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Functional Colonography of *Min* Mice Using Dark Lumen Dynamic Contrast-Enhanced MRI

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Abstract

Dark lumen MRI colonography detects colonic polyps by minimization of the intestinal lumen signal intensity. Here we validate the use of perfluorinated oil as an intestinal-filling agent for dark lumen MRI studies in mice, enabling the physiological characterization of colonic polyps by dynamic contrast-enhanced MRI. In control and *Min* (multiple intestinal neoplasia) mice with and without pretreatment with oral dextran sodium sulfate (DSS), polyps as small as 0.94 mm diameter were consistently identified using standard 2D gradient echo imaging (voxel size, $0.23 \times 0.16 \times 0.5$ mm). In serial studies, polyp growth rates were heterogeneous with an average $\approx 5\%$ increase in polyp volume per day. In DSS-treated control mice the colon wall contrast agent extravasation rate constant, K^{trans} , and extravascular extracellular space volume fraction, v_e , values were measured for the first time and found to be $0.10 \pm 0.03 \text{ min}^{-1}$ and 0.23 ± 0.09 , respectively. In DSS-treated *Min* mice, polyp K^{trans} values ($0.09 \pm 0.04 \text{ min}^{-1}$) were similar to those in the colon wall but the v_e values were substantially lower (0.16 ± 0.03), suggesting increased cellular density. The functional dark-lumen colonography approach described herein provides new opportunities for the noninvasive assessment of gastrointestinal disease pathology and treatment response in mouse models.

Keywords

dark lumen MRI; *Min* mouse; DCE-MRI; perfluorinated oil

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The in vivo assessment of mouse colorectal anatomy and function is challenging for all imaging modalities but could potentially provide new insights into disease progression and aid in the evaluation of new therapeutics for clinical use. Computed tomography (CT) colonography has shown great promise in the detection of colorectal polyps in both humans and mice and serves as an accurate high-resolution anatomical screening tool but provides no functional information relevant to the underlying tumor physiology (1,2). Conversely, optical and positron emission tomography (PET) imaging can interrogate the underlying biochemical processes of tissue function with the use of targeted contrast agents but have a limited spatial resolution (3-5). MRI has only recently been used to study the mouse colon but could potentially bridge the gap between these imaging paradigms by providing high-resolution anatomical and dynamic contrast-enhanced functional images of the colon. In contrast to conventional imaging-based colonography, which yields a morphological assessment of the colon, a “functional colonography” could provide a physiological characterization of the colon tissue. The use of dynamic contrast-enhanced (DCE) MRI methods and standard gadolinium (Gd)-based agents, such as Gd-DTPA, has the potential to measure colonic tissue contrast agent kinetic parameters that have been shown to differentiate malignant from benign tumors (6), aid in tumor staging (7), and monitor treatment response (8) in other disease states. With targeted Gd-based agents this approach could potentially report on gene activity, cell receptor expression, and/or other biochemical processes in colon tissue (9-11).

Functional MRI colonography has great potential for the physiological assessment of murine models of colon cancer. One such model, the *Min* (multiple intestinal neoplasia) mouse, is an appropriate model for the study of early stage colon tumor progression as a result of an *APC* mutation similar to the human Familial Adenomatous Polyposis Coli (FAP) syndrome that predisposes to colon cancer (12). These mice contain a truncating mutation of the *Apc* tumor suppressor gene and spontaneously develop adenomas in both the small and large intestine. This murine model is increasingly utilized to evaluate the efficacy of colorectal cancer treatments (13,14). Treatment of *Min* mice with the colitis-inducing agent dextran sodium sulfate (DSS) results in a greater number specifically of colonic as opposed to small intestinal polyps (15). Additionally, the DSS-elicited polyps are generally larger and more advanced than colonic polyps in non-DSS treated mice. The noninvasive, serial, and functional characterization of the microenvironment of these polyps could offer new insights into their progression and potentially provide a means to identify polyps that will undergo malignant transformation.

