

Molecular imaging of the atherosclerotic plaque using positron emission tomography

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ABSTRACT Accurately assessing an individual's risk of myocardial infarction or stroke using currently available risk stratification tools remains a challenge, even in patients with symptomatic disease. Inflammation, micro-calcification and intra-plaque angiogenesis occur during the development and ultimate rupture of vulnerable plaques. Molecular imaging techniques such as combined positron emission tomography and computed tomography (PET/CT) offer the opportunity to target these key cellular processes within atheroma and identify high-risk lesions. In this review we will set out the studies that have demonstrated the feasibility of PET/CT imaging in assessing atherosclerotic plaque inflammation, micro-calcification and angiogenesis. We will also discuss the potential of PET/CT molecular imaging as both a screening tool for novel therapeutic interventions and as a means of improving cardiovascular risk stratification.

KEYWORDS angiogenesis, inflammation, micro-calcification positron emission tomography, vulnerable plaque,

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INTRODUCTION

Atherosclerosis is dependent on a number of cellular processes from initial endothelial injury to sequestration of lipid in the subendothelial space and activation of immune cells.¹ Risk factors for predicting the development of atherosclerosis are well established, as are the various imaging techniques available to assess the burden of atherosclerosis in patients with symptomatic disease.² However, accurately assessing an individual's risk of acute complications even in patients with symptomatic atherosclerosis remains a challenge.

Knowledge of the cellular processes involved in atherosclerosis has been used to identify blood borne biomarkers in order to risk stratify patients.³ A similar approach is currently being applied to molecular imaging modalities such as positron emission tomography (PET).⁴ With the additional anatomical information provided by imaging modalities, there is potential to direct risk stratification to the level of the individual atherosclerotic plaque. Molecular imaging of atherosclerosis using PET is currently at the stage where it is being applied as a biomarker in drug development and has potential to make the transition into clinical medicine to aid cardiovascular risk stratification.^{5,6}

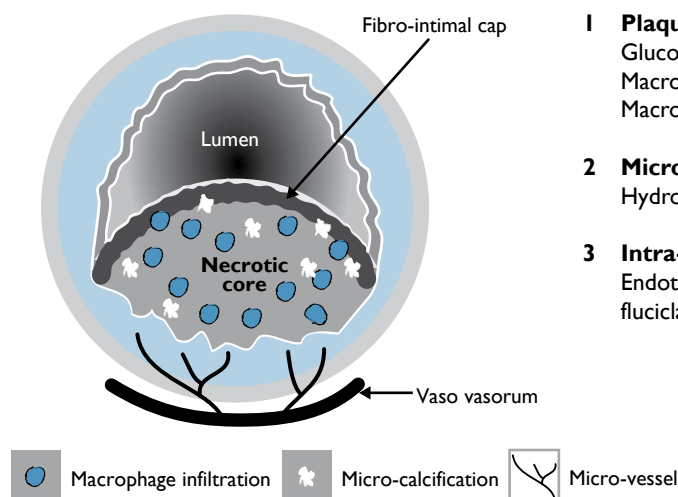
POSITRON EMISSION TOMOGRAPHY IMAGING

Positron emission tomography imaging uses ionising radiation to localise specific cellular processes in the body with a spatial resolution of ~4 mm. It combines a positron emitter with a molecular vehicle targeting a cellular process of interest. ¹⁸F-Fluoride is the most practical and commonly used positron emitter because it has a half-life of 110 min. A PET image is formed by the detection of two photons with a specific energy emitted following the collision of an electron with a positron arising from the fluoride nucleus.⁷ This annihilation event produces two photons at 180 degrees from each other. Their simultaneous detection helps to build a 3D image of positron emission events and localise the anatomical site where the tracer has accumulated.⁷ Such PET images are then fused with CT allowing activity to be localised to even small structures within the body.⁸

The dominant features of vulnerable atherosclerotic plaque include a thin fibrous cap and large necrotic core with a predominance of foam cells, macrophages and apoptotic bodies.¹ There is also evidence of micro-calcification associated with the necrotic core and intra-plaque angiogenesis, which may lead to haemorrhage and plaque instability. By targeting plaque macrophage density, micro-calcification and angiogenesis, it is hoped that *in vivo* molecular imaging with

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1 Plaque inflammation:

Glucose analogue: fluorodeoxyglucose
 Macrophage translocator protein: PK11195
 Macrophage mannose receptor: fluorodeoxymannose

2 Micro-calcification:

Hydroxyapatite: sodium fluoride

3 Intra-plaque neovascularisation:

Endothelial $\alpha_v\beta_3$ integrin receptor: galacto and flucilatide

FIGURE 1 Characteristics of vulnerable atherosclerotic plaque alongside potential positron emission tomography radiotracers capable of identifying such lesions.

