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An unmet clinical need: The history of thrombus imaging

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Abstract

Robust thrombus imaging is an unresolved clinical unmet need dating back to the mid 1970s. While early molecular imaging approaches began with nuclear SPECT imaging, contrast agents for virtually all biomedical imaging modalities have been demonstrated in vivo with unique strengths and common weaknesses. Two primary molecular imaging targets have been pursued for thrombus imaging: platelets and fibrin. Some common issues noted over 40 years ago persist today. Acute thrombus is readily imaged with all probes and modalities, but aged thrombus remains a challenge. Similarly, anti-coagulation continues to interfere with and often negate thrombus imaging efficacy, but heparin is clinically required in patients suspected of pulmonary embolism, deep venous thrombosis or coronary ruptured plaque prior to confirmatory diagnostic studies have been executed and interpreted. These fundamental issues can be overcome, but an innovative departure from the prior approaches will be needed.

Keywords

Thrombus; fibrin; platelet; biomedical imaging

INTRODUCTION

Effective thrombus formation is an essential facet of life required to maintain normal vascular hemostasis and to respond to injury whether accidental or surgical. Yet thrombosis can be the sine qua non of pathological events with serious or mortal consequence, such as acute myocardial infarction (AMI), deep venous thrombosis (DVT), pulmonary embolism (PE), or cerebral vascular arterial stroke (CVA). Indeed, fibrin deposits are the fertile matrix into which the neovasculature of cancers expand to fuel metastatic tumor progression. The pathological significance of thrombus has long been recognized and its specific diagnosis

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led to contrast agent development efforts dating back to at least 1974. Although many targeted probes across a variety of imaging modalities have been proposed, no thrombus-specific diagnostics are commercially marketed within the US.

THROMBOSIS: VASCULAR INFLAMMATION

Thrombosis is a complicated physiological process. Commonly, vessel wall injury triggers thrombus development, which involves the interplay of platelet activation and a cascade of enzymatic clotting proteins. Counter biochemical mechanisms by natural anti-coagulants and the fibrinolytic system modulate to prevent an over-exuberant progression. Thrombus formation is vascular inflammatory reaction wherein activated platelets adhere to the injured vessel wall and release cytokines, growth factors, and other pro-inflammatory mediators. Leukocytes, recruited to the site of vascular injury, adhere to P-selectin expressed on the activated platelet surface via P-selectin Glycoprotein Ligand-1 (PSGL-1).[1] Circulating microvesicles rich in tissue factor (TF) from leukocytes bind to platelet surface PSGL-1, sustaining the coagulation process triggered by vascular TF from endothelial injury.

Cross-communications between inflammation and coagulation biochemical networks are extensive. The local release of inflammatory cytokines induces leukocytes and endothelial cells to express TF.[2] Complexation of TF and the coagulation factor FVIIa or Xa incite coagulation on the negatively charged cell membranes. Thrombin, the major hemostatic coagulation enzyme, activates protease-activated receptors (PAR-receptors) highly expressed in platelets and endothelial cells, myocytes and neurons, which amplify the inflammatory pathway activity well beyond the acute coagulation process.[3] Fibrin, the mortar of clots, recruits and activates platelets through the platelet receptor $\alpha_{IIb}\beta_3$,[4] as well as attracts leukocytes through interaction with the $\alpha_M\beta_2$ (mac-1) integrin receptor.[5] Leukocyte binding to fibrin increases phagocytosis, NFκB-mediated transcription, production of chemokines and cytokines, and degranulation. Importantly, the inter-relationship between inflammation and coagulation in the pro-coagulant process also exists for the modulating anti-coagulant pathways centered around Tissue Factor Pathway Inhibitor (TFPI),[6] Antithrombin (AT),[3] Protein C/S.[3] The pro- and anti-coagulation and inflammatory pathways are counter balanced by endothelial cell responses to other physiological or pathological stimuli broadly including bacteria, toxins, trauma, and cytokines. While thrombus formation appears to be a simple process *en face*, it is, like other biological processes, a complicated and tightly regulated event.