In both humans and animals the detection of colon polyps using MR colonography requires clear delineation of the colon wall from bowel lumen and the capability to differentiate between polyps and artifacts of residual stool or air bubbles. With T_1 -weighted imaging, high contrast between the colon wall and the lumen can be achieved by enhancing either the colon lumen using Gd-doped enemas (bright lumen MRI) or the colon wall by intravenous administration of paramagnetic contrast agents and water enemas (dark lumen MRI). With bright lumen MRI, colonic polyps appear as dark filling defects within the bright colon lumen but differentiating these masses from air bubbles or residual stool is difficult and often requires multiple scans with the patient in different positions (16). To overcome this limitation while maintaining high contrast between the colon wall and lumen, dark lumen colonography was proposed and has been shown to be highly accurate for the detection of colorectal masses (17,18). Dark lumen MRI relies on minimizing the signal intensity of the intestinal lumen and its contents while enhancing the signal from the lumen wall and any masses using intravenous injections of paramagnetic contrast agents. Clinically, two dark lumen strategies have been developed to enhance the contrast between the colon wall and the lumen on T_1 -weighted images, including backfilling the colon with water or air following an enema (17,19,20) or alternatively adding contrast agents (typically barium sulfate) to the food that reduces the MRI signal from feces (fecal tagging) (21).

Currently, two studies have adapted these methods for use in mouse models. Using the fecal tagging approach with barium sulfate, Larsson et al. (22) were able to differentiate between healthy and inflamed colon tissue in a mouse model of colitis. Hensley et al. (23) identified colon polyps in a *Min* mouse model as small as 1.5 mm in diameter using a 7T animal scanner by first cleansing the bowels and then inserting a polyurethane tube filled with Gd-DTPA fully into the colon, which assisted in the identification of the colon on the MRI images. Despite the success of these early studies, these methods are not optimal for functional colonography. To serially assess tumor growth and evaluate treatment response, it is important to identify lesions and changes in their size on a scale smaller than 1.5 mm. Also, given that the location and number of polyps is unknown prior to a study it would be advantageous to clearly and repeatedly delineate all portions of the colon. Without distending the colon this is a challenging problem given that mucosal folds and redundancies of the colon can be unpredictably flattened, hindering both the detection of polyps and the accurate determination of their dimensions. We propose that these issues can be minimized if the colon is fully distended using a filling agent prior to each scan, similar to the original dark lumen MRI methods that require patients to retain 2-2.5 L of enema solution during the examination (19). Furthermore, although water enemas do reduce the lumen signal intensity, we propose that the residual signal seen using water enemas can be effectively eliminated with the use of nonhydrogenous perfluorinated oil enemas.

Therefore, the goals of this study were to demonstrate: 1) the potential of perfluorinated oils as colon-filling agents for dark lumen MRI mouse studies, 2) the feasibility of dark lumen MRI to serially assess polyp growth, and 3) the feasibility of dark lumen DCE-MRI to characterize the physiology of colon polyps and the colon wall.

MATERIALS AND METHODS

In Vivo Murine Model Preparation

Thirteen C57BL/6 mice (10 $Apc^{Min/+}$ and 3 controls [$Apc^{+/+}$]), between 20-24 g, were studied using dark lumen MR colonography. To enhance colonic polyp growth, per oral dextran sodium sulfate (DSS), which has been shown to induce colonic mucosal inflammation and increase the frequency of colonic polyp occurrence, was administered to six $Apc^{Min/+}$ mice (24,25). For comparison, three $Apc^{+/+}$ mice were also treated with DSS. Mice were treated with DSS according to Cooper et al. (15) except that treatment for 4×24 hr with 4% (w/v) DSS in the drinking water was initiated at 70 days after birth followed by 17 days on regular drinking water and finally 3×24 hr on 4% DSS in drinking water. Mice were monitored throughout for rectal bleeding and weight loss or other signs of serious illness and MRI was begun 5 to 7 days following the second period of DSS treatment (100 days old).

Prior to imaging, animals were anesthetized using 2%/98% inhaled isoflurane/oxygen and were administered three successive warm saline enemas to clear the colon of solid fecal material. Two milliliters of perfluorinated oil (Fomblin Y LVAC 06/6 perfluoropolyether oil [average MW = 1800], Sigma-Aldrich, St. Louis, MO) was introduced into the colon via a rectal catheter consisting of microbore tubing (Tygon Tubing, Lima, OH). The tubing was then removed and the anus ligated using a 6-0 Prolene suture (Ethicon, Somerville, NJ). At the completion of imaging the suture was removed and the perfluorinated oil allowed to pass. All animal procedures adhered to our Institutional Animal Care and Use Committee standards and were approved by this committee.