PET tracers will help identifying vulnerable plaques prior to rupture (Figure 1).

FLUORODEOXYGLUCOSE AS A BIOMARKER OF PLAQUE INFLAMMATION

Positron emission tomography imaging with the tracer 18F-fluorodeoxyglucose (18F-FDG) is currently used in clinical practice to assess myocardial viability.⁹ 18F-FDG is a glucose analogue that accumulates in cells proportional to their glycolytic activity. Macrophages are the most abundant cell within the atherosclerotic plaque and have a higher glucose metabolism than surrounding plaque cells. Therefore, the 18F-FDG signal from atherosclerotic plaques is regarded as a marker of plaque macrophage inflammation.¹⁰

Macrophages are implicated in every stage of atherosclerosis from its initiation and progression through to clinical plaque rupture events. Histological examination of ruptured plaques demonstrates an abundance of macrophages, particularly in the shoulder regions where they secrete matrix metalloproteinases, weakening the fibrous caps and predisposing these sites to rupture. Indeed, Rudd et al. first demonstrated increased focal 18F-FDG uptake within culprit atherosclerotic plaques in patients with symptomatic carotid disease.¹¹ Carotid plaques ipsilateral to the neurological event were found to have 27% higher 18F-FDG uptake relative to the asymptomatic contra-lateral artery.¹¹ *Ex vivo* examination using carotid plaque autoradiography confirmed selective accumulation of deoxyglucose within macrophage-rich areas of plaques.¹¹

There is also a close correlation between 18F-FDG uptake and both the absolute and percentage of macrophage accumulation within plaque sections.¹² Moreover, gene expression analysis from carotid plaques

demonstrates an association between 18F-FDG uptake and markers of plaque instability including CD68, GLUT-1, HK-12 and cathepsin K.¹³ However, recent data also indicate that 18F-FDG accumulation may reflect the degree of hypoxia within plaques, which encourages glycolytic rather than oxidative metabolism and increased tracer uptake.¹⁴

18F-FDG uptake is associated with traditional cardiovascular risk factors, including age, male gender and metabolic syndrome, as well as inflammatory biomarkers.^{15,16} Moreover, vascular 18F-FDG uptake is elevated in patients infected with human immunodeficiency virus, perhaps accounting for their increased cardiovascular event rate.¹⁷⁻¹⁹ A retrospective review of vascular 18F-FDG uptake on PET scans performed in patients with cancer has demonstrated an association between 18F-FDG uptake and subsequent vascular events.²⁰

However, the predictive power of 18F-FDG uptake over and above that of conventional cardiovascular risk factors remains to be established prospectively. This is being addressed in studies such as High Risk Plaque Initiative and BiImage, which will assess whether imaging biomarkers including 18F-FDG imaging are able to risk stratify patients beyond that achieved with traditional cardiovascular risk factors.^{5,21} However, using 18F-FDG imaging as a longitudinal marker of cardiovascular risk may be limited by the variation of the vascular 18F-FDG signal over time (perhaps reflecting waxing and waning of plaque inflammation) and by the associated radiation exposure making its less attractive as a screening tool in low-risk populations.²²

18F-FDG PET does hold considerable promise as a method of evaluating the efficacy of novel anti-atherosclerotic treatments. Small studies have demonstrated that statin therapy and lifestyle

modification reduces plaque 18F-FDG activity.^{23,24} Moreover, 18F-FDG imaging has been used as a safety end-point to demonstrate the feasibility of novel lipid treatments and demonstrated their lack of impact on vascular inflammation.²⁵ Currently, 18F-FDG is being used in multiple ongoing clinical trials of novel agents with the aim of evaluating vascular safety, detecting early signs of biological effect and understanding the mechanism of action of novel therapies.

