The utilization of ultrasound and microbubbles for therapy in acute coronary syndromes

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Ultrasound has become a useful high resolution imaging modality for examining the cardiac microcirculation. With the use of microbubbles as an ultrasound contrast agent, ultrasound can be utilized to image the microcirculation and detect capillary flow abnormalities in acute ischaemia. A wide range of ultrasound frequencies (including those used for diagnostic transthoracic imaging) have also been utilized therapeutically to augment the effectiveness of fibrinolytic therapy in ST-segment elevation myocardial infarction (STEMI). Ultrasound and microbubbles are now being explored as methods of improving both microcirculatory and epicardial flow in acute STEMI. This article will review the mechanisms by which ultrasound and microbubbles assist in thrombus detection and dissolution. In addition, the pre-clinical studies utilizing transthoracic ultrasound as a therapeutic entity in acute STEMI will be reviewed. Clinical studies, completed and ongoing, will also be presented.

KEYWORDS
Ultrasound; Microbubbles; Thrombus

An estimated number of patients discharged with the diagnosis of acute coronary syndrome (ACS) from hospitals in 2004 was 840 000.1 This diagnosis carries with it significant morbidity and mortality.1 Pathological and angioscopic examinations have demonstrated that acute thrombosis on top of ulcerated lipid-rich plaque plays a major role in the development of ACS.2–4 Patients with ST-segment elevation myocardial infarction (STEMI) usually have a completely occluded coronary artery with thrombus, whereas varying degrees of thrombotic occlusions are seen with non ST-segment elevation ACS.5 In all of these settings, restoring coronary patency with pharmacological or interventional strategies as promptly as possible has been shown to be an important factor in determining short- and long-term outcomes. Several hospitals have made percutaneous coronary interventions (PCI) available 24 h a day to provide emergent recanalization of the epicardial vessel in acute STEMI.6 However, the time required to open a coronary vessel successfully with PCI still requires ~90 min after presentation to the Emergency Department, at even the most experienced centres.7 Nonetheless, this approach appears to be more successful at achieving early coronary artery patency than fibrinolytic therapy, where effective recanalization is achieved in less than 60% of those treated within 4 h of symptom onset.8 Although each of these therapies (PCI, thrombolytic therapy) is focused on restoring patency in the epicardial vessel, another critical component in ACS has been downstream microthrombi, which occlude the microcirculation. Epicardial recanalization frequently does not result in restoration of flow of these smaller vessels, and patients exhibit no reflow into these zones. The detection of no-reflow indicates a poor prognosis, with increased risk for both heart failure and death.9–11

With the use of microbubbles as an ultrasound contrast agent, ultrasound can be utilized to image the microcirculation and detect capillary flow abnormalities in acute ischaemia.11 Ultrasound has also been shown in pre-clinical studies to be a useful therapeutic procedure to augment the effectiveness of fibrinolytic therapy in STEMI.12 This augmentation of lysis may be aided by microbubbles, especially in microcirculatory thrombi.13 This article will review the diagnostic and therapeutic potential of ultrasound and microbubbles in detecting and treating intravascular thrombi, and how we could exploit these tools to change the paradigm by which we emergently manage ACSs.

1. Utilization of ultrasound and microbubbles to image thrombi

Commercially available microbubbles are manufactured in a manner that allows them to serve as free intravascular tracers, and therefore they do not adhere to endothelium and are not taken by the endothelial cells. However, platelet or fibrin-targeting ligands have been attached to the surface of a lipid or albumin encapsulated microbubble, resulting in their binding to the surface of intravascular thrombi. This has resulted in improved visualization of the
thrombus with diagnostic ultrasound imaging.\textsuperscript{14,15} For example, investigators have demonstrated that microbubbles with an attached glycoprotein 2b/3a target can increase the acoustic intensity of thrombi in vitro.\textsuperscript{16,17} Subsequent in vivo studies have confirmed that glycoprotein 2b/3a targeted microbubbles improve the visualization of carotid artery thrombi, when compared with non-targeted microbubbles.\textsuperscript{16}