THROMBUS DIAGNOSTIC IMAGING

Despite the complexities of thrombus formation, medical imaging of the pathology has evolved with a more simplistic perspective based on the accumulation of fibrin or platelets. Both targets are richly present in both venous and arterial clots and offer the potential for bound contrast agents to provide requisite high contrast to blood/tissue signal for noninvasive imaging. Since the early 1970s, the development of thrombus-specific diagnostics has evolved along these two pathways with the earliest technologies being nuclear medicine approaches. Later, with expanding interest in molecular imaging, the most relevant modalities include ultrasound, magnetic resonance imaging (MRI), optical and

photoacoustic or optoacoustic imaging, and computed tomography (CT) including the newer Spectral or Multicolored CT.

Fibrin Imaging

Fibrin is a major constituent of arterial and venous thrombi as well as thrombotic accretions to biomaterials such as stents, valves, wires, and other mechanical devices. Fibrinogen is a soluble, large, and complex 340 kDa glycoprotein that is converted by thrombin into fibrin during thrombus formation.[7,8] Synthesized by liver hepatocytes, fibrinogen circulates in plasma at levels between 200 and 400 mg/dL. During normal blood coagulation, prothrombin is converted into thrombin, a serine protease, which subsequently converts soluble fibrinogen into insoluble fibrin strands. These insoluble fibrin fibrils become further cross-linked strands by circulation clotting factor XIIIa (FXIIIa) to yield a stabilized clot.[9] Beyond cross-linking, FXIIIa further stabilizes the fibrin clots by entrapment of fibrinolysis inhibitors alpha-2-antiplasmin[10] and TAFI (thrombin activatable fibrinolysis inhibitor, procarboxypeptidase B),[11] and by interacting with cell adhesive receptors presented by inflammatory cells. Activated coagulation factors such as Factor Xa and thrombin also bind to fibrin and become transiently inactivated by entrapment within the fibrin fibril network. Fibrin simultaneously triggers the pro-coagulant pathway via activation of factor XIII by thrombin and the anti-coagulant pathway through plasminogen activator, such as tissue plasminogen activators (TPA).[12]

Early probes sought to label and incorporate natural constituents of clot formation, the first one being ^{125}I radiolabeled fibrinogen.[13–15] This gamma nuclear probe produced promising increases in detection results relative to negative controls, but the data were only loosely correlated with thrombus fibrin content; imaging was delayed 24 to 72 hours, and after clot lysis significant intramural nuclear signal remained in the vessel walls creating frequent false positive interpretations. Shortly after the first ^{125}I fibrinogen probes, the development of ^{111}In-platelets was pursued creating the second general pathway to thrombus detection.[16] In overview, this approach dominated investigator interest for a decade. However, around this time Niels Jerne's earlier theories concerning the specificity in development and control of the immune system[17,18] were combined with the discovery of the principles for production monoclonal antibodies by Köhler and Milstein.[19] These discoveries and investigators were recognized with a shared Nobel Prize for Physiology or Medicine in 1984[20] and presented the opportunity to pursue dedicated antibodies specific for fibrin and later platelets.

Before the wider availability of monoclonal antibodies, ^{131}I anti-fibrin rabbit polyclonal antibodies were studied in patients with thrombophlebitis or chronic varicosities. The new technique detected formed or developing clots and helped to discriminate between thrombophlebitis, best detected 24 to 72 hours post-injection, and thrombus-free varices, discerned optimally after 6 hours.[21] Ten years later, Grossman et al reported thrombus imaging with ^{111}In-labeled anti-fibrin monoclonal antibodies and $F_{(ab)2}$ fragments.[22] They achieved better CNR using the more rapid clearing $F_{(ab)2}$ probes than the more circulatory persistent antibodies. Subsequently, the question of fibrin vs platelet imaging was addressed for the first of several times with ^{131}I-anti-fibrin antibodies injected simultaneously

with ^{111}In-platelets.[23] The anti-fibrin approach demonstrated 60% higher clot uptake vs the ^{111}In-platelets reference.[23] Similarly, comparisons between murine monoclonal antibody ^{123}I $F_{(ab)}$ and $F_{(ab)2}$ fragments were performed to determine the optimal balance between maximum thrombus binding and rapid blood pool clearance in rabbits and not unexpectedly the higher avidity $F_{(ab)2}$ fragment proved superior to $F_{(ab)}$. [24]