Ex Vivo Colon Preparation

Mice were euthanized according to procedures approved by our Institutional Animal Care and Use Committee. Through a midline incision the colon was resected from the level of the cecum

to the anus. Colons were rinsed until clear by gentle intraluminal injection of phosphate-buffered saline (PBS). The proximal end of the colon was ligated and 2-3 mL of perfluorinated oil was instilled via the distal end, which was later ligated following full distention of the colon. The specimen was fixed overnight in 10% buffered formalin and transferred to 70% ethanol for storage.

Magnetic Resonance Imaging

The in vivo and ex vivo MRI scans were performed on a Varian 7T scanner equipped with a 38 mm quadrature birdcage coil. A high-resolution gradient echo sequence (TR/TE/ α = 1000/3 ms/20°, 30 slices, slice thickness = 0.5 mm, field of view [FOV] = 60 mm × 40 mm, matrix = 256 × 256, NEX = 4, scan time = 17 min) was performed to identify polyps and measure their volume. In addition to the 2D scan used in all mice, 3D gradient echo images (TR/TE/ α = 15/3 ms/20°, NEX = 4, FOV = 60 mm × 40 mm × 40 mm, matrix = 256 × 128 × 128, scan time = 16 min) were acquired in one of the non-DSS-treated *Min* mice for comparison. For all mice scanned with the DCE-MRI protocol, the high-resolution 2D sequence was used to identify five slices that contained polyps. A variable flip angle spoiled gradient echo approach (TR/TE = 200/4.85 ms, flip angles = 12°, 24°, 36°, 48°, 60°, FOV = 60 mm × 40 mm, matrix = 128 × 128, NEX = 4, 5 slices, slice thickness = 0.5 mm) was employed to produce precontrast 2D T_1 -maps. The DCE-MRI protocol used a similar 2D gradient echo sequence (TR/TE/ α = 200/4.85 ms/36°, NEX = 2, temporal resolution = 51.2 sec) to obtain 35 serial images. The DCE-MRI protocol was performed on one untreated and three DSS-treated *Min* mice and on two untreated *Apc^{+/+}* control mice. Five images were acquired before a bolus of 0.2 mmol/kg Gd-DTPA was injected over a 30-sec period via a jugular catheter. The temperature of the animal was maintained at 37°C by circulating warm air through the bore of the magnet. The animal's respiratory rate was monitored throughout the experiment and maintained at a rate of 20-40 breaths per minute.

In five of the *Min* mice (four DSS-treated, one untreated) polyp growth was evaluated over time using the high-resolution gradient echo scan. In initial studies, DSS-treated mice ($n = 2$) were scanned twice but after observing that the mice recovered well from the surgical preparation subsequent studies included two additional imaging scans ($n = 2$). All repeat imaging studies were separated by 7 days. The untreated *Min* mouse was scanned twice over a month period. Growth rates of polyps were computed as the ratio of the volume change between imaging sessions and the number of days between them. For the in vivo and ex vivo studies polyp volume was measured by manually drawing a region of interest around the polyp in the high-resolution 2D gradient echo images.

DCE-MRI Data Analysis

To minimize the effects of peristalsis, the dynamic images were registered to the first precontrast image using a rigid registration technique preferentially weighted to each polyp. The multiframe angle data were fit to the following equation to construct precontrast T_1 maps (T_{10}):

$$S = S_0 \left[\sin \alpha (1 - \exp(-TR/T_{10})) / (1 - \exp(-TR/T_{10}) \cdot \cos \alpha) \right], \quad [1]$$

where α is the flip angle, S_0 is a constant describing the scanner gain and proton density, and it is assumed that $TE \ll T_2^*$. The T_{10} map was then used to estimate R_1 ($R_1 \equiv 1/T_1$) time courses for each voxel from the raw signal intensity time courses (26). The reference region (RR) model was used to describe the contrast agent pharmacokinetics. Details of the RR theory are described elsewhere (27). Briefly, a tissue of interest (TOI) R_1 time course, R_1^{TOI} , can be modeled as the following:

$$R_1^{TOI}(T) = R \cdot (R_1^{RR}(T) - R_1^{RR}) + R \cdot \left[\left(K^{trans,RR} / v_{e,RR} \right) - \left(K^{trans,TOI} / v_{e,TOI} \right) \right] \cdot \int_0^T (R_1^{RR}(t) - R_1^{RR}) \cdot \exp \left(\left(-K^{trans,TOI} / v_{e,TOI} \right) \cdot (T - t) \right) dt + R_1^{TOI},$$