2. Mechanisms for ultrasound induced thrombus dissolution

Ultrasound was first studied as a stand alone technique for enhancing fibrinolysis. The first description of the effect of ultrasound in enhancing intravascular thrombus dissolution was performed in 1976 in the femoral or iliac veins of dogs.\textsuperscript{18} Pfaffenberger et al.,\textsuperscript{19} in their thorough review of ultrasound thrombolysis, have detailed the many subsequent in vitro and peripheral vascular in vivo studies that have confirmed the efficacy of ultrasound in augmenting fibrinolysis. Controlled in vivo studies have utilized high frequency catheter-based or transcutaneous ultrasound to produce clot lysis with ultrasound alone.\textsuperscript{20,21} The frequencies, intensities, and duty cycles for in vivo studies examined have ranged from 27 kHz to 1.0 MHz frequencies, 0.13–160 W/cm\textsuperscript{2} intensity, and pulsed to continuous wave duty cycles.\textsuperscript{19}

One of the main reasons for this potentiating effect of ultrasound appears to be induction of cavitation. Cavitation is defined as the rapid growth and collapse of gas bodies. It has generally been classified into two sub-types: stable and inertial, with the stable form being induced at a lower peak negative pressure.\textsuperscript{22} The cavitation process leads to axial fluid acceleration, resulting in acoustic streaming and the creation of high-velocity gradients at the thrombus surface.\textsuperscript{23} This microstreaming appears to be one of the main mechanisms by which ultrasound potentiates thrombolysis in vitro.\textsuperscript{24,25} In vitro studies have implied that the fluid jets created from streaming penetrate the thrombus and lead to better fibrinolytic therapy exposure, and de-stabilization of the thrombus.\textsuperscript{19}

Since microbubbles serve as a nucleus for cavitation, the ultrasonic peak negative pressure threshold required to induce cavitation is lowered when they are present within the field of insonation.\textsuperscript{26} Microbubbles have consistently been shown to potentiate the effect of ultrasound in causing clot lysis.\textsuperscript{19,27} In vitro studies have shown that ultrasound and microbubbles can fragment thrombi and cause their dissolution even in the absence of a fibrinolytic agent.\textsuperscript{23} In vivo studies have also shown that microbubbles produce thrombus dissolution in the presence of surface applied ultrasound even in the absence of fibrinolytic agents.\textsuperscript{28–31} Furthermore, these studies also demonstrated no serological evidence of activation of the fibrinolytic system, as d-dimer levels do not increase following ultrasound and microbubble-induced thrombus dissolution in the absence of a fibrinolytic agent.\textsuperscript{28,32}

A method of further enhancing the effects of cavitating microbubbles would be to attach ligands to microbubbles which increase their affinity to the surface of thrombi, increasing the potential for both ultrasonic detection of thrombus and enhanced dissolution during cavitation.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Targeting ligands attached to microbubbles to enhance binding to intravascular thrombi involved in the pathogenesis of acute coronary syndromes</th>
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</thead>
<tbody>
<tr>
<td>Target</td>
<td>Targeting ligand</td>
</tr>
<tr>
<td>Platelets</td>
<td>Glycoprotein 2b/3a</td>
</tr>
<tr>
<td>Fibrin</td>
<td>Tissue plasminogen activator</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>Phosphatidyl serine, Echistatin</td>
</tr>
</tbody>
</table>

Glycoprotein 2b/3a targeted microbubbles, when combined with intermittent high mechanical index (MI) diagnostic ultrasound, improve microvascular reflow in pigs with acute STEMI, when compared with non-targeted microbubbles.\textsuperscript{13} This targeting technique has been used to improve recanalization rates with intravenous microbubbles in animal models of stroke and carotid artery thrombi without the aid of a fibrinolytic agent.\textsuperscript{33,34} Table 1 lists the potential ligands that could be attached to microbubbles to increase their presence within the risk area in ACS.