However, given the close relationships between fibrinogen and fibrin, efforts to produce ligands with higher fibrin specificity were pursued. The best monoclonal antibody candidates created in this quest were developed by Patrick Gaffney at the National Institute of Biological Standards and Controls (NIBSC) in London[25–28] and by Bohdan Kudryk at the New York Blood Center.[29–32] While these monoclonal antibodies were used in several subsequent molecular imaging studies with multiple modalities discussed subsequently, neither fibrin ligand achieved clinical translation.

Another highly specific family of monoclonal antibodies were raised to D-dimer, a site formed when adjacent fibrin D-domains cross-link.[31] This neo-epitope and its degradation product were not present in monomeric fibrin fibrils or fibrinogen and immunoscintigraphy and biodistribution studies in rats confirmed high thrombus specificity with little normal tissue background for this probe. Subsequent preclinical and eventual clinical studies were conducted with $F_{(ab)}$ fragments of this antibody, most notably DD/3B6, but the probe never became clinically established.[33–35] One challenge for the DD/3B6 target was its spontaneous liberation from the clot into blood, which variably reduced the thrombus retained antigen and concurrently generated circulating D-dimer that could potentially interfere with targeted imaging. Today D-dimer assays in blood are routinely used to exclude thrombotic disease, such as pulmonary embolus or deep venous thrombosis.[36]

Developments in phage display recombinant peptide panning and selection led to the discovery and use of smaller molecular weight fibrin binding peptides.[37] For fibrin targeting, the most effective probes were identified and developed by Epix Pharmaceuticals of Cambridge, MA. Epix had produced an MRI contrast Gadofosveset trisodium, which spontaneously coupled with circulating albumin to increase relaxivity and lengthen blood pool contrast persistence. A complicated and evolving set of corporate arrangements with Mallinckrodt Chemical Co, Siemens, Schering, Berlex, and lastly Lantheus Medical Imaging, led to product launch in the US and then worldwide as ABLAVAR® in 2010. During this timeframe, Epix Pharmaceutical became interested in the development of a fibrin paramagnetic MR molecular imaging probe based on the coupling of chelated gadolinium, typically 4 Gd-DOTA per peptide[38] and applied their MR contrast development expertise to this goal. Epix developed families of well-characterized fibrin-binding peptides, [39] which were extensively tested in the preclinical[40–47] and later clinical setting.[48] Yet, despite the strength of this seminal product concept, the technology failed to reach the market and clinical use.

Another commercial approach included Tc-NC100668, a peptide partial analogue mimic of α_2-antiplasmin.[49–51] Uniquely, this ligand was effective for targeting venous thrombosis in the presence of anticoagulants and thrombolytics, which led others to later adopt this homing strategy.

Platelet Imaging

While radiolabeled fibrinogen imaging initiated the quest to develop thrombus-specific imaging agents, this approach was quickly superseded by ^{111}In-labeled platelet imaging.[16,52] Both radiolabeled fibrinogen and platelets in patients showed relatively low signal initially but platelet imaging had modestly better results with acute clots less than 24 hours.[53] Retrospectively this finding was prescient and points to a key limitation impacting most published thrombus imaging probes to date. The inadequate uptake of ^{111}In platelets by aged thrombus compared with acute thrombus was further validated in canines.[54] Since common clinical practice for patients suspected of DVT or PE dictates anti-coagulation until the diagnoses are ruled out, Fedullo et al assessed the impact of heparin on ^{111}In platelet imaging.[55] Their experiment in dogs demonstrated that heparin anti-coagulation markedly inhibited ^{111}In platelet uptake until it effects were reversed by protamine.[55] This was another prophetic result,[56] which continues to haunt the development and translation of thrombus imaging agents. Despite these limitations, ^{111}In platelet imaging research continued robustly for a decade in the context of LV thrombus,[57–60] acute cerebrovascular thrombosis,[61] iliac arterial mural thrombosis,[62] and DVT.[63]