While 18F-FDG imaging has now been validated in the aorta and carotid, iliac and femoral arteries,^{26,27} attempts to use this tracer in the coronary arteries have been less successful. Initial studies were encouraging, with 18F-FDG activity in the proximal vessels and adjacent aorta demonstrating increased activity in patients following acute coronary syndromes compared with those with stable angina.^{28,29} However, attempts to examine the coronary vasculature in more detail have been limited by the glycolytic activity of the myocardium, which uses glucose as its predominant energy source and therefore avidly takes up 18F-FDG obscuring any signal in adjacent structures. High-fat, low-carbohydrate diets have been used in an attempt to switch myocardial metabolism to free fatty acid and reduce ventricular uptake.³⁰ However, these measures have proved only modestly effective, with background myocardial 18F-FDG activity remaining a significant problem in almost half of patients.^{29,30}

We believe that this lack of specificity is likely to limit the use of 18F-FDG as a biomarker of coronary artery disease risk and that more macrophage specific tracers are required. The novel radio-ligand IIC-PK11195 holds promise in this regard, targeting a translocator protein specific to human macrophages and demonstrating increased uptake in atherosclerotic plaques that correlates with macrophage infiltration.³¹ Targeting cell surface receptors distinct to macrophage subtypes may enhance the specificity of imaging plaque inflammation. This approach to refinement has been taken with 18F-fluorodeoxymannose (FDM), which is an analogue of glucose but also binds to mannose receptors present on alternatively activated (M2) macrophages.³² The M2 macrophages are associated with high-risk plaque features such as neovascularisation and intra-plaque haemorrhage.³² By targeting mannose receptors expression in addition to plaque glucose metabolism, 18F-FDM is potentially able to both quantify and characterise plaque inflammation.

SODIUM FLUORIDE IMAGING AS A BIOMARKER OF MICRO-CALCIFICATION

Calcification is a key hallmark of the atherosclerotic plaque that was previously thought to be a generalised passive process associated with age-related degeneration.¹ However, extra-skeletal calcification in other areas of the body is commonly seen in conjunction with chronic

inflammation such as granulomatous infections and chronic inflammatory connective tissue disorders. Similarly, calcification in atherosclerosis is now believed to occur as a healing response to the potent inflammatory stimulus in the necrotic core.³³ While ultimately the latter stages of macro-calcification seen in atherosclerosis are believed to impart stability to the plaque, the early stages of microcalcification are associated with increased plaque vulnerability and tendency to rupture.³⁴

Computed tomography coronary artery calcium score (CAC) is a widely used measure of vascular calcification.³⁵ Calcium in the coronary arteries is pathognomonic of atherosclerosis, so CAC scoring provides a useful surrogate of a patient's coronary atherosclerotic burden.² Moreover, it is a useful predictor of cardiovascular events since the more plaques a patient has, the more likely they are to have a clinical plaque rupture event. It is not, however, capable of identifying high-risk vulnerable lesions directly.³⁶

Indeed, intravascular ultrasound and CT studies demonstrate that culprit coronary artery lesions in patients with acute coronary syndromes have less extensive coronary calcification in comparison with lesions associated with chronic stable angina.^{37,38} This would suggest that macro-calcification of a plaque, which is measurable by the CAC score, is protective and has a role in plaque stability. However, biomechanical modelling indicates that regions of micro-calcification have the opposite effect and predispose to plaque rupture. Areas of micro-calcification within the fibrous cap can create an interface between the rigid and compliant structures of the cap that intensifies the effect of incident haemodynamic forces resulting in plaque rupture.³⁹ With progressive calcification the areas of interface would decrease and so at this point the risk of rupture would be expected to decrease as plaque calcification becomes increasingly confluent and the cap more rigid.

It has also been demonstrated that hydroxyapatite micro-particles are able to activate human macrophages. Macrophages have a greater capacity to endocytose hydroxyapatite micro-particles with a more complex nanotopography and larger surface area for attachment and phagocytosis.⁴⁰ Micro-calcification would therefore appear an excellent marker of high-risk atherosclerotic plaque at risk of rupture. Indeed histological studies have demonstrated an association of micro-calcification with both ruptured plaques and also high-risk lesions with large necrotic cores.³⁴ Consequently, techniques capable of detecting micro-calcification non-invasively and differentiating it from more advanced regions of macro-calcification could potentially prove useful in identifying vulnerable regions of atheroma.

