Exploration and application of nanomedicine in atherosclerotic disease

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Three-dimensional dynamic contrast-enhanced MRI for the accurate, extensive quantification of microvascular permeability in atherosclerotic plaques

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Abstract

Atherosclerotic plaques that cause stroke and myocardial infarction are characterized by increased microvascular permeability and inflammation. Dynamic contrast enhanced (DCE) MRI has been proposed as a method to quantify vessel wall microvascular permeability in vivo. Until now, most DCE-MRI studies have been limited to 2 dimensional (2D), multi-slice imaging. While providing the high-spatial resolution required to image the arterial vessel wall, these approaches do not allow quantifying plaque permeability with extensive anatomical coverage, an essential feature when imaging heterogeneous diseases, such as atherosclerosis. To our knowledge, we present the first systematic evaluation of 3 dimensional (3D), high-resolution, DCE-MRI for the extensive quantification of plaque permeability along an entire vascular bed, with validation in atherosclerotic rabbits. We compare two acquisitions: 3D turbo field echo (TFE) with MSDE preparation (motion sensitized driven equilibrium), and 3D TSE (turbo spin echo). We find 3D TFE DCE-MRI to be superior to 3D TSE DCE-MRI in terms of temporal stability metrics. Both sequences showed good intra and inter-observer reliability, and significant correlation with ex vivo permeability measurements by Evans Blue near infrared fluorescence (NIRF). Additionally, we explore the feasibility of using compressed sensing to accelerate 3D DCE-MRI of atherosclerosis, to improve its temporal resolution and therefore the accuracy of permeability quantification. Using retrospective under-sampling and reconstructions we show that compressed sensing alone may allow accelerating 3D DCE-MRI up to 4 folds. We anticipate that the development of high spatial resolution 3D DCE-MRI with prospective compressed sensing acceleration may allow for the more accurate and extensive quantification of atherosclerotic plaque permeability along an entire vascular bed. We foresee that this approach may allow for the comprehensive and accurate evaluation of plaque permeability in patients, and may be a useful tool to assess therapeutic response to approved and novel drugs for cardiovascular disease.
Introduction

Atherosclerotic plaques at high risk of causing severe clinical events, such as stroke or myocardial infarction, are characterized by increased microvascularization and endothelial permeability, associated with active vessel wall inflammation. In the past 10 years, efforts to develop and validate non-invasive imaging techniques to quantify plaques microvascular permeability in both humans and animal models have intensified. Being able to quantify plaque permeability may not only help identifying high-risk lesions and/or patients, but may also allow us to i) follow plaque progression in longitudinal studies, ii) evaluate the efficacy of approved and novel treatments, and iii) identify candidate patients that may benefit from specific treatments. Dynamic contrast enhanced (DCE) MRI is a non-invasive imaging method originally developed to quantify tumors' vascularity and permeability. This technique involves the rapid acquisition of T1-weighted MR images before and during the injection of a gadolinium (Gd) based contrast agent. Signal enhancement curves in the tissue of interest are extracted from the MR acquisition and processed using kinetic models to derive quantitative estimates of fractional microvascular volume ($v_p$) and permeability ($K^\text{trans}$). Moreover, non-model based parameters such as area under the curve (AUC), upslope, time to peak, or peak concentration can also be calculated as surrogates of microvascular volume or permeability. DCE-MRI has been employed in a variety of studies to quantify microvascularity and permeability in atherosclerotic plaques. A correlation between the fractional microvascular volume, $v_p$, by DCE-MRI and plaque neovessels in human carotid specimens has been demonstrated. Moreover, a relationship between $v_p$, $K^\text{trans}$ (permeability) and plaque macrophages, neovessels and loose matrix, as well as risk factors for atherosclerosis (such as smoking, and elevated C reactive protein) was found. In atherosclerotic rabbits, the non-model based parameter AUC was found to correlate with aortic plaque neovascularization. In the same animal model, AUC was able to capture therapeutically induced changes in plaque microvasculature/ permeability. Despite these successful results, current DCE-MRI protocols do not allow for the accurate and extensive quantification of microvascular permeability in vulnerable plaques. Imaging the arterial wall requires high in-plane spatial resolution (0.5-0.7 mm) to capture the complex structure of plaques to minimize partial volume artifacts and to provide good vessel wall/ lumen delineation. Adequate anatomical coverage is also necessary, to map this heterogeneous disease along a whole vessel. Both these requirements make it difficult to acquire DCE-MRI with fast time resolution, which is necessary to capture contrast agent kinetics in the blood plasma (the so called arterial input function, AIF), and its extravasation in atherosclerotic plaques. Since it is challenging to satisfy all these requirements simultaneously, almost all vascular DCE-MRI studies have focused on the use of high spatial resolution, 2D, single or multi-slice acquisitions, thereby sacrificing temporal resolution and anatomical coverage. A goal is to improve the quantification of plaque microvasculature and permeability by using 3 dimensional (3D) DCE-MRI with high spatial resolution and extensive anatomical coverage. In this study we compare two different approaches (3D TFE, turbo field echo, and TSE, turbo spin echo) for 3D DCE-MRI. To allow for accurate validation we use a pre-clinical rabbit model of aortic atherosclerosis, where in vivo permeability imaging with 3D DCE-MRI is compared with ex vivo Evans Blue (EB) near infrared fluorescence (NIRF) measurements of endothelial permeability, used as gold standard. Moreover, we investigate the feasibility of using compressed sensing to accelerate 3D DCE-MRI of atherosclerosis, with the aim to improve the time resolution of 3D DCE-MRI and the quantification of microvascular permeability in atherosclerotic plaques.