The use of radiolabeled platelets gave way to platelet binding ligands that were adopted to circumvent issues with limited platelet uptake in aged clot and during anti-coagulation, to overcome the inconvenience of harvesting, washing, and labeling of patient platelets for study, and to accelerate image acquisition following treatment. In 1985, initial results with anti-platelet (anti-GPIIbIIIa) monoclonal antibodies (7E3) were reported in vitro and in dogs.[64] Again, acute thrombus could be imaged but 48h thrombus was not detectable. Another monoclonal antibody (SZ-51) was developed against a platelet epitope, alpha granule membrane protein (GMP-140), which was later characterized to be P-selectin. $^{65–131}$I-SZ-51 was initially shown to be effective against acute dog thrombus[66] and was later modified into a mouse-human chimeric ligand that was characterized in vitro.[65,67] However, both monoclonal approaches failed to progress significantly. In parallel with the events in fibrin targeting, platelet homing ligands targeting the GP IIbIIIa receptor took many forms over the years including: radiolabeled small molecule receptor antagonists,[68–70] disintegrins,[71–73] and peptides.[74–78] Unfortunately, none have translated to the clinic.

ALTERNATIVE MODALITIES FOR MOLECULAR IMAGING OF THROMBUS

Gamma emitter-based nuclear imaging approaches dominated thrombus imaging from the mid-70s into the mid-90s. Beginning in the mid-90s, thrombus contrast agent development for alternative imaging modalities emerged involving nanoparticles, microbubbles, optical, and PET.

Ultrasound

The first ultrasound contrast agent for thrombus was demonstrated in dogs in 1996 (Figure 1). This ligand-targeted nanoparticle utilized perfluorocarbon (PFC) emulsions, which offered negligible acoustic contrast in circulation at low doses, but nanoparticle binding to arterial thrombus generated enhanced acoustic reflectivity.[79] One can imagine the PFC particles acting like silver grains accumulating on a glass plate to create a mirror.[79–81] The

echogenicity of clot bound PFC nanoparticles was easily appreciated within 30 to 60 minutes because the blood pool background signal was negligible. This was in stark contrast to the high background noise of nuclear approaches that could delay immunoscintigraphic imaging for days. Continued research by Hughes et al developed new and improved acoustic detection approach based on entropy detection that optimized ultrasonic PFC molecular imaging. Entropy imaging is a statistical spatial and temporal index wherein the reflected RF signal variation in each ultrasound line of data is projected as a histogram and the characteristics of the histogram are mapped as an image as opposed to the common approach of using reflected RF power.[82–86] While these experimental approaches to ultrasound imaging offered significant advancements in general, the fundamental techniques used by echocardiographic equipment better complemented microbubble use, which was gaining effectiveness for blood pool imaging of the heart and later molecular imaging as discussed below.

In 1999, echogenic liposomes (ELIP), which had been reported as an early concept for acoustic thrombus imaging,[87,88] were demonstrated in vivo for intravascular clots.[89] These submicron liposomal particles were developed to avoid the transpulmonary particle entrapment issues experienced by microbubbles being developed for intracardiac blood pool imaging in the early 1990s. While the acoustic reflectivity of ELIP was less than microbubbles and circulatory stability less than perfluorocarbon nanoparticles, ELIP particles have found therapeutic applications involving intravascular gas and thrombolytic delivery.[90–94]