[2]

where R_1^{RR} and R_1^{TOI} are the longitudinal relaxation rate constants in the RR and TOI, respectively; $K^{trans,RR}$ and $K^{trans,TOI}$ are the contrast agent extravasation rate constants for the RR and the TOI, respectively; $v_{e,RR}$ and $v_{e,TOI}$ are the extravascular extracellular space (EES) volume fractions for the RR and the TOI, respectively; and $R \equiv K^{trans,TOI} / K^{trans,RR}$. Equation [2] was used with a nonlinear least squares curve fitting routine to extract $K^{trans,TOI}$ and $v_{e,TOI}$ if R_1^{TOI} and R_1^{RR} can be measured. For region of interest (ROI) analysis, a TOI time course was generated by selecting tumor voxels with obvious signal enhancement (i.e., no necrotic tissue was included in the TOI). The RR was selected from an ROI placed in the leg muscle and contained a similar number of voxels as the TOI. The RR and TOI curves were then input into curve-fitting software (28) to extract the pharmacokinetic parameters described in Eq [2]. The parameters $K^{trans,TOI}$, $K^{trans,RR}$, and $v_{e,TOI}$ were allowed to vary while $v_{e,RR}$ was set to 0.08, which is reasonable for muscle tissue (29). For the voxel-by-voxel analysis $K^{trans,RR}$ was fixed at the value returned from the ROI analysis and a two-parameter fit was used to extract $K^{trans,TOI}$ and $v_{e,TOI}$ (30).

RESULTS

Figure 1 shows example high-resolution 3D (Fig. 1a,b) and 2D (Fig. 1c,d) gradient echo images of a control mouse colon filled with saline (Fig. 1a) and non-DSS treated (Fig. 1c) and DSS-treated (Fig. 1b,d) *Min* mice colons filled with perfluorinated oil. By contrast with images obtained from a mouse with a saline-filled colon, distending the mouse colon with a perfluorinated oil, either ex vivo (data not shown) or in vivo, virtually eliminated the lumen signal, which enabled the identification of all portions of the rectum and colon from the anus to the cecum. Numerous polyps are easily identifiable in the imaging datasets acquired from *Min* mice. The three enemas performed prior to the imaging studies were effective at completely cleansing the colon of feces. Thus, practically all visualized protrusions into the colon could be classified as polyps even prior to contrast agent enhancement validation. To demonstrate the feasibility of performing typical imaging-based colonography, we also acquired 3D gradient echo images in one of the non-DSS treated *Min* mice as illustrated in Fig. 2, which shows a polyp detected on axial (Fig. 2a), coronal (Fig. 2b), and sagittal (Fig. 2c) views of the colon. Although the scan times for the 2D and 3D acquisitions were ≈ 17 min, motion artifacts due to peristalsis were only observed in a few ($n = 3$) of the mice. Peristalsis artifacts were more of an issue when the colon was not completely distended with oil. Respiratory gating was not used for these studies, which resulted in some image degradation in the most cephalad portions of the colon.

To determine the inherent limits of detecting polyps with this approach we compared the in vivo and ex vivo imaging-derived measures of polyp volume in five *Min* mice. As shown in Fig. 3, excised colon tissue suspended in perfluorinated oil provided MR images suitable for virtual endoscopic rendering and easy detection of colonic polyps. We were unable to directly compare the in vivo and ex vivo volume of each respective polyp due to the tortuosity of the in vivo colon and the lack of complete 3D information. Instead, the number of detected polyps and the tumor burden or the sum of the detected polyp volumes for all mice was compared as

shown in Table 1. The in vivo tumor burden was found to be 14.8% higher than that measured in the ex vivo colons. In three of the five mice the number of polyps detected on the in vivo and ex vivo images were in agreement. In the other two mice the number of polyps detected on the in vivo images was less than that observed on the ex vivo images. In these two mice several polyps were in very close proximity to one another, making the boundaries between them ambiguous on the in vivo images.