Methods

Animal model. Atherosclerosis was induced in 4 New Zealand White (NZW) rabbits by a combination of a high cholesterol diet (4.7% palm oil and 0.3%, weeks 1 through 8, and 0.15%, weeks 8 through 16, cholesterol; Research Diet Inc., New Brunswick, NJ) and double balloon injury of the abdominal aorta. Three additional NZW rabbits were kept on chow diet and used as non-atherosclerotic controls. Experiments were conducted 6 months after diet initiation. All animal experiments were approved by the Icahn School of Medicine Institutional Animal Care and Use Committee (IACUC).

MR acquisition. Animals were imaged 3 times with DCE-MRI on a 3T clinical MR scanner (Philips Achieva), using a commercial knee coil (Philips 3T 8 channel) for signal reception. The average time in between sessions was 4.8 days.
The order of the 3 imaging sessions was randomized for each rabbit. In all sessions, localizers and time-of-flight (TOF) non-contrast enhanced angiography were performed, to identify anatomical landmarks, such as the renal arteries and the iliac bifurcation. DCE-MRI was performed following these sequences. After the acquisition of three pre-contrast DCE-MRI frames, 0.2 mmol/kg of Gd-DTPA (gadopentetate dimeglumine, Magnevist, Bayer Schering Pharma) was injected with a power injector, at the rate of 1 ml/s, followed by a 15 ml saline chase. During each imaging session either one of the following sequences was used for DCE-MRI: 1) 3D T1W turbo field echo (TFE) with motion sensitized driven equilibrium (MSDE) for blood suppression; 2) 3D T1W turbo spin echo (TSE); 3) previously validated 2D double inversion recovery (DIR) T1W TSE, used as a gold standard. 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curves derived from ROIs traced in the same axial slices showed in Fig. 1C-E, from all acquisitions, as a representative example of the signal and noise expected in the uptake curves. For each curve, the gray vertical bar indicates frames 6 and 8, between which tSNR and tCNR were calculated. To facilitate comparisons between different sequences, tSNR and tCNR were normalized by slice thickness and by the square root of the acquisition time per frame of each sequence (Table 1), as previously validated.\(^\text{[5]}\)

**Quantification of in vivo permeability.** ROI curves were converted to contrast agent concentration assuming linearity with MR signal as previously validated\(^\text{[6,41]}\):

\[
C^\text{VW}(T) = \frac{C^\text{VW}_\text{US}}{C^\text{VW}_\text{FO}} T^{p(T)} \left( \frac{C^\text{VW}_\text{US}}{C^\text{VW}_\text{FO}} \right)
\]

where \(C^\text{VW}(T)\) and \(C(T)\) are respectively the contrast agent concentration and signal intensity in the vessel wall at time \(T\), \(C^\text{VW}_\text{US}\) and \(C^\text{VW}_\text{FO}\) are respectively the average signal intensity in the vessel wall and in muscle in the first 3 frames (pre-contrast), is the pre-contrast T1 relaxation time of the vessel wall, and \(r1\) is the longitudinal relaxivity of the contrast agent. Pre-contrast T1 was taken to be 1.5s,\(^\text{[42]}\) while \(r1\) was taken to be 4.3 (mM*s)\(^\text{[1,43]}\). Additional details about the method used to convert MR signal to contrast agent concentration can be found in the Supplemental Materials section.