In 2002, microbubble development by ImaRx Therapeutics, which invented, developed and licensed Definity® microbubbles for cardiac imaging, reported modification of their bubbles to target platelet GP IIbIIIa receptors in vitro. Later, the company developed abciximab-targeted microbubbles, which targeted the GP IIbIIIa receptors on platelets, and demonstrated the formulation in vitro and in rats with carotid occlusions.[95] ImaRx continued to pursue microbubble-based thrombus imaging and compared fibrin- and platelet-directed bubbles in vitro concluding that fibrin-targeted microbubbles produced greater signal.[96] This outcome was reminiscent of the conclusion previously advanced by Bosnjakovic et al comparing ^{111}In platelets with ^{131}I anti-fibrin antibodies.[23] Wang et al targeted GP IIbIIIa platelet receptor with a single-chain antibody-microbubble construct, which was shown to target thrombus and provide imaging confirmation of thrombolysis.[97] During the same time period, Hu et al reported and RGD-peptide-microbubble agent studied its binding under varying shear conditions, and demonstrated enhanced acoustic contrast against aortic thrombus compared with control microbubbles.[98,99] In addition to imaging, microbubbles offer a theranostic component to the treatment of thrombus through mechanical disruption by particle cavitation and local release of fibrinolytic agents.[100,101]

Magnetic Resonance Imaging

Thrombus-specific MR imaging was first reported in 1998 using fibrin-specific monoclonal antibodies produced by Patrick Gaffney at the NIBSC to target paramagnetic perfluorocarbon nanoparticles, as previously used for acoustic thrombus imaging.[102] The lipid-encapsulated PFC particles incorporated lipid-anchored Gd-DTPA at high payloads

that markedly increased the ionic (Gd-based) and particulate (NP-based) relaxivity and overcame partial volume signal dilution (Figure 2).[102] Further development of these nanoparticles for thrombus imaging optimized Gd-chelate loading and the relative particle surface positioning of the metal.[103] In the mid-2000s, further imaging and gadolinium stability optimization with these particles were achieved with the use of Gd-DOTA prior to Australian clinical trials. Unexpectedly, these Phase 1 clinical studies were paused then eventually discontinued when some early patients complained of transient back pain occurring at very low contrast dosages of the imaging agent. Despite extensive safety testing in vitro and in mice, rats, rabbits, dogs, and baboons without adverse complications, recognition of this issue was only triggered late by patient comments. The problem was identified as acute complement activation and was extensively characterized.[104] Subsequent advancements in clinical ^{19}fluorine imaging spurred a return of this technology without gadolinium to the clinic in the context of angiogenesis MR molecular imaging.

A similar perfluorocarbon particle targeted to thrombus in the IVC of mice via an α_2-antiplasmin peptide was imaged successfully using high-field ^{19}F MRI (9.4T).[105] The results corroborated the thrombus ^{1}H imaging earlier in dogs in 1998[102] and later in vitro and then in vivo during 2000 to 2001[106,107] with 4.7T and clinical 1.5T scanners, respectively. As previously mentioned, the first of many reports involving the Epix paramagnetic peptides agent reached the scientific literature in 2004, representing the first modern MR thrombus imaging program to reach Phase II clinical studies.[40]

In 2001, working with Nycomed (Norway), Johansson reported the first platelet-targeted MR approach using ex vivo and in vivo models based on an RGD-USPIO probe (ultrasmall superparamagnetic iron oxide).[108] Subsequently in 2008, MPIO (microparticles of iron oxide) targeted with single-chain antibodies directed to platelet GP IIbIIIa in mice were reported.[109] The thrust of using large iron oxide particles was to improve contrast but importantly take advantage of rapid macrophage phagocytic system clearance of the large particles in order to shorten the time from injection to MR imaging, overcoming the 24 to 72 hours delays typically required before imaging long circulating USPIO particles.

Recently, a new form of MR-related tomographic imaging was developed and studied known as Magnetic Particle imaging.[110] MPI varies from MRI by utilizing changing magnetic fields to produce a single magnetic field free region. Using shape optimized SPIO (super-paramagnetic iron oxide) nanoparticles, signal is generated only in this region, which is moved systematically to produce an image that avoids the interfering iron contrast background that delays post-injection image acquisition. The MPI molecular imaging concept was tested in 2013 using a fibrin-peptide functionalized SPIO in vitro to produce about 200 times more signal than traditional MR magnetic particle spectroscopy.[111] This unique opportunity for ultrahigh MR molecular imaging sensitivity is very attractive, but both the instrumentation and iron oxide contrast development need to progress much further.