Mechanistic studies have also indicated ultrasound may enhance thrombus dissolution by processes other than inertial cavitation.\textsuperscript{22,25} Everbach and Francis\textsuperscript{24} have demonstrated up to 50% of thrombus dissolution that occurs from ultrasound still occurs in the absence of inertial cavitation. Others have monitored IC activity within a thrombus during 1.0 MHz ultrasound application in vitro and found that the induction of IC only slightly increases the degree of thrombus dissolution.\textsuperscript{35} Holland and colleagues have demonstrated with 1.0 MHz ultrasound that the efficacy of enhancing rt-PA mediated thrombolysis is dependent on SC rather than IC.\textsuperscript{22} These studies have shown no additional beneficial effect of ultrasound in dissolving thrombus at higher peak negative pressures. Additional work utilizing microbubbles\textsuperscript{27} to promote and sustain the cavitation induced with 120 kHz pulsed ultrasound has shown a significant correlation between thrombus dissolution and ultraharmonic signals (a marker of stable cavitation).

3. Ultrasound parameters to induce thrombus dissolution with microbubbles in vivo

The optimal ultrasound parameters (frequency, pulse duration, peak negative pressure) for inducing thrombus dissolution with transthoracic ultrasound and microbubbles are not known. Intuitively, it has been thought that a lower frequency would be expected to improve thrombus dissolution for transthoracic applications, because of the lower threshold for inducing cavitation.\textsuperscript{19} In addition, a lower frequency would attenuate less across the chest wall, and thus would appear better suited for deeply located intravascular thrombi. At higher frequencies (1 MHz or higher), there may be potential problems if the therapeutic peak negative pressure and duty cycle are too long. Nilsson et al.\textsuperscript{16} exposed human blood clots to fibrinolytic agents and either 1 MHz ultrasound or 170 kHz ultrasound. At power outputs of less than 2.0 W/cm\textsuperscript{2}, the degree of thrombolysis achieved at either frequency was not different. When
higher power outputs were used with a 1 MHz continuous wave frequency, a paradoxical increase in thrombus formation occurred (Figure 1). Using continuous wave ultrasound at a high peak negative pressure may actually increase thrombus formation via platelet activation. An increase in platelet accumulation on acute femoral artery thrombi was demonstrated by Riggs et al., using continuous wave 1 MHz ultrasound at 2 W/cm². In vivo studies have confirmed this, where acute femoral artery thrombi in rabbits treated with transcutaneous 1 MHz continuous wave ultrasound at a power output of 6.3 W/cm² resulted in a higher rate of reocclusion, when compared with fibrinolytic agents alone. It should be noted that these same frequencies and power outputs at shorter duty cycles were still effective at augmenting thrombolysis.

From a clinical perspective, there are advantages in using higher frequencies for ultrasound-mediated thrombus dissolution. If higher frequencies (1–2 MHz) are nearly as effective as lower frequencies, it is possible that diagnostic imaging frequencies could be utilized. This would have the advantage of permitting simultaneous imaging of the area of interest and detection of whether microbubbles are present prior to applying higher peak negative pressure pulses required to induce thrombus dissolution. This imaging-guided approach has been shown to achieve a greater success rate in recanalizing intravascular thrombi, when compared with non-imaging-guided approaches with the same transducer. In this setting, a diagnostic 1.5 MHz transducer, applying high-MI impulses transcutaneously only when the microbubbles were visualized within the thrombus with the same transducer, was as effective as a therapeutic 1 MHz transducer in recanalizing acutely thrombosed arteriovenous grafts (Figure 2).