Normalization to skeletal muscle as a reference tissue was chosen based on its similar pre-contrast T1 to the vessel wall.\(^\text{[42]}\) After conversion to concentration, the area under the concentration versus time curve (AUC) was calculated to quantify the extravasation of contrast agent in the vessel wall, in the first 2 minutes after injection.\(^\text{[4,12]}\) AUC is a non-model based parameter and a validated measure of plaque microvasculature/permeability in rabbits.\(^\text{[4,9,12,23]}\)

Furthermore, ROI curves were analyzed to calculate kinetic parameters according to the following modified Tofts-Kermode model,\(^\text{[3]}\) under Patlak assumption,\(^\text{[3,44]}\) as previously validated\(^\text{[10,21,22]}\):

\[
[C(0) - C\text{plasm}]{T} = \frac{Vp}{[C(0) - C\text{plasm}]} {\int} C(t)\text{dt}
\]

where \(C^\text{VW}(T)\) and \(C(T)\) are respectively the contrast agent concentration in the vessel wall and in the blood plasma compartment at time \(T\), \(Vp\) is the fractional microvascular volume and \(K\text{trans}\) is a measure of the extravasation of contrast agent from the plasma to the tissue compartment (in this case indicating endothelial permeability). Fitting to the kinetic model was implemented using the function \(lsqnonneg\) (linear least squares with non-negativity constraints) in Matlab. The use of a black blood acquisition for DCE-MRI, precludes sampling of \(C(T)\) from the MR images themselves.\(^\text{[43]}\) Therefore, a population input curve derived from the literature was used.\(^\text{[45]}\) Figure S2 shows examples of ROI concentration curves from corresponding axial slices from all acquisitions. Dashed lines indicate experimental data, while continuous lines indicate data fitting to the kinetic model.

**Compressed sensing acceleration.** Based on its better performance in regards to temporal stability metrics, 3D TFE DCE-MRI data were used for this analysis. Retrospective reconstructions were performed by Fourier transforming magnitude images back into k-space data, which were under-sampled using a pseudo-random \(k\)-space pattern, in order to generate incoherent aliasing artifacts (Fig. S3). This approach does not take into account information deriving from phase, different elements of the receiving coils, as well as previous under-sampling during acquisition, but simply relies on the anatomical characteristics of the images and of the temporal enhancement to evaluate the application of compressed sensing. A sixth-order polynomial function, defined as \((1 - k)^d + d\) (with \(-1<k<1\), while \(d\) is a constant parameter), was used to generate a different variable-density random under-sampling pattern along \(k\)-space for each DCE-MRI frame. This pattern ensures a higher density at the center of \(k\)-space than the edges, and provides a better starting point for the iterative reconstruction algorithm than uniform random sampling, as previously described.\(^\text{[9-12,46-48]}\) Retrospectively under-sampled data were reconstructed using a previously validated \(k\)-SPACE-SENSE algorithm, using temporal principal component analysis (PCA) as the sparsifying transform\(^\text{[46-48]}\):

\[
\hat{m} = \text{argmin}_{m} \{\|Fm - y\|_2 + \lambda\|Pm\|_1\}
\]

