-C: representive proton slice(grayscale) overlaid by corresponding ^{19}F signal(red-hot scale) at different time points (A: 1 hour; B: 3 hour; C:15 hour); D. Quantitative ^{19}F signal of a representive slice over monitoring and for different pHs, signal is normalized to its peak value.

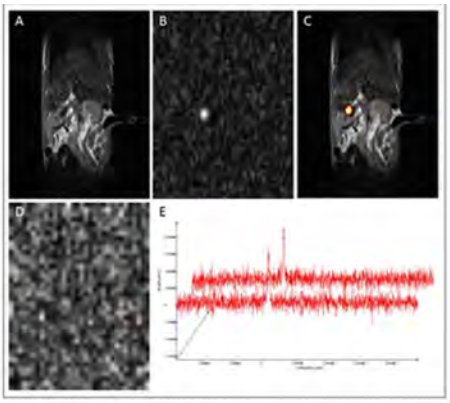

Fig.2. In vivo MRI images of capsule disrupted in stomach. A: proton slice; B: corresponding ^{19}F image at 2 hours time point; C: composite A and B; ^{19}F image is red-hot scaled; D. corresponding ^{19}F image at 5 hours time point; E. ^{19}F spectrums at different time points(below: 2 hours; upper: 5 hours).

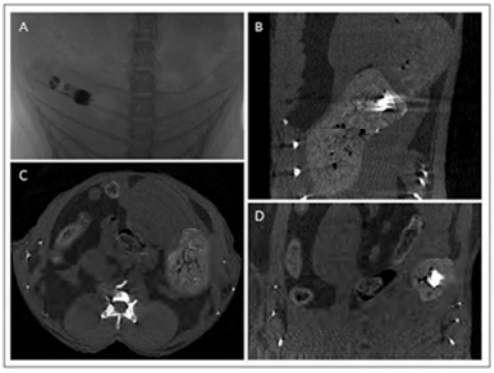

Fig.3. In vivo CT images of capsule disrupted in stomach. A: 2D projection image at 2 hour; B-D: 3D reconstructed image at 5 hour time point (coronal view, axial view and sagittal view).

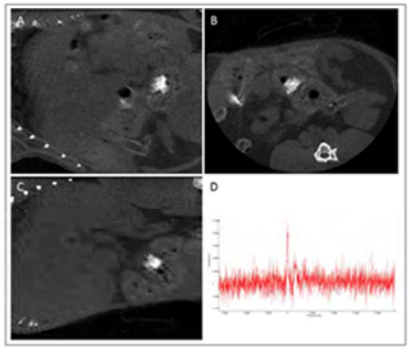

Fig.4. In vivo CT images and ^{19}F spectrum of capsule disrupted in bowel. A-C: 3D reconstructed image (coronal view, axial view and sagittal view); D: ^{19}F spectrum of the same animal

Results: We confirmed the low pH resistance of the coated capsules by in vitro time lapse MRI and CT of capsules filled either with ^{19}FDG or $BaSO_4$. The appearance and intensity of the ^{19}F signal was correlated with the pH values (fig.1). The lower the pH, the longer time it needed for the solution to get into the capsule and dissolved the ^{19}FDG. The time point where the ^{19}F signal reached its maximal could be considered as the moment when the capsule was getting porous. Ultimately, the ^{19}FDG was released from the disrupted capsule to the surrounding solution, which diluted the ^{19}F signal until it was evenly distributed. In vitro CT data also gave similar results and confirmed that the capsules have a better resistance at low pHs (data not shown). During in vivo studies (fig.2A-C and fig.3A), we could clearly localize the capsule in the stomach 2 hours after administration both by using MRI and CT. Furthermore, in vivo studies confirmed the in vivo resistance to degradation of the capsule at low pH in the stomach for up to 4-5 hours (fig.2D E and fig.3B-D) until the capsule disintegrated and contrast agents dissolved in the tissues. In one out of four administrations, the capsule passed the stomach and reached the small intestine which was visualized by CT and confirmed by ^{19}F spectroscopy (fig.4).

Conclusion and Discussion: We were able to show that combined application of ^{19}F MRI and CT provide information on the location, integrity and release of material from the capsule into the gastrointestinal tract of small animal models for the first time. This forms the basis for further studies on the delivery of antibodies against *Clostridium difficile*. Further technical improvements are required in terms of size and coating quality of the capsule to demonstrate the repeated release of the material from the capsule in the small intestine. Using our approach, CT contrast agents have the advantage of providing anatomical information with high spatial and temporal resolution and good sensitivity. Thanks to the high specificity and quantitative feature of ^{19}F MRI, it could offer more detailed information to better understand the process of capsule disruption. We believe this imaging approach could be applied to other applications where controlled release mechanisms need to be understood.