The versatility of MR imaging can be used to exploit the inherent MR magnetic properties of thrombus using techniques like magnetization transfer (MT) and diffusion imaging (DI) techniques.[112] Serial imaging of IVC thrombus in mice demonstrated that older densely organized thrombus, which is typically resistant to fibrinolytic therapy, was visualized well

with MT signal and poorly with DI. For younger lytic responsive thrombus, the converse was noted: high DI efficacy but low MT signal.

Some investigators have pursued other clever targeting approaches to thrombus, such as Myerson et al who used an irreversible thrombin inhibitor (PPACK) to bind thrombin on carotid clots in vivo for MR imaging of PFC particles.[113] These results further suggested a theranostic potential to the construct, which also interfered with continued thrombus progression.

Computed Tomography

The bromine atom associated with each molecule of perfluorooctyl bromide used in some PFC nanoparticles inherently provides CT contrast. The accumulation of fibrin-targeted nanoparticles on clots can impart significant x-ray opacity to thrombus.[114] In this regard, PFC nanoparticles offer multi-multimodal-targeted contrast consistent with early blood pool observations by Mattrey in the early 1980s.[115–117]

CT imaging has made many advances in both detector hardware and software reconstruction. Perhaps one of the newest and most exciting involves Spectral or Multicolor CT based on K-edge imaging.[118] Every element has electrons that inhabit energy “shells” which surround the nucleus. The shell closest to the nucleus is designated the K-shell. K-edge imaging exploits an absorption discontinuity created when an emitted x-ray photon with energy identical to or larger than the energy of the K-shell electron fuse. An abrupt rise in attenuation at that energy is noted within the x-ray spectrum, the K-edge. CT scanners with K-edge imaging capability can be engineered with different hardware x-ray tube and detector configurations; one advanced system uses a standard x-ray tube and a new multilayered photon counting detector that counts and characterizes the energy of each photon striking it. These clinical instruments utilize advanced reconstruction techniques to calculate and deconvolve three types of x-ray energy on a voxel by voxel basis: Compton, photoelectric, and K-edge data. The Compton and photoelectric energies are used to create the standard x-ray CT image on which the K-edge data can be superimposed without registration issues because all the data are derived simultaneously. Molecular imaging contrast agents for CT, particularly Spectral CT, can be achieved by incorporating sufficient metal loads of elements ranging from iodine to bismuth on the periodic table into nanoparticles. The K-shell electron energies of these elements correspond to the photon energies emitted by a typical x-ray tube. The first example of fibrin imaging with a Spectral CT contrast agent and scanner was reported by Pan et al based on bismuth organometallic complexes and demonstrated in vitro and in femoral-iliac thrombus in rabbits (Figure 3).[119] This was followed by studies with Spectral CT particles based on ytterbium organo-complexes or minute gold nanoparticles suspended in “oil” and encapsulated with phospholipids.[120–122] Although not specifically addressing fibrin imaging, Cormode et al have spent considerable energy developing contrast agents for Spectral CT imaging.[123–126] For thrombus imaging, the potential of Spectral CT rests in its development of speed of acquisition, which 1-day could reach a level to permit ruptured plaque imaging in coronary beds and pulmonary embolus imaging.

Optical-Related Imaging

In 2004, Jaffer et al reported a near-infrared (NIR) optical imaging approach to thrombus detection and characterization conducted in vitro and in rats that utilized a peptide homing to Factor XIIIa.[127] Jaffer utilized Factor XIIIa as an acute thrombus marker with the potential to assess thromboembolic risk and susceptibility to fibrinolysis. The next year, this team demonstrated the development of a fluorescent probe targeted via a branched peptide against platelet GP IIbIIIa.[77] The lab continued development of optical probes using one of the Epix Pharmaceutical fibrin binding peptides conjugated to a Cy7 NIR dye-to detect acute DVT in rats.[128] Like the use of Factor XIIIa targeting this probe could stratify venous thromboembolism for fibrinolysis.[129]