where \(F\) is the under-sampled 3-dimensional spatial Fourier transform, \(m\) is the reconstructed image, \(y\) is the acquired k-space data from the scanner, \(P\) is the temporal principal component analysis (PCA)\(^\text{[46-48]}\), \(\|\cdot\|_2\) is the \(L_2\)-norm used to enforce sparsity, while \(\|\cdot\|_1\) is the \(L_1\)-norm used to enforce data consistency. \(\lambda\) (in this case taken to be 0.075) is a weighting parameter that controls the balance between sparsity in the temporal PCA domain (right hand term) and data consistency (left-hand term). Six different under-sampling rates (R= 2,4,6,8,10 and 15) were used for off-line reconstructions (Fig. S3). Compressed sensing reconstructions were evaluated using 2 metrics: 1) image quality, in terms of vessel wall/lumen delineation; 2) root mean square error between ROI signal curves derived from under-sampled and fully sampled reconstructions, normalized by the average signal intensity of the fully-sampled curve (N,RMSE). Vessel wall/lumen delineation was quantified as:

\[
D = \frac{\text{SI}_{\text{VW}}^t_{\text{US}} - \text{SI}_{\text{VW}}^t_{\text{US}}}{\text{SI}_{\text{VW}}^t_{\text{US}}}
\]

where \(\text{SI}_{\text{VW}}^t_{\text{US}}\) and \(\text{SI}_{\text{VW}}^t_{\text{US}}\) are respectively the average signal intensity of the vessel wall and lumen after contrast agent injection. This metric is expected to decrease with increasing acceleration, due to blurring of the vessel wall/lumen contours in the under-sampled reconstructions. N,RMSE for each curve was calculated as:

\[
\text{N,RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} \frac{(\text{SI}_{\text{US}} - \text{SI}_{\text{US}})^2}{\text{mean}(\text{SI}_{\text{US}})}}
\]

where \(\text{SI}_{\text{US}}\) and \(\text{SI}_{\text{US}}\) are respectively the signal intensity of the fully sampled and under-sampled curves, and \(N\) is the number of data points in each curve. N,RMSE is expected to increase with increasing acceleration.
**Statistical analysis.** For fully sampled data, $t$SNR and $t$CNR were compared among sequences using paired $t$-tests. For each rabbit, slice-by-slice AUC, $v_p$, and $K_{trans}$ were averaged in segments from the left renal to the aortic bifurcation, corresponding to the number of segments used for EB analysis. Parameters derived from DCE-MRI acquisitions and EB fluorescent radiant efficiency were correlated using Pearson’s correlation. A $p$ value less than 0.05 was considered significant. Intra- and inter-observer reliability (see Supplemental Materials section) was evaluated using intra-class correlation coefficients (ICCs) and Bland Altman analysis of DCE-MRI parameters in each segment. For under-sampled data, only analysis by observer 1 was taken into account. The highest under-sampled rate yielding both vessel wall/lumen delineation and average signal curves N_RMSE within 10% of the fully sampled scanner reconstruction was considered suitable for further investigation.

**Figure 1.** A and B, curved maximum intensity projection (MPR) of the abdominal aorta of one rabbit imaged using 3D TFE and TSE DCE-MRI, respectively. Orange lines indicate the left renal artery and the iliac bifurcation. C and D, one representative axial slice of the abdominal aorta of the same rabbit shown in A and B, imaged using 3D TFE and TSE DCE-MRI, respectively. E, one representative axial slice of the same rabbit, imaged using 2D TSE DIR. Red arrow indicates the abdominal aorta. F, time-intensity curves for an aortic ROI in the same axial slice showed in C-E for all 3 sequences. Blue line, 3D TFE DCE-MRI. Red line, 3D TSE DCE-MRI. Green line, 2D DIR TSE DCE-MRI. Gray bars indicate the data points from which temporal SNR and CNR were calculated. X axis, time (min). Y axis, signal intensity (a.u.).

**Results**

**Comparison between 3D TFE, 3D TSE and 2D DIR TSE DCE-MRI.** $t$SNR and $t$CNR normalized by slice thickness and temporal resolution were significantly higher in both 3D DCE-MRI sequences compared to 2D DIR TSE ($p<0.05$), with 3D TFE being significantly higher than 3D TSE ($p<0.05$, Fig. 2). This indicates an average higher temporal stability, and vessel wall enhancement (compared to the signal in the lumen) in ROI curves derived from 3D TFE DCE-MRI compared to 3D TSE and 2D DIR TSE DCE-MRI. Figure 2 shows temporal...
stability results derived from the tracings by observer 1. Tracings from the two observers and repeated tracings from observer 2 did not yield significantly different results from the ones described here ($p \geq 0.4$).