In the 13 mice used in this study we identified 94 polyps, including those imaged in mice scanned at multiple timepoints. A histogram of the polyp sizes (as measured by their largest dimension) detected by dark lumen MRI colonography is shown in Fig. 4. The largest dimension of the smallest polyp detected on the high-resolution 2D gradient echo images was 0.94 mm ($n = 7$), which is the equivalent to the length of 4 voxels. Polyps 1.5 mm or greater were readily discerned within the colon. Polyps less than 1.5 mm in longest dimension were more difficult to identify on the 2D images (slice thickness = 0.5 mm) in regions where the colon folds or turns. Polyps were most reliably detected in the relatively straight portion of the colon extending from the rectum to the splenic flexure, a distance that ranged up to ≈ 3 cm.

In the five mice used for the polyp growth study, 33 polyps were detected for which the volumes of 21 polyps were determined at two or more timepoints. In the three mice scanned twice, all the polyps were identified at both timepoints. All the polyps in one of the two mice scanned four times were discernable at each timepoint, while in the other the polyps were detected either two or three times in the experiment. Polyps that were undetected at each of the four timepoints were located in cephalic regions of the colon where the inherent tortuosity of the colon and respiratory artifacts made it more difficult to reliably identify polyps. Figure 5 shows representative images of a DSS-treated *Min* mouse colon that were acquired 7 days apart. Three polyps that showed a marked increase in tumor volume are delineated by the white rectangle. Polyp growth curves for four polyps selected from two DSS-treated *Min* mice are shown in Fig. 6. The absolute polyp growth rates (mm^3/day) were heterogeneous both within a single mouse and among the mice, as illustrated in the plot of growth rates versus initial polyp volume (Fig. 7a). Polyps that presented with an initial larger volume on the MRI scan generally grew at higher rates with an average growth rate, calculated from the slope (0.05) of the growth rate plot, corresponding to an average of $\approx 5\%$ increase in the volume per day of individual polyps. When expressed as a percent volume change, the growth rates were found to generally be less than 10% per day, distributing around $\approx 5\%$ per day, with a few showing much faster growth (Fig. 7b). While polyp volume was found to always increase over time the growth rates of individual polyps were temporally variable. Most polyps with a high initial growth rate ($>1.4 \text{ mm}^3/\text{day}$ between days 12 and 19 post-DSS treatment) were found to grow more slowly at later timepoints ($<1 \text{ mm}^3/\text{day}$ between days 19 and 28 post-DSS treatment). Polyps with a lower initial growth rate were typically found to grow at a relatively constant rate.

The DCE-MRI protocol was successfully implemented in six mice producing time course data suitable for tracer kinetic analysis in the colon wall of all mice and in 10 of the 18 polyps identified. Again, the relatively straight portion of the colon extending from the rectum to the splenic flexure provided time series data that were most suitable for analysis by model fitting. Intermittent peristaltic events that occurred in this region of the colon lasted between 5-10 min often resulted in displacement of a polyp (or polyps) from its original position (or positions) in the unregistered images. However, the rigid registration algorithm preferentially weighted to the polyp ROI yielded time series suitable for DCE-MRI analysis. Figure 8 illustrates a typical $R_1(t)$ time curve taken from a non-DSS *Min* mouse polyp region of interest that has been corrected for motion, the reference region and the results of the RR model fit to the polyp data. Table 2 summarizes the K^{trans} and v_e parameters computed using the RR model. The $K^{trans,RR}$ values for skeletal muscle (also shown in Table 2) were comparable to reported values in mice (29,31,32). The K^{trans} values in the colon wall of DSS-treated $Apc^{+/+}$ mice were found

to be similar to that typically measured in muscle while the DSS-treated colon wall v_e values were generally elevated, 2.8 times greater than that found in skeletal muscle (26). As with the growth rate results, the DCE-MRI parameters in the polyps were found to vary both within individual mice and across the group of mice. In this small cohort of animals the polyp and DSS-treated colon wall K^{trans} values were similar to one another, while the polyp v_e values appeared to be somewhat lower than those measured in the DSS-treated colon wall.

