Diagnostic and Therapeutic Utilization of Microbubbles

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Myocardial contrast echocardiography (MCE) is rapidly becoming a technique that can be utilized with intravenous microbubbles to detect myocardial perfusion abnormalities during stress echocardiography. Real-time techniques with low mechanical index pulse sequence schemes have been developed that allow for the simultaneous detection of wall motion and perfusion during pharmacologic stress echocardiography. MCE is also very helpful in determining the viability of the dysfunctional myocardium in the setting of acute myocardial infarction, and in the setting of chronic coronary artery disease. Microbubbles normally pass freely through the large and small vessels, but do adhere to the endothelium in the setting of endothelial dysfunction. This may be one method of concentrating the delivery of drugs or genes bound to microbubbles to selective areas of endothelial dysfunction. Ultrasound-mediated destruction of microbubbles may also be useful for targeted drug delivery and enhancing intravascular thrombolysis.

Key Words: Myocardial contrast echocardiography • Drug delivery • Thrombolysis

INTRODUCTION

Besides the well-established application of contrast echocardiography for left ventricular opacification and endocardial border enhancement in patients with suboptimal images both at rest and during stress,[1] this technique has emerged as a useful tool for the assessment of myocardial perfusion. The currently used ultrasound contrast agents are composed of perfluorocarbon-filled microbubbles encapsulated in a thin shell made of albumin or lipid, which confers high stability to them. Following an intravenous injection of contrast agent, the microbubbles fill the left ventricular cavity, improving the delineation of endocardial borders, and reach the aortic root, epicardial coronary arteries and the microcirculation. Importantly, the microbubbles exhibit properties similar to those of red blood cells, remaining totally in the intravascular space, and thus can be characterized as blood flow tracers.

The concentration of microbubbles in the microcirculation reflects the blood volume in the different regions of the myocardium, and forms the basis for the assessment of perfusion by myocardial contrast echocardiography (MCE). The clinical applications of MCE include the evaluation of patients with suspected or known coronary artery disease, determination of risk area and the efficacy of reperfusion therapies in patients with acute myocardial infarction (AMI), and assessment of myocardium viability either following AMI (identification of no-reflow phenomenon) or in the setting of chronic coronary artery disease (identification of hybernating myocardium). In addition, new applications have emerged that expand the utility of microbubbles for the noninvasive detection of tissue inflammation and angiogenesis (Table 1).

Role of MCE in the Detection of CAD

Due to the autoregulatory mechanisms of coronary circulation, the myocardial blood flow is maintained

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Table 1. Potential diagnostic and therapeutic applications of intravenous microbubbles

•	Detection of coronary artery disease
√	Stress echocardiography
√	Evaluation of collateral blood flow
√	Measurement of coronary flow reserve
•	Acute myocardial infarction
√	Determination of area at risk during coronary occlusion
√	Efficacy of reperfusion
√	No-reflow phenomenon
•	Assessment of myocardial viability
√	Following therapy in acute myocardial infarction
√	Chronic coronary artery disease
•	New potential applications of microbubbles
	√ Assessment of endothelial Integrity
	√ Targeted drug delivery
	√ Thrombus dissolution

constant at resting conditions even in areas supplied by arteries with significant stenosis (50 to 85% of luminal diameter), and is decreased only when the stenosis exceeds approximately 85% in severity. However, there is a reduction of blood flow reserve in the presence of stenosis > 50% because the arterioles have already dilated to varying degrees in order to maintain the resting blood flow. Therefore, although both myocardial perfusion and wall motion are maintained normal under resting conditions even in regions supplied by a non-limiting flow stenosis, this decrease in coronary blood flow reserve is unmasked during pharmacological or exercise stress.

The relative difference in the myocardial blood flow between normal regions and those supplied by stenotic arteries can be detected by MCE. Regional myocardial perfusion abnormalities induced by different pharmacological stress (dobutamine, dipyridamole, and exercise) have been identified with intermittent contrast harmonic imaging, and clinical studies have demonstrated that they correlated well with regional radionuclide tracer distribution and with coronary angiography.[2,3] However, intermittent harmonic imaging is time-consuming and does not permit the simultaneous analysis of wall motion. In addition, the need for increasing triggering intervals in order to evaluate the myocardial blood flow is challenging during stress, particularly when using dobutamine or exercise.