**Figure 2.** Average temporal signal-to-noise ratio ($t$SNR, panel A) and temporal contrast-to-noise ratio ($t$CNR, panel B) for 3D TFE (blue bar), 3D TSE (red bar) and 2D DIR (green bar) DCE-MRI. Black lines represent the standard deviation of the mean. Black stars indicate significant difference ($p<0.05$).

**Correlation between in vivo and ex vivo permeability.** AUC and $K_{\text{trans}}$ (permeability) from 3D TFE and TSE, and 2D DIR TSE DCE-MRI were significantly different between atherosclerotic and control animals ($p<0.05$, Fig. 3A, B). This difference was corroborated by ex vivo results, where microvascular permeability was quantified using Evans Blue NIRF. This measure was also significantly different between atherosclerotic and control rabbits ($p<0.05$, Fig. S4). The parameter $v_p$ (microvascular volume) was not significantly different between atherosclerotic and control rabbits for all acquisitions. Figure 4 shows representative AUC pixel-by-pixel maps for one representative atherosclerotic and one control rabbit. For 2D DIR, one axial slice is shown, while for 3D acquisitions 3D curved MPRs are represented. In addition, ex vivo EB NIRF for both rabbits is shown. Yellow lines in the 3D curved MPRs and EB NIRF indicate the left renal artery and the iliac bifurcation. In both AUC maps and EB images, hotter colors indicate higher permeability. The higher permeability in the EB NIRF of the atherosclerotic rabbit is mirrored in the “hotter” AUC maps for all acquisitions. On the contrary the control animal shows modest Evans Blue extravasation and very low AUC maps indicating low endothelial permeability. In atherosclerotic animals, AUC from 3D TFE ($R^2 = 0.204$), TSE ($R^2 = 0.150$) and 2D DIR ($R^2 = 0.286$) was significantly correlated with ex vivo permeability by EB NIRF ($p<0.05$, Fig. 5). For 3D TFE, $K_{\text{trans}}$ was also significantly correlated with EB uptake ($R^2 = 0.144$, $p<0.05$). No significant correlation was found between $v_p$ and EB uptake for all acquisitions.
Compressed sensing acceleration. Vessel wall/lumen delineation (quantified as difference in vessel wall and lumen signal, normalized by the vessel wall signal itself) was found to decrease with increasing acceleration, from 35.1% for the scanner reconstruction, to 28.3% for an under-sampling rate of 15. Figure 6A shows a reduction in this parameter with increasing acceleration rate. Correspondingly, increased blurring in the images can be found with increased under-sampling (Fig. 6B). The highest under-sampling rate with vessel wall/lumen delineation within 10% of the scanner reconstruction was R=8 (gray dashed line, Fig. 6A). The N_RMSE between fully sampled and under-sampled signal curves was instead found to increase with increasing acceleration, ranging from 9.2% for R=2, to 14.3% for R=15 (Fig. 7). Representative signal intensity curves reconstructed with increasing degrees of under-sampling show increasing difference with curves derived from the reference images (Fig. 7B). The higher acceleration rate with N_RMSE within 10% of the scanner reconstruction signal intensity curves was R=4 (gray dashed line, Fig. 7A). Based on these results we foresee that compressed sensing alone may allow accelerating 3D DCE-MRI up to four-fold.
Figure 4. Difference between permeability in atherosclerotic (A) and control (B) animals. Hotter colors in AUC maps and EB images indicated higher permeability. Yellow lines indicate the left renal artery and the iliac bifurcation. The higher ex vivo permeability of the atherosclerotic rabbit (EB) is mirrored in the “hotter” AUC maps. On the contrary the control animal shows poor Evans Blue uptake and very low AUC maps.