DISCUSSION

We have shown that dark lumen DCE-MRI can be used to assess anatomical and functional properties of colon tissue in mouse models of gastrointestinal (GI) disease when perfluorinated oil is used as a colon-filling agent. Specifically, we have demonstrated that this MRI approach enables 1) the assessment of morphological and anatomical features of the colon from the anus to the cecum, 2) the consistent detection of colonic polyps as small as 1 mm, 3) the evaluation of individual polyp growth rates, and 4) for the first time, as far as we are aware, the derivation of the contrast kinetic parameters K^{trans} and v_e for the colon wall and colonic polyps. To the best of our knowledge this represents the first application of DCE-MRI to the characterization of contrast kinetics in mouse colon polyps.

Immersing excised tissue samples into perfluorinated oil is a commonly employed method to improve ex vivo imaging by removing extraneous MRI signals and reducing susceptibility effects near air-tissue interfaces (33-35). These same features have been exploited for high-resolution in situ imaging of the pulmonary airways and parenchymal and vascular structures in the mouse lung (36) and for the detection of small tumors (1 mm in diameter) in mouse models of lung cancer (37). To our knowledge, perfluorinated oil has not been previously evaluated as a colon background or distending agent for imaging applications. Although the approach used in this study required administration of enemas and anal suturing, no adverse side effects were observed throughout the duration of the experiments in any of the mice. Perfluorinated oil was chosen as a safe alternative that provided superior image quality. Furthermore, given that perfluorinated oil is immiscible in water it can be maintained in the colon for long periods of time, presumably without changing the colon tissue osmolality.

As a colon-filling agent for dark lumen MRI, perfluorinated oil provides several advantages over currently utilized methods for imaging the murine colon. Alternative approaches such as enemas containing only water or feces containing barium sulfate (fecal tagging) only partially reduce the MRI signal from the colon lumen, whereas a perfluorinated oil-based enema can eliminate this signal, thereby enhancing the contrast between the colon lumen and wall. Although there can be issues with patient compliance, MR dark lumen colonography that uses cleansing and distention of the lumen has been shown to produce a more sensitive approach for the detection of polyps as compared to fecal tagging. The former has been shown to reliably detect polyps as small as 5 mm in patients, whereas the latter is limited to polyps 8 mm or greater (21,38). In both humans and animals alike, distention of the colon prior to imaging improves the visualization of the tortuous colonic anatomy as compared to fecal tagging. In humans the use of barium for fecal tagging has resulted in poor patient compliance due to the side effects of abdominal pain and constipation (39). In addition to enhancing the colon contrast and anatomic delineation in the mouse studies reported here, cleansing the colon, distending it with perfluorinated oil, and suturing the anus for the duration of the imaging experiment are optimal methods for assuring high-quality MRI images.

The technical advances presented here should translate into an improved characterization of mouse colonic pathology. For example, even without utilizing 3D imaging methods polyps as small as 1 mm were readily detected. All of the polyps identified in the excised colon tissue of DSS-treated mice were found to be 1 mm or greater in size. In conjunction with 3D imaging

sequences, smaller polyps might have been detected if the imaging studies had been implemented either sooner after initiating DSS-treatment or in younger animals that were not DSS-treated. By enabling a fly-through visualization of the colon, a 3D sequence might also improve the detection of polyps in serial imaging studies, especially in the cephalad portions of the colon where folds made it difficult to repeatedly identify unique polyps in the 2D images. With regard to the polyp growth rates measured with this approach, we found that the growth rates for mice undergoing identical DSS-treatment regimes were markedly heterogeneous. It is not clear from the data whether either the growth rates or the number of developing polyps varied between anatomical regions of the colon, issues that might be resolved by collecting larger image sets such as with the 3D MRI scanning sequence. If such a spatial dependency exists it would be of some interest, as it might reflect differences in the local tissue microenvironment for tumor growth. The observed heterogeneity also depends on the precision of the proposed approach. Further studies utilizing multiple concurrent measures of the same polyp on repeated imaging sessions are needed to determine the precision and reproducibility of the proposed method.