4. Pre-clinical studies examining the potential of transthoracic ultrasound to treat ACSs

Pre-clinical studies in peripheral artery thrombi have demonstrated that ultrasound and microbubbles can dissolve intravascular thrombi, even in the absence of a thrombolytic agent. Birnbaum et al. have demonstrated that intravenous perfluorocarbon-filled microbubbles and low-frequency (less than 40 kHz) transcutaneous applications of ultrasound achieved recanalization rates of 100% in a rabbit model of ilio-femoral occlusion after 60 min of...
therapy. Similar results were obtained by others in peripheral vessels or arteriovenous grafts using 1 MHz ultrasound and intravenous or intra-arterial microbubble infusions or injections.\textsuperscript{30–32}

Although the efficacy of transcutaneous ultrasound and microbubbles appears excellent in peripheral vessels, where the ultrasound is applied directly over the thrombosed vessels, the effectiveness of this technique in more deeply located vessels has not been as successful. The initial studies with a 27 kHz transthoracic probe in canines demonstrated that over 85% of acute left anterior descending thrombotic occlusions could be recanalized when combined with 1.2 mg/kg intravenous tissue plasminogen activator (Table 1). These thrombi were discrete and in otherwise normal vessels.\textsuperscript{12} However, initial studies with a 1 MHz therapeutic transthoracic ultrasound and intravenous PESDA microbubbles angiographically recanalized only seven of 14 (50%) acute left circumflex occlusions in farm-bred pigs.\textsuperscript{39} The most likely explanation for the reduced effectiveness of ultrasound and microbubbles in these settings is the attenuation of the ultrasound peak negative pressure and reduced delivery of microbubbles to the region of interest. Nonetheless, in these experiments an additional intriguing finding was observed. Treatment with ultrasound and microbubbles was consistently effective at improving myocardial blood flow to the risk area even in the absence of angiographic recanalization.\textsuperscript{39} Both 40 kHz and 1 MHz ultrasound with PESDA improved regional wall thickening and electrocardiographic abnormalities ($P < 0.05$ compared with control or ultrasound alone), suggesting possible benefit of ultrasound and microbubbles in the microcirculation even in the absence of epicardial recanalization. Siegel et al.\textsuperscript{40} has demonstrated a myocardial perfusion bed subtended by an occluded coronary artery experiences an improvement in tissue perfusion when 27 kHz ultrasound (30% duty cycle) was applied to the bed. This improvement in tissue perfusion was prevented by pre-treatment with an inhibitor of nitric oxide.\textsuperscript{40} Additional studies using a same frequency and duty cycle transthoracically have shown rapidly reversible coronary vasodilation is achieved with this transducer, similar to what is seen with intracoronary nitroglycerin.\textsuperscript{41} The effectiveness of higher ultrasound frequencies, or of microbubbles combined with ultrasound, was not examined in these studies.

Because higher frequencies (1–2 MHz) have also been effective in inducing thrombus dissolution with microbubbles, more recent investigations have examined the effect of diagnostic ultrasound in this setting. As stated previously, the advantage of diagnostic ultrasound is that simultaneous imaging can be performed with contrast specific ultrasound imaging techniques, utilizing sensitive low-MI pulse sequence schemes to detect the presence of microbubbles within the thrombus and guide the timing of high-MI impulses. This would be especially helpful in ACS, where only small concentrations of intravenously infused microbubbles reach the thrombosed coronary artery and downstream microvasculature. Myocardial contrast echocardiography has been clinically useful in both defining the risk area and eventual infarct size,\textsuperscript{42} and detecting where microvascular no-reflow is still present after epicardial perfusion therapy.\textsuperscript{42} Current therapies in acute STEMI are focused on rapid recanalization of the epicardial vessel using either emergency PCIs or intravenous fibrinolytic agents. With these interventions, however, successful epicardial recanalization is often not accompanied by functional recovery due to microvascular no-reflow.\textsuperscript{44,45} This phenomenon is in large part due to capillary plugging by \textit{in situ} thrombi, as well as thrombi and inflammatory cells propelled downstream from the ruptured plaque. The commercially available low-MI pulse sequence schemes on most diagnostic transthoracic transducers are exquisitely sensitive for the detection of microbubbles channelling through the microcirculation, and identifying the circumferential extent of microvascular no reflow.\textsuperscript{46} Recent pre-clinical studies have examined whether high-MI impulses directed at both the microvascular thrombi and epicardial thrombosis in ACS, could be utilized to improve the recanalization rates from these sites. In a recent porcine study examining the effect of either intravenous glycoprotein 2b/3a targeted or non-targeted microbubbles with guided diagnostic high-MI ultrasound impulses following left anterior descending thrombotic occlusion, epicardial recanalization, and microvascular recovery were significantly improved in the pigs treated with glycoprotein 2b/3a targeted microbubbles and guided high-MI impulses from the diagnostic transducer.\textsuperscript{13} Even if epicardial recanalization did not occur, microvascular recanalization was evident in 40% of the pigs treated with ultrasound and microbubbles, in which there was ST-segment elevation and wall motion recovery at 1 h into treatment (Figure 3).