Figure 5. Correlations between in vivo permeability by DCE-MRI and ex vivo permeability by Evans Blue near infrared fluorescence. Panel A, 3D TFE DCE-MRI (blue). Panel B, 3D TSE DCE-MRI (red). Panel C, 2D DIR TSE DCE-MRI (green). Black dashed line, regression line.
Figure 6. A, vessel wall/lumen contrast is shown to decrease with increasing acceleration rate, indicating blurring and poorer wall/lumen delineation with increasing under-sampling. Red dashed line, vessel wall/lumen contrast within 5% of the fully sampled acquisition. Gray dashed line, vessel wall/lumen contrast within 10% of the fully sampled acquisition. Vertical black lines, standard deviation. B, representative images of the dynamic series (averaged in the time dimension) reconstructed with compressed sensing after different degrees of under-sampling. Vessel wall is bright after contrast agent injection. Images show increased blurring at high accelerations. S, fully sampled scanner reconstruction, used as the reference image. Based on data shown in panel A, reconstruction from data under-sampled eight-fold show vessel wall/lumen delineation within 10% of the reference images (S).

Figure 7. A, normalized root mean square error between curves derived from under-sampled and fully sampled (scanner) reconstructions. N_RMSE is shown to increase with increasing acceleration. Gray dashed line, N_RMSE within 10% of scanner reconstruction. Vertical black lines, standard deviation. B, representative signal intensity curves from acceleration 2, 4, 6, and 15. S, scanner reconstruction.
**Discussion**

To our knowledge, this study describes the first validation of 3D black blood DCE-MRI to quantify microvascular permeability in the abdominal aorta of atherosclerotic rabbits. Microvascular permeability, together with plaque inflammation, is one of the recognized hallmarks of vulnerable atherosclerotic plaques at high-risk for causing severe cardiovascular events.\(^1,2\)

Previous studies have employed 2-dimensional (2D), multi-slice DCE-MRI to quantify microvascular permeability in the vessel wall.\(^3,6,8,10,20,22,27,34\) These approaches have yielded high, in-plane spatial resolution, to accurately delineate the atherosclerotic wall. However, these strategies provided limited anatomical coverage (necessary to evaluate this heterogeneous disease along a whole vessel), and relatively slow time resolution (thereby potentially compromising the accuracy of permeability quantification). To extend anatomical coverage, several groups have already proposed the use of 3D, high-resolution, isotropic, black blood acquisitions to characterize atherosclerotic plaque burden and composition in humans. To our knowledge these techniques have never been applied compared in atherosclerotic rabbits for DCE-MRI of the arterial vessel wall. These previous studies have focused on two types of acquisitions: i) gradient echo based, with Motion Sensitized Driven Equilibrium (MSDE) for black blood imaging, so called 3D MERGE (MSDE prepared rapid gradient echo);\(^28,37\) ii) spin echo based, using 3D SPACE (Sampling Perfection with Application optimized Contrast using different flip angle Evolutions).\(^29\)

In this manuscript, we built upon these approaches and compared 3D turbo field echo (TFE) with MSDE preparation (equivalent to 3D MERGE) and 3D turbo spin echo (TSE, equivalent to 3D SPACE) between each other and with gold standard multi-slice, 2D double inversion recovery (DIR) turbo spin echo (TSE)\(^4\) for DCE-MRI of aortic atherosclerosis in rabbits. We find a positive, significant correlation between in vivo microvascular permeability calculated from both 3D and 2D DCE-MRI and permeability calculated from ex vivo NIRF with Evans Blue. For both 3D and 2D sequences, we evaluated temporal stability metrics,\(^39\) intra and inter-observer reproducibility\(^49\) (see Supplemental Materials) and correlation with ex vivo permeability.

Among 3D sequences, 3D TFE DCE-MRI showed superior tSNR and tCNR, while both 3D TFE and TSE DCE-MRI showed significant correlation with ex vivo NIRF and good intra and inter-observer reliability.

Despite the higher steady state signal with a flip angle of \(90^\circ\) and TR = 500 ms enjoyed by 3D TSE compared to values of \(20^\circ\) and 6.2 ms of 3D TFE, the signal for 3D TSE can be degraded by many factors. Predominantly, contamination of the T1 weighted signal by residual T2 weighting is more significant compared to that of the MSDE preparation and TFE readout. Furthermore, the variable refocusing flip angles train used in 3D TSE results in a lower flip angle at the center of k-space, which reduces the signal amplitude. Receiver bandwidth was also higher for 3D TSE (777 Hz/pixel) with respect to 3D TFE (360 Hz/pixel), therefore affecting the noise levels in the acquisition. Last but not least, the 3D TFE acquisition was acquired with 3 signal averages, while 3D TSE was averaged only once in the same frame duration. Take together, all these factors may have contributed to a lower signal enhancement and higher noise levels in the 3D TSE acquisition with respect to 3D TFE, thereby explaining our finding of lower temporal SNR and CNR for this sequence.