Photoacoustics or optoacoustics invoke the use of endogenous proteins, cells, or contrast agents to adsorb gated laser light pulses to create minute levels of heat (nanojoules) that dissipates the energy as acoustic emissions. These emissions are detectable with standard single element or even clinical ultrasound transducers. As an example, gold nanobeacons (GNB) incorporate a multitude of tiny (4 to 5 nm) gold nanoparticles into an oil suspension and target thrombus using fibrin antibodies in vitro.[130] The dense incorporation of gold particles within the lipid-encapsulated particles generated a very strong photoacoustic signal effectively similar to a much larger gold particle. However, because the human renal clearance threshold for particles is about 8 nm, larger non-degradable particles will be retained in the body indefinitely, potentially posing future safety issues, while particles below this size will be eliminated in urine. As opposed to technologies where local injection of gold particles limits treatment cost, systemic therapies for thrombus detection will require orders of magnitude more gold and increase treatment cost substantially. To address this issue, high-density organometallic suspensions of divalent copper in lipid-encapsulated nanoparticles were developed for fibrin imaging with MRI[131] and then used for systemic photoacoustic imaging and anti-angiogenic drug delivery (fumagillin prodrug) targeted to the sparse $\alpha v\beta 3$-integrin receptor expressed by nascent neovessel sprouts.[132] These copper-rich nanoparticles demonstrated NIR photoacoustic reflectively similar to gold GNB.

Positron Emission Tomography

While the vast abundance of early nuclear medicine probes for fibrin or platelet imaging involved gamma emitters, such as ^{99m}Tc, ^{125}I, ^{131}I, or ^{111}In, recent PET probes based on ^{64}Cu[133] have been reported for thrombus imaging. These investigators described one peptide, called FBP8, as an effective fibrin-specific probe, originally described within the Epix Pharmaceutical IP estate, but its efficacy was greatest towards acute thrombus and diminished quickly as the clot aged. The loss of thrombus binding paralleled a reduction in fibrin content as the clots organized.[134,135]

SUMMARY

Thrombus imaging has a rich history dating back to the mid 1970s and continuing to the present. This continued effort is fueled by an unresolved clinical unmet need, perhaps greatest for pulmonary embolism, ischemia-reperfusion injury microthrombus, and unstable ruptured or eroded carotid and coronary atherosclerotic plaques. While early molecular

imaging approaches began with nuclear SPECT imaging, contrast agents for virtually all biomedical imaging modalities have been reported, each offering some unique strengths and weaknesses. Despite years of study, platelets and fibrin remain the dominant thrombus targets. Acute thrombus is readily imaged with all probes, but aged thrombus remains difficult to target. Similarly, anti-coagulation continues to interfere and often negate thrombus imaging probe efficacy, yet the use of this therapy is clinically demanded when pulmonary embolism, DVT or coronary ruptured plaques are suspected. The lack of a clinical thrombus imaging contrast agent reflects the difficulty of overcoming these fundamental challenges that will require an “out of the box” design approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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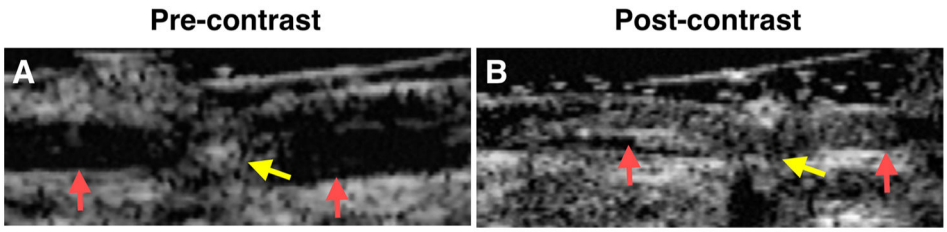

Figure 1.