Real-time MCE are newly developed pulse sequence schemes that utilize a low mechanical index and permit the simultaneous analysis of myocardial perfusion and wall motion following intravenous ultrasound contrast agent injections or infusions. This technique can potentially increase the sensitivity of the test in identifying patients with coronary artery disease.[4,5] Using real-time low mechanical index MCE, the assessment of microbubbles kinetics can also be used for quantification of the myocardial blood flow and, therefore, coronary flow reserve. The myocardial contrast enhancement that occurs after an intravenous injection of microbubbles reflects the cappilary blood volume. High-energy ultrasound bursts can be briefly applied to produce complete bubble destruction in the capillaries, followed by low mechanical index imaging of the subsequent cardiac cycles to delineate the replenishment of myocardial flow by the microbubbles. The replenishment rate and peak intensity reflect the velocity of flow and myocardial blood volume, respectively, and their quantification has allowed for the measurement of myocardial blood flow.[6] Recent studies have confirmed the value of quantitative real-time MCE using dipirydamole for the detection of coronary artery disease and for the definition of its severity.[7]

MCE in acute myocardial infarction

There are several potential applications of MCE in the setting of acute myocardial infarction (AMI). During acute coronary occlusion, intravenous injection of microbubbles could assist in the risk stratification of patients by determining the extent of myocardium at risk for necrosis (Figure 1). The final infarct size is resultant of the duration of coronary occlusion, the total area of myocardium supplied by the infarct-related artery, and the presence or absence of collateral flow.[8] The perfusion defect size early after a flash destruction impulse represents the risk area, while the perfusion defect size late after the same impulse represents the eventual infarct size.[9] Therefore, MCE may be used to identify the patients at relatively lower risk (high degree of collateral flow) from those with potentially extensive infarct size if reperfusion is not established (large area at risk), and this identification is important for the management of patients with AMI. Furthermore, MCE can be used for the assessment of recanalization therapy. The patency of the infarct-related artery can potentially be determined by comparing the extent of the myocardial defect by MCE performed before and after interventional or pharmaco-

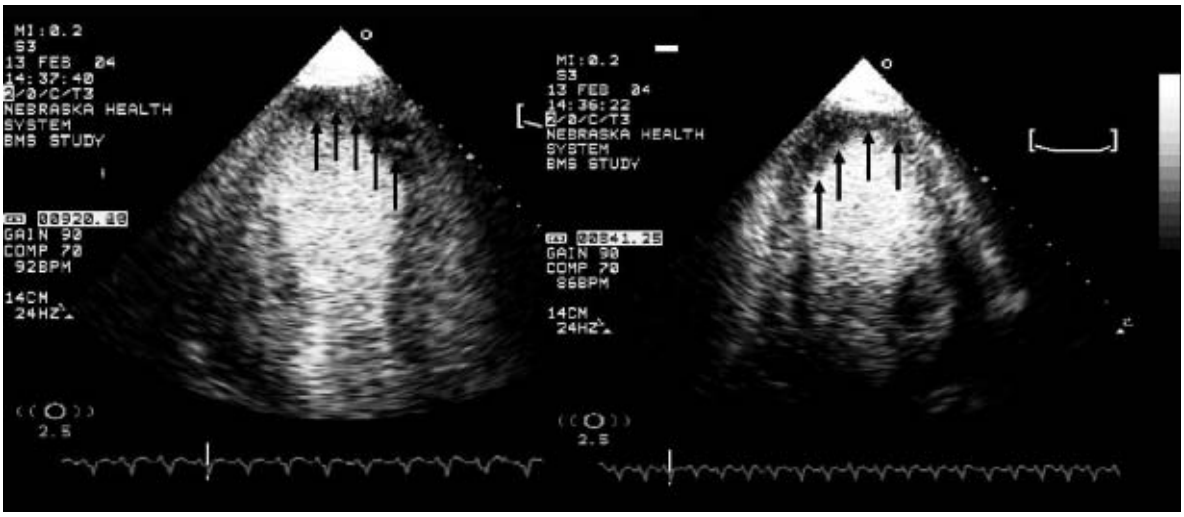

Figure 1. *Delineation of the risk (arrows) area using a real-time low mechanical index pulse sequence scheme in a patient presenting to the emergency room with an acute anteroseptal myocardial infarction.*

logic therapy.