Contrarily to other studies\(^3\) we have not found a positive correlation between the DCE-MRI parameter \(v_p\) (microvascular volume) and ex vivo permeability. This may be attributed to difference in the subjects examined (human carotid plaques\(^3,21,23\) versus aorta of atherosclerotic rabbits)\(^4,5,9,10\), MR acquisition parameters and sequences (bright versus black blood, 2D versus 3D), different time resolutions (~15s versus ~30s) and direct AIF sampling versus use of a population AIF, or non model based analysis methods. While they do not allow for direct AIF sampling, two dimensional black blood acquisitions, have been successfully employed in the past to quantify vessel wall microvasculature and permeability in atherosclerotic rabbits, either using non model-based approaches, or kinetic modeling.\(^4,5,8,10,11,20,23,50\) Our group has recently corroborated these previous findings using AUC calculated from 3D black blood DCE-MRI in two additional independent studies.\(^12,50\) While these results are encouraging, to mitigate possible inaccuracies in the quantification of vessel wall permeability when using a population AIF, our group and others are investigating either hybrid acquisition approaches\(^8\) or relative...
modeling methods\textsuperscript{52} to directly derive the AIF from the DCE-MRI data. Last but not least, the ex vivo validation method used in our study (ex vivo Evans Blue NIRF), is different than immunohistochemistry based methods used conventionally in other studies, a factor that may also have impacted the strength of the correlation between in vivo and ex vivo metrics. Evans Blue (EB) is a dye that binds to albumin and extravasates in tissues with increased microvascular permeability. EB has been previously validated as a measure of endothelial permeability in atherosclerotic mice\textsuperscript{38} and rabbits.\textsuperscript{12,50} Better than immunohistochemistry, which is limited to few representative tissue samples, EB NIRF allows quantifying permeability along the whole rabbit abdominal aorta, thus closely matching the in vivo quantification of permeability by 3D DCE-MRI. However, EB has significantly higher molecular weight (66.5 kDa) than Gd-DTPA (0.547 kDa)\textsuperscript{53}, the contrast agent used for 3D DCE-MRI. This property affects the extravasation rates of the two molecules, with EB-albumin being slower with respect to Gd-DTPA, and requiring a highly permeable endothelium to extravasate, and may explain the fairly low correlation between ex vivo NIRF and 3D DCE-MRI. Ex vivo validation methods that more closely mimic the uptake of Gd-DTPA in the vessel wall, while maintaining the extensive anatomical coverage provided by NIRF are the subject of future investigations.

The second goal of our study was to explore the feasibility of using compressed sensing\textsuperscript{54} to accelerate and improve the time resolution of 3D DCE-MRI, for a more accurate quantification of plaque microvasculature and permeability in atherosclerotic plaques. Compressed sensing is a novel acceleration technique, that can be used alone or in combination with parallel imaging, to accelerate the acquisition of images that are “sparse” in a given transform domain.\textsuperscript{14} Using a previously validated compressed sensing algorithm, with temporal principal component analysis (PCA) as the sparsifying transform,\textsuperscript{46,47,48} we performed retrospective reconstructions using different acceleration rates (different levels of under-sampling).\textsuperscript{48} Other studies have investigated the feasibility of using compressed sensing to accelerate the acquisition of 3D black blood vessel wall imaging,\textsuperscript{28,55,56} although none of these studies involved DCE-MRI. These studies employed spatial sparsifying transforms, different from the temporal PCA used in this study. We foresee that a combination of spatial and temporal sparsifying transforms may help accelerating 3D DCE-MRI acquisitions even further. A limitation of the compressed sensing feasibility analysis in this work is the retrospective nature of the reconstructions and the lack of validation with prospective acquisitions. As mentioned in the methods section, retrospective reconstructions were performed by Fourier transforming magnitude data therefore not taking into account information deriving from phase and different elements of the receiving coils, as well as previous under-sampling with SENSE parallel imaging. Using this approach alone we find that acceleration rates up to 4 fold can be achieved without significantly compromising vessel wall/lumen contrast or the quality of the tissue enhancement curves (within 10% of the scanner reconstructions). We anticipate that developing a prospective under-sampled acquisition and reconstruction including information from multiple receiving channels, and employing a combination of compressed sensing and parallel imaging\textsuperscript{44} may yield even higher acceleration rates.