The PET tracer 18F-sodium fluoride (18F-NaF) has been used as a bone tracer for more than 40 years. It acts as

a biomarker for hydroxyapatite, which is a key structural component of both bone and vascular calcification. It forms a chemical bond to exposed hydroxyapatite by replacing the hydroxyl group with fluoride to form fluoroapatite.⁴¹ The degree of ¹⁸F-NaF uptake is dependent on the surface area of the hydroxyapatite crystalline structure. Transmission electron microscopy demonstrates that during the early stages of vascular calcification formation hydroxyapatite crystals are nano-sized, very thin and long.⁴² This results in an enormous surface area of nanocrystals to which ¹⁸F-NaF can bind. As a consequence of this surface area effect ¹⁸F-NaF preferentially binds to regions of powdery micro-calcification as opposed to field macroscopic calcification where much of the hydroxyapatite is internalised and not available for binding.

Initial evidence for vascular uptake of ¹⁸F-NaF was provided by retrospective reviews of PET scans performed in patients with cancer. Its uptake was found in the large vessels, including the aorta and carotid, iliac and femoral arteries.⁴³⁻⁴⁶ Our group demonstrated cardiac uptake of ¹⁸F-NaF in patients with calcific aortic valve disease.⁴⁷ Areas of ¹⁸F-NaF uptake in calcific aortic stenosis were frequently remote from established calcification identified on CT imaging.⁴⁷ This was the first indication that ¹⁸F-NaF could be used to differentiate between vascular micro- and macro-calcification. This finding is supported by our recent observations using serial ¹⁸F-NaF PET/CT imaging of patients with asymptomatic aortic stenosis. We found baseline valvular ¹⁸F-NaF uptake predicted the development of new areas of macro-calcification and the progression in aortic valve calcium scores on follow-up CT after one year.⁴⁸

We demonstrated the feasibility of imaging coronary ¹⁸F-NaF uptake in a prospective study of 119 patients with and without aortic valve disease.⁴⁹ ¹⁸F-NaF imaging of coronary arteries was not hindered by myocardial uptake and so could be reliably measured with excellent intra- and inter-observer variability. The ¹⁸F-NaF signal was higher in patients with coronary artery atherosclerosis and discrete areas of ¹⁸F-NaF uptake could be localised to individual coronary plaques.⁴⁹ Interestingly, ¹⁸F-NaF uptake correlated with the Framingham risk score, suggesting its association with a higher cardiovascular risk profile. Indeed, stratification of the patients with coronary artery disease using ¹⁸F-NaF uptake demonstrated that those with increased uptake were more likely to have symptomatic coronary artery disease and a history of major adverse cardiovascular events.⁴⁹

Our group has recently gone on to investigate whether ¹⁸F-NaF activity in patients with unstable atherosclerotic disease localises to ruptured culprit lesions.⁶ In this study patients with recent acute myocardial infarction (MI) underwent both invasive coronary angiography and ¹⁸F-NaF imaging. The ¹⁸F-NaF signal localised to the site

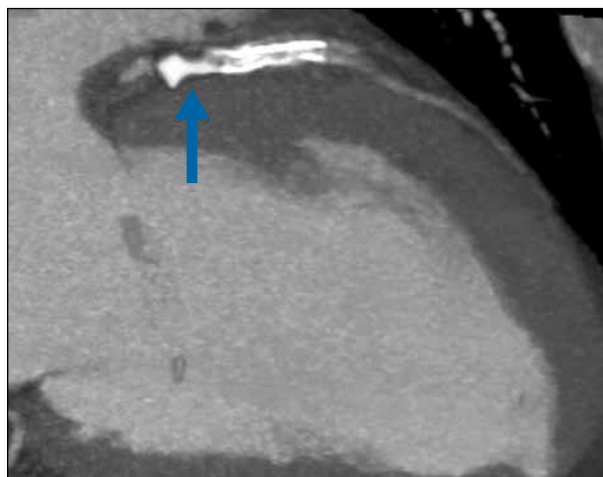


FIGURE 2 Focal ¹⁸F-NaF uptake localising to the culprit atherosclerotic plaque responsible for a patient's acute myocardial infarction. Intense focal ¹⁸F-NaF (¹⁸F-NaF, tissue-to-background ratios, culprit 2.17 versus reference segment 1.18 [184% increase]) uptake (yellow-red) at the site of the culprit plaque (red arrow) on the combined PET-CT image.