MCE can also be useful for the evaluation of microvascular reperfusion following thrombolytic therapy or primary angioplasty. Complete patency of the infarct-related coronary artery does not necessarily result in restoration of adequate myocardial perfusion at the tissue level. Therefore, prolonged ischemia may result in failure to establish microvascular reperfusion despite restoration of epicardial coronary patency, and the absence of microvascular reperfusion is termed no-reflow phenomenon.[10] The no-reflow phenomenon detected by MCE is a marker of myocardial necrosis and has consistently been associated with impaired recovery of contractile function and a worse clinical outcome.[11-13]

However, it has been demonstrated that contrast enhancement in the reperfused regions may change with time following the acute phase after infarction, and should be interpreted with caution.[14,15] First, coronary hyperemia occurs in the immediate reperfusion period, resulting in underestimation of myocardial necrosis. For this reason, the determination of infarct size by MCE should be performed after abatement of hyperemia (generally after 24 hours). Furthermore, the extent of the no-reflow phenomenon varies over time. Although the exact time of evaluation is not established, some studies suggest that improvements in perfusion defect size at a later time after the AMI relates better to recovery of function and the presence of myocardial viability.

Several studies have shown the value of MCE for evaluating myocardial viability after AMI. In general, MCE presents high sensitivity (62% to 90%) but poor specificity (18% to 67%) in predicting functional recovery after AMI, with high negative predictive value.[16-18] Senior et al.[19] have recently demonstrated that the sensitivity and negative predictive value of low-dose dobutamine for the assessment of functional recovery of akinetic segments three months after AMI improved significantly when contrast opacification was observed in the dobutamine non-responsive segments. The combination of MCE and low-dose dobutamine was an independent predictor of functional recovery, suggesting that MCE is a technique that should be employed for the optimum assessment of viability after AMI.

Identification of hibernating myocardium

In the setting of chronic coronary artery disease, the myocardium may respond to the state of severe hypoperfusion by downregulating the contractile function. The term hibernating myocardium describes such a state of dysfunctional but viable myocardium, in which restoration of adequate coronary flow allows for recovery of ventricular function. The identification of hibernating myocardium is of utmost importance for the management of patients with ischemic cardiomyopathy, since revascularization may result not only in improvement of ventricular function and symptoms but has also been demonstrated to decrease the risk for future cardiac events.[20]

Studies have shown that MCE is a useful method for identification of myocardial hibernation and for the predic-

tion of functional recovery after coronary revascularization, with accuracy similar to that of radionuclide techniques.[21] When compared with biphasic response during dobutamine stress echocardiography, MCE generally presents a slightly higher sensitivity and a lower specificity. In other words, contractile reserve seems to be more specific for functional recovery than the analysis of myocardial perfusion.[22,23]

Shimoni et al.[24] recently demonstrated the value of quantitative MCE in comparison with DSE and thallium 201 scintigraphy for the detection of myocardial viability in patients with suspected myocardial hibernation. The accuracy of quantitative MCE was higher than the qualitative interpretation, and the quantitative measurement of myocardial blood flow during a continuous infusion of microbubbles was the best parameter for predicting myocardial functional recovery three to four months after surgical revascularization. Also, quantitative MCE had similar sensitivity (90% versus 92%; p = NS) but higher specificity (63% versus 45%, respectively, $p < 0.05$) than thallium scintigraphy, and improved the accuracy of DSE for the detection of myocardial viability.

Therapeutic applications

The recent advances in gene therapy and molecular biology have increased the interest in methods of noninvasive delivery of therapeutic agents. Besides the well-known application of microbubbles as contrast agents for diagnostic ultrasound, microbubbles have also been demonstrated an effective technique for targeted delivery of drugs and genes.[25-30] Drugs can potentially be incorporated into the microbubbles in a number of different ways, including binding of the drug to the microbubble shell and attachment of site-specific ligands. As perfluorocarbon-filled microbubbles are sufficiently stable for circulating in the vasculature as blood pool agents, they act as carriers of these agents until the site of interest is reached. Ultrasound applied over the skin surface can then be used to burst the microbubbles at this site, causing localized concentrated delivery of the drug.[31-34] This technique then permits using lower concentrations of drugs systemically, and site-specific targeting of the drug only where it is needed. This improved therapeutic index may be extremely advantageous in cases of drugs with hazardous systemic side effects, like cytotoxic agents. Albumin-encapsulated microbubbles have also demonstrated to adhere to the vessel walls in the setting of endothelial dysfunction.[35] This also may be a method of targeting delivery with microbubbles but without the application of ultrasound (Figure 2).