### 4.1 Potential clinical role for microbubble-augmented ultrasound thrombolysis in ACSs

Table 2 lists the pre-clinical and clinical studies that have used ultrasound as a therapeutic tool in ACS. After a successful pre-clinical study in canines which demonstrated that a 27 kHz transthoracic probe may significantly augment the effectiveness of full-dose fibrinolytic therapy in acute STEMI,\textsuperscript{12} a randomized controlled study comparing...
the effectiveness of this in humans was performed. A total of 360 patients with acute STEMI were randomized to receive the 27 kHz transthoracic TIMI 3 sonoperfusion device in the emergency department as a supplement to thrombolytic therapy. Over 60% of the STEMI’s were in a non-anterior location. Treatment with the sonoperfusion device did not increase the frequency of TIMI 3 flow in the culprit vessel (49% in the conventional therapy group vs. 41% in the ultrasound treated group). Therefore, the success that was achieved in the pre-clinical canine studies was not seen in a prospective randomized clinical trial. There are several potential explanations for this. First, humans would be expected to attenuate and scatter the ultrasound beam more than canines, especially in the 60% or greater STEMI’s located in the non-anterior coronary artery territories. Furthermore, the thrombus burden that occurs

<table>
<thead>
<tr>
<th>US frequency (outputs)</th>
<th>Duty cycle</th>
<th>Microbubbles</th>
<th>Pre-clinical study angiography (90 min)</th>
<th>Microvascular recovery rate pre-clinical study</th>
<th>Clinical study</th>
</tr>
</thead>
<tbody>
<tr>
<td>27–29 kHz (0.58 W/cm²)</td>
<td>100%</td>
<td>None</td>
<td>Angiographic success 90%</td>
<td>Not assessed</td>
<td>Not successful</td>
</tr>
<tr>
<td>1.6 MHz (3D) (1.18 W/cm²)</td>
<td>&lt;1%</td>
<td>Luminity</td>
<td>Angiographic success 60%</td>
<td>Not done</td>
<td>Ongoing</td>
</tr>
<tr>
<td>1.7–1.9 MHz (MI &gt; 1.0)</td>
<td>&lt;1%</td>
<td>MRX 815</td>
<td>Angiographic success 33%</td>
<td>73%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Not done</td>
</tr>
<tr>
<td>1.7–1.9 MHz (MI &gt; 1.0)</td>
<td>&lt;1%</td>
<td>MRX 802</td>
<td>Angiographic success 60%</td>
<td>67%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>

<sup>a</sup>For two-dimensional ultrasound, it was 1.9. As assessed by ST-segment recovery at 60 min into treatment.  
<sup>b</sup>For three-dimensional ultrasound, it will be 1.1. As assessed by ST-segment recovery at 60 min into treatment.