of coronary plaque rupture in 37 of the 40 (93%) patients (Figure 2). Of the remaining three patients without culprit lesion uptake, two were young smokers who may have suffered an event due to plaque erosion rather than rupture. The third patient had a co-dominant circulation, and while the right coronary artery was adjudicated on angiography as the culprit, focal increased ¹⁸F-NaF activity was instead observed in the left circumflex artery that might have equally explained the clinical presentation. The localisation of ¹⁸F-NaF to ruptured atherosclerotic plaques was then confirmed in symptomatic patients undergoing carotid endarterectomy following transient ischaemic attack or stroke. *Ex vivo* ¹⁸F-NaF imaging of excised carotid plaques from nine patients demonstrated tracer uptake localised to the site of macroscopic plaque rupture. Both of these studies suggest that ruptured atherosclerotic plaques in patients with symptomatic coronary and carotid disease appear to have increased focal ¹⁸F-NaF uptake.

It is not feasible to perform histological examination of coronary plaques in patients with recent MI. Therefore, excised carotid plaques were again used to correlate vascular ¹⁸F-NaF uptake with histological findings in order to get an understanding of the mechanism of tracer uptake.⁶ Carotid plaques were sectioned into areas with and without ¹⁸F-NaF uptake. Plaque sections with increased ¹⁸F-NaF uptake had evidence of increased calcification activity, inflammation as determined by macrophage infiltration, and cell death (both necrosis and apoptosis).⁶ This would support the hypothesis that micro-calcification occurs as a healing response to the intense inflammation in the necrotic core that also triggered the plaque rupture event. An alternative interpretation is that calcification occurs as healing response to the plaque rupture event itself. However, we do not believe this is the

explanation because of the short time interval between MI and the PET imaging, and the fact that iatrogenic plaque rupture induced by stenting of bystander lesions did not result in increased 18F-NaF activity.

While it is interesting that 18F-NaF can identify culprit atherosclerotic plaque, the key question is whether it can identify these lesions prospectively before they cause clinical events. We therefore examined coronary 18F-NaF uptake in a group of high-risk patients with stable disease.⁶ Focal regions of increased coronary 18F-NaF uptake were observed in more than two out of five (18/40; 45%) patients with stable angina, most commonly (72%) in non-obstructive (<70% stenosis) lesions on coronary angiography. Following 18F-NaF coronary imaging, the morphology of coronary plaques with 18F-NaF uptake was evaluated using radio-frequency intravascular ultrasound and CT coronary angiography.⁶ Plaques with 18F-NaF uptake were associated with high-risk features including micro-calcification (73% versus 22%; $p=0.002$), a larger necrotic core (24.6% [interquartile range (IQR) 20.5–28.8] versus 18.0% [14.0–22.4]; $p=0.001$) and positive remodelling (remodelling index 1.12 [IQR 1.069–1.19] versus 1.01 [0.94–1.06]; $p<0.001$). In contrast 18F-NaF negative plaques had features associated with a stable morphology such as being composed of more fibrous (51.0% [IQR 46.3–56.6] versus 58.1% [51.6–65.5]; $p=0.015$) and fibro-fatty tissue (10.9% [6.0–13.8] versus 12.6% [9.3–17.8]; $p=0.09$) and greater confluent areas of calcification (12.6% [9.1–18.1] versus 10.2% [4.0–14.9]; $p=0.09$).

18F-NaF imaging holds promise as a non-invasive imaging technique that can risk stratify atherosclerotic plaque by identifying active micro-calcification occurring in response to intense necrotic inflammation. However prospective studies are now required to confirm whether those plaques with increased 18F-NaF activity go on to cause future cardiovascular events and whether they can identify high-risk patients who would benefit from aggressive medical therapy.