On average, the in vivo and ex vivo tumor burden measurements were very similar. Given that $\approx 10\%$ of the distal end of the colon is lost in the ex vivo preparation the similarity between these tumor burden measurements indicates that the current in vivo approach is capable of detecting the majority of colonic polyps. However, for two of the mice (#3 and #5) there was a marked discrepancy between the in vivo and ex vivo tumor burden measurements. A number of factors likely contributed to this difference. Tumor burden could be reasonably underestimated if motion artifacts from the intrinsic peristalsis of the colon wall prohibited the resolution of small polyps (<0.5 mm), especially those in the cephalad regions of the colon. In the one case where tumor burden was substantially overestimated (mouse #5) the abnormal morphology (e.g., nonspherical) of several larger polyps (>3 mm) yielded ambiguous boundaries between the tumor and normal-appearing tissue on the in vivo images. In general, it is also possible that some structures were incorrectly identified as polyps (false-positives), which resulted in the overestimation of tumor burden. The use of 3D imaging would have likely enabled the quantification of sensitivity and specificity for the new approach since polyps detected on in vivo and ex vivo images could have been individually compared.

In addition to an improved anatomical dark lumen approach we have demonstrated that this procedure can be used in conjunction with DCE-MRI. This combination could aid in the assessment of drug efficacy in preclinical models of colon cancer as an increasing number of treatment options are becoming available that target the tumor vasculature. Surprisingly, peristalsis-induced artifacts rarely rendered the DCE-MRI datasets unsuitable for analysis. In general, the duration and frequency of peristaltic events was sufficiently low to enable high-resolution imaging scans (duration ≈ 15 min) and dynamic imaging for DCE-MRI. However, these longer duration studies could potentially benefit from the use of spasmolytic agents, respiratory gating, and nonrigid registration algorithms, which may also aid in visualizing the upper GI tract.

The DCE-MRI derived parameter, K^{trans} , measured in colonic polyps was substantially lower than the values reported in a subcutaneous murine MC-38 colon carcinoma model (mean $K^{trans} = 0.66 \text{ sec}^{-1}$) (40), possibly reflecting the differences between in situ and implanted tumor models. The v_e values reported for the MC-38 colon model (mean $v_e = 0.17$) were similar to those found in this study. Both parameters were found to have a large range of standard deviation within and across polyps, suggesting that the physiology of these tumors is at least as heterogeneous as that observed in other types of tumors. In the small number of DSS-treated mice included in this study, the polyp v_e values tended to be substantially lower than those measured in the normal-appearing colon wall, suggestive of an increased cellular density within the polyp. In a similar experimental mouse colitis model, Larsson et al. (22) have previously

reported DSS-dependent MRI signal increases when using a T_1 -weighted sequence, which is indicative of tissue edema. A limited set of quantitative T_1 maps generated as part of these studies (not shown) also found a trend toward higher T_1 relaxation times in DSS-treated animals as compared to controls. Similarly, the v_e values measured in the colon wall of DSS-treated mice was relatively high compared to previously reported values in normal tissue types, such as that found in muscle, and is possibly indicative of local tissue edema. Further studies will be required to determine whether these values are significantly different from those found in non-DSS-treated control animals. Using perfluorinated oil to distend the colon should improve the characterization of the colon wall on 3D images for most regions of the colon and in combination with T_1 and v_e measurements could provide an improved quantitative assessment of colonic inflammation.

The quantitative functional colonography approach described herein could potentially provide new opportunities for the assessment of GI disease physiology and treatment response in mouse models in addition to improving upon existing high-resolution anatomical mouse colon imaging methods. With targeted MRI-detectable contrast agents, this approach could become a practical alternative to PET or SPECT-based methods for high-resolution molecular imaging of murine colonic polyps. Similarly, cellular infiltration into the colon, such as occurs within the inflamed colon wall, could be evaluated by merging existing MRI-based cell labeling methods with the proposed dark lumen MRI scan. These techniques could be useful for elucidating the underlying mechanisms driving disease development and progression, measuring the efficacy of novel drugs targeted to GI disease, and provide the preclinical development and validation studies necessary for the clinical translation of these advanced noninvasive GI imaging methods.