Acoustic enhancement of canine femoral artery thrombus, targeted with biotinylated anti-fibrin antibody, before (**A**) and after (**B**) exposure to targeted perfluorocarbon emulsion. Pre-contrast the acute arterial thrombus is poorly visualized with a 7.5-MHz linear-array, focused transducer. The transmural electrode (*yellow arrow*) and the wall boundaries of the femoral artery are clearly delineated. Post contrast the thrombus is easily recognized (*red arrows before and after*) exposure. Reproduced with permission Reference.[79]

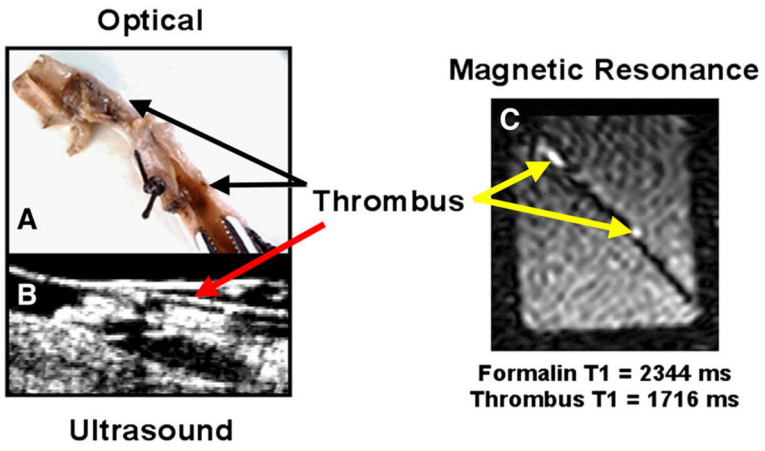

Figure 2.

A White light image of canine femoral artery with recovered segmented thrombus, following recovery, formed as described in Figure 1 with electrical current to draw platelets toward anodal needle tip to propagate clot. **B** Ultrasound imaging obtained in vivo in the canine femoral artery prior to excision showing thrombus. **C** Ex vivo T1-weighted MRI image (1.5T) of excised artery in beaker of buffer. These data present the first demonstration of in vivo targeting of thrombus with a dual modality US–MR perfluorocarbon nanoparticle in canines showing close correspondence between the light, ultrasound, and MR images.[102]

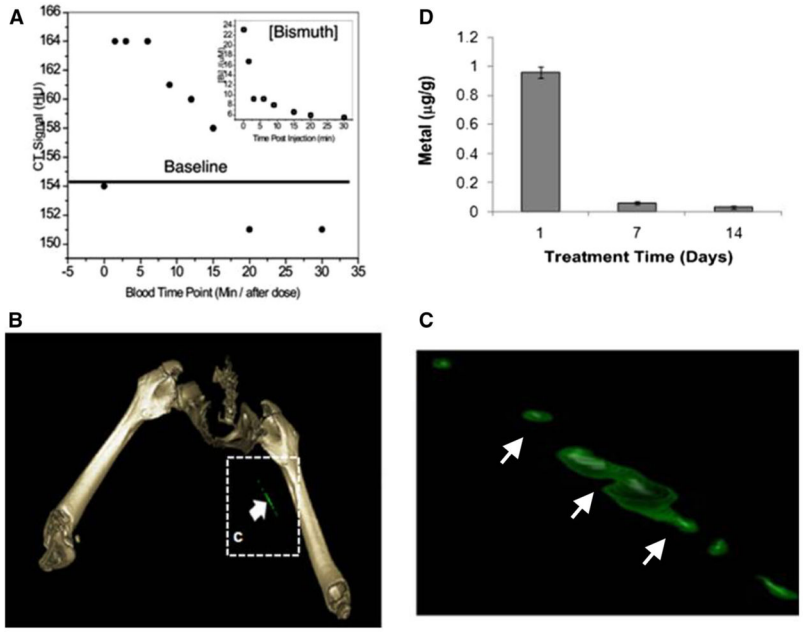

Figure 3.

(**A**) CT blood pool signal in rabbits following IV injection of NanoK. CT scan imaging parameters were thickness 0.8, increment 0.8, kV 90, mAs 1500, resolution HIGH, collimation 4 × 0.75, pitch 0.35, rotation time 1.5 seconds, FOV 75 mm. *Inset* The concentration of bismuth (ICP) in blood vs time post-injection. Note that the background signal is at baseline in less than 30 minutes; (**B, C**) targeting in situ clot (thrombus) in rabbits; (**D**) 2 weeks clearance profile of bismuth from mice. Reproduced with permission.[119]