ATHEROSCLEROTIC PLAQUE NEOVASCULARISATION

Medium and large size arteries are accompanied by *vasa vasorum*, which form a network of microvasculature in the adventitial layer and supply oxygen and nutrients to the vessel wall. Proliferation of adventitial *vasa vasorum* occurs in areas of atherosclerosis and leads to neovascularisation of the media and intima.⁵⁰ While neovascularisation can occur even before endothelial dysfunction (perhaps suggesting that this may be an initiating event in the development of atherosclerosis) the predominant trigger is believed to be the progressively hypoxic conditions that develop within the plaque as it enlarges.⁵¹ Indeed once the plaque thickness exceeds a critical limit of ~100 μm , the capacity for diffusion of

blood oxygen and nutrients from the lumen is limited and new vessels are required.⁵² As a consequence while microvessels are observed in around 40% of coronary atherectomy specimens, they are much more frequently observed in thicker carotid lesions.⁵³

Intra-plaque angiogenesis is an independent predictor of plaque rupture and associated with intra-plaque haemorrhage.⁵⁴ It therefore has potential as an imaging biomarker for identification of vulnerable plaques. Newly formed microvessels do not have supporting cells and so are fragile and permeable. Intra-plaque haemorrhage following the extravasation of erythrocytes from fragile new microvessels is relatively common and microvessel density correlates with the extent of intra-plaque haemorrhage.^{55,56} The cell membrane of extravasated erythrocytes contributes a rich source of free cholesterol and cholesterol ester which leads to enlargement of the necrotic core and rapid progression of atherosclerosis.^{56,57} However, intra-plaque angiogenesis is not the only source of plaque haemorrhage. It can also result from plaque fissuring following rupture of a fibrous cap with the subsequent formation of an intra-intimal thrombus.⁵⁵

As mentioned previously, inflammation and infiltration of macrophages are prominent features of the vulnerable plaque. There is a strong correlation between microvessel density and histological content of macrophages in carotid artery plaques.⁵⁸ It is believed that the microvessels are used by inflammatory cells to enter the atherosclerotic plaque.⁵⁹ Indeed, unstable coronary atherosclerotic plaques are associated with a higher microvessel density with a two-fold increase in vulnerable plaques and a four-fold increase in ruptured plaques in comparison with stable lesions.^{56,57} Furthermore, microvessel density is found to be higher in the vulnerable shoulder region of atherosclerotic plaques.⁵⁴ Overall, intra-plaque angiogenesis appears to correlate with key features of the vulnerable plaque that are associated with plaque rupture.

How then can we image angiogenesis? New blood cells express the cell surface integrin receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$, which recognise the arginine-glycine-aspartate (RGD) motif.⁶⁰ On this basis the RGD-containing PET tracers 18F-galacto and 18F-fluciclatide, have been developed to target the $\alpha_v\beta_3$ integrin receptor and detect angiogenesis.^{61,62} At present 18F-galacto has been validated as a biomarker for $\alpha_v\beta_3$ integrin expression in atherosclerotic plaques of patients with significant carotid artery stenosis.⁶³ *Ex vivo* histological studies on carotid endarterectomy specimens demonstrated that 18F-galacto correlated with both angiogenic endothelial cells and macrophages expressing $\alpha_v\beta_3$ integrin. It therefore appears to act as a marker of both plaque inflammation and angiogenesis holding promise as a method of risk stratifying carotid plaque, although whether it will be detectable in the coronary arteries remains to be established.⁶⁴

CONCLUSION

Our understanding of plaque biology is directing the development of imaging biomarkers. The currently available PET tracers offer the potential to assess in vivo the pathological processes that underlie the vulnerable plaque. A direct measure of disease activity with 18F-FDG imaging could potentially be used to screen novel therapeutic interventions and assess their efficacy before investing in large multi-centre clinical trials. Newer radiotracers are attempting to target the distinct cellular constituents of the vulnerable plaque and so enhance

the specificity of imaging atherosclerotic plaques. This will advance our understanding of plaque biology and the effects of therapeutic interventions at the cellular level. With regard to delivering tailored patient care, the key advantage of imaging biomarkers is the additional anatomical data which helps localise and risk stratify individual atherosclerotic plaques. At present 18F-NaF imaging is the most promising prospect for being able to achieve the goal of prospectively identifying coronary plaques at high risk of rupture. This vision will hopefully be realised in the coming years with more widespread research in this area.

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