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FIG. 1.

High-resolution 3D (**a,b**) and 2D (**c,d**) coronal images of a control mouse (**a**) and DSS-treated (**b,d**) and non-DSS-treated (**c**) *Min* mice whose bowels have been cleansed with a saline solution and distended using saline (**a**) or perfluorinated oil (**b-d**). Using perfluorinated oil as the distending agent virtually eliminated the lumen signal and enabled the identification of all parts of the rectum and colon from the anus to the cecum. Arrows indicate the location of polyps.

FIG. 2. Axial (a), coronal (b), and sagittal (c) dark-lumen MRI images of DSS-treated *Min* mouse colon acquired using a 3D signal acquisition. Circles indicate the location of a polyp visible on each orientation.

FIG. 3.
The high contrast between the bright colon wall and the dark lumen enabled a high-resolution virtual endoscopic rendering of excised colon suspended in perfluorinated oil.

FIG. 4. Histogram of the polyp sizes (as measured by the largest dimension) detected by dark lumen MRI colonography (number of polyps = 94). The largest dimension of the smallest polyp detected was 0.94 mm.

FIG. 5. Dark lumen MRI images of a DSS-treated Min mouse taken 7 days apart. The white squares denote the location of three polyps that were detected at both timepoints. Such images were used to measure polyp volume at each timepoint and to determine polyp growth rates.

FIG. 6. Dark-lumen MRI-derived measures of polyp growth for four polyps selected from two DSS-treated *Min* mice. These curves illustrate the heterogeneity of growth rates found in the 21 polyps tracked over time.

FIG. 7.

Absolute polyp growth rates versus the initial polyp volume (a) and histogram of growth rates expressed as percent volume change(b) as determined from successive dark lumen MRI imaging studies of DSS-treated mice. These are the rates from all the acquired data ($n = 32$) and include rates for polyps that were measured at multiple intervals (e.g., between days 7 and 14 and between days 14 and 21). Linear regression analysis (excluding the two outliers [growth rate ≈ 1.4 mm³/day]): slope = 0.05/day, r-value = 0.89.

FIG. 8.

Typical $R_1(t)$ time curve taken from a polyp region of interest and the muscle reference region (RR) in an untreated mouse. The solid line represents the results of the RR model fit to the polyp data.

Table 1
Comparison of Colon Tumor Burden as Determined Using the In Vivo and Ex Vivo Dark Lumen Imaging Methods

Mouse #	In vivo (# of polyps)	Ex vivo (# of polyps)	In vivo (mm ³)	Ex vivo (mm ³)
1	3	3	18.2	13.6
2	8	13	46.5	41.3
3	8	8	92.3	130.8
4	6	6	57.5	41.2
5	7	9	111	56.6
Pooled	32	39	65.1 ± 36.9	56.7 ± 44.2

Table 2

Summary of the DCE-MRI Region of Interest Analysis Performed on Polyps and DSS-Treated Colon Wall

Polyp #	K^{trans} (min^{-1})	v_e
1 ^a	0.14 ± 0.09	0.16 ± 0.06
2 ^a	0.14 ± 0.07	0.20 ± 0.06
3	0.06 ± 0.02	0.10 ± 0.03
4	0.05 ± 0.04	0.18 ± 0.11
5	0.06 ± 0.05	0.18 ± 0.11
6	0.15 ± 0.28	0.17 ± 0.14
7	0.05 ± 0.05	0.11 ± 0.05
8	0.07 ± 0.05	0.15 ± 0.11
9	0.11 ± 0.16	0.15 ± 0.04
10	0.06 ± 0.05	0.18 ± 0.11
Pooled ^b	0.09 ± 0.04	0.16 ± 0.03
Colon Wall ^c	0.10 ± 0.03	0.23 ± 0.09
Muscle ^d	0.09 ± 0.03	N/A

^a Polyps located in non-DSS-treated mice.^b Computed from the 10 polyps identified in four mice.^c Average colon wall parameters from two DSS-treated Apc^{+/+} mice.^d Average $K^{trans,RR}$ from these six mice as determined using the Reference Region method.