In conclusion, in this study we present the first systematic validation of 3D DCE-MRI to quantify microvascular permeability in the entire abdominal aorta of atherosclerotic rabbits. We compare two three dimensional sequences (3D TFE and 3D TSE DCE-MRI) among themselves and against 2D DIR TSE DCE-MRI, a previously validated acquisition. We find 3D TFE DCE-MRI to be superior to 3D TSE DCE-MRI in terms of temporal stability metrics. Both sequences showed good intra and inter-observer reliability, and significant correlation with ex vivo permeability measurements by Evans Blue NIRF. Furthermore, we perform retrospective reconstructions to explore the feasibility of improving 3D DCE-MRI of atherosclerosis by using compressed sensing acceleration. Our retrospective reconstructions suggest that compressed sensing alone may yield up to 4 fold acceleration rates. We anticipate that combining 3D DCE-MRI with prospective compressed acceleration, may allow for the improved quantification of both plaque microvascular volume ($v_p$) and permeability ($K_{trans}$) along an extensive anatomical region. This approach may allow for the improved evaluation of heterogeneous diseases (such as atherosclerosis) along an entire vessel. Furthermore, it may be useful as a tool for development of new anti-atherosclerotic compounds, whose impact on plaque permeability could be evaluated extensively and accurately in longitudinal in vivo studies.
References


Acknowledgements

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Supplementary information

Additional supporting information may be found in the online version of this article at the publisher’s website.
Supplementary figures

Table 1S. Intra-class correlation (ICC) coefficient for inter- and intra-observer variability. O1, observer 1. O2a, observer 2, first tracing. O2b, observer 2, second tracing.

<table>
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<th>Sequence</th>
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<th>r^2</th>
<th>R^2corr</th>
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<tr>
<td>3D TSE</td>
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<td>3D TSE</td>
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Figure 1S. Images showing different enhancement phases are showed for an atherosclerotic and a control rabbit for all 3 sequences. Numbers at the top left of each image indicated the dynamic frame displayed.

Figure 2S. Concentration versus time curves for all three acquisitions. Blue dashed and full lines, data and data fitting for 3D TFE DCE-MRI. Red dashed and full lines, data and data fitting for 3D TSE DCE-MRI. Green dashed and full lines, data and data fitting for 2D TSE DCE-MRI.

Figure 3S. Schematics of compressed sensing reconstructions. K-space of fully sampled dynamic data (S, left).
is under-sampled with different acceleration rates (2 to 15). White pixels in the schematic k-space matrix represent sampled data, while black pixels represent data that are not acquired. From the schematics it can be appreciated that with increasing under-sampling (i.e. increasing acceleration rate in a prospective acquisition), less data are acquired (the number of white pixels decreases).

**Figure 4S.** Difference in Evans Blue uptake between atherosclerotic (blue) and control (red) rabbits. Data are represented as radiant efficiency \([p/s/cm^2/sr]/(\mu W/cm^2)\). Data are multiplied by 10^9.

**Figure 5S.** Bland-Altman plots for inter-observer reliability (O1-O2a). X axis, average parameter value. Y axis, percentage difference. Panel A, 3D TFE DCE-MRI (blue). Panel B, 3D TSE DCE-MRI (red). Panel C, 2D DIR TSE DCE-MRI (green). Black line, average percentage difference. Gray lines, confidence intervals.

**Figure 6S.** Bland-Altman plots for intra-observer reliability (O2a-O2b). X axis, average parameter value. Y axis, percentage difference. Panel A, 3D TFE DCE-MRI (blue). Panel B, 3D TSE DCE-MRI (red). Panel C, 2D DIR TSE DCE-MRI (green). Black line, average percentage difference. Gray lines, confidence intervals.