Figure 4 An example of replenishment within the risk area of a pig (arrows) with an acute thrombotic occlusion of the left anterior descending artery using a guided three-dimensional high mechanical index approach. The images represent the plateau intensity during a continuous infusion of microbubbles early on in the treatment period (left panel), and after 20–25 min of treatment in the right panel. Note the microcirculation within the risk area replenishes at 20–25 min into treatment. This pig also exhibited epicardial recanalization at 60 min into treatment.
from an ulcerative plaque (unlike the focal electrical-created occlusion in canines) would be greater especially in the downstream microvasculature. Finally, microbubbles were not used with ultrasound in this study, which reduce the threshold for ultrasound-induced cavitation. One clinical trial is underway utilizing intravenous commercially available microbubbles and high-MI impulses being delivered with a three-dimensional diagnostic transducer. In this trial, the ultrasound impulses are directed at the coronary artery and not the microvasculature.\(^4^\)

More attention may need to be focused on the importance of microcirculatory flow in ACS. Thirty day mortality is five-fold higher in patients with TIMI 3 flow in the epicardial vessel, if microvascular recanalization is not achieved.\(^4^\) Low-MI pulse sequence schemes and intravenous microbubbles are uniquely suited to examine microcirculatory flow in ACS, and thus serve as an emergent diagnostic tool to assess this in the emergency department.\(^4^\) The pre-clinical studies cited above indicate that high-MI impulses applied to the risk area (not the coronary artery) may be a rapid method of restoring microcirculatory flow in this setting.\(^1^\) Commericially available diagnostic three-dimensional transducers are now available which broaden the coverage of the guided high mechanical impulses to ensure the entire microcirculatory risk area is being treated. Unpublished preliminary studies from our laboratory have shown the effectiveness of this guided three-dimensional approach in improving microcirculatory flow in acute STEMI using non-targeted intravenous microbubbles (Figure 4). Indeed, the portability of ultrasound is such that such treatment measures could be ongoing even while a patient is being prepped for angiography. The availability and low cost of ultrasound and microbubbles make this treatment measure available to remote regions where interventional techniques are not accessible within short-time periods.

4.2 Microvascular damage with transthoracic ultrasound and intravenous microbubbles

Recent investigations have also demonstrated the potential for ultrasound and microbubbles to increase microvascular damage.\(^5^\)\(^-\)\(^4^\)\(^\) These studies have mainly been performed where there was minimal attenuation between the ultrasound transducer and the exposed heart. Furthermore, the doses of microbubbles administered were higher than what would be expected to reach the myocardium in clinical settings. Nonetheless, it is evident that when one utilizes higher mechanical indices (more than 1.0), the potential for microvascular damage exists. Although the degree of microvascular damage appears small when using transthoracic ultrasound, it is critical to further explore the MI that is required to augment thrombus dissolution with ultrasound and microbubbles. It is possible that stable cavitation of the microbubbles can be achieved with minimal microvascular damage, while still achieving clinically successful thrombus dissolution.

4.3 Future directions

There is an urgent need for more pre-clinical studies to determine the optimal ultrasound parameters and whether microbuble targeting ligands are needed to improve the efficacy of ultrasound facilitated thrombus dissolution. Other than glycoprotein 2b/3a receptor targeting, it is possible that targeting to fibrin or even inflammatory cells within the microcirculation might improve microbubble adhesion within the microcirculation.\(^5^,\)\(^6^\) Such targeting would increase microbubble proximity to thrombus within the microcirculation prior to therapeutic high-MI impulses, leading to increased thrombus dissolution with each high-MI impulse. Secondly, there is a need to determine the type of cavitation (inertial vs. stable) that is necessary to improve thrombi dissolution in vivo. These additional studies will further elucidate the clinical potential for ultrasound and microbubbles as a new emergent treatment in ACS that focuses on restoring both epicardial and microcirculatory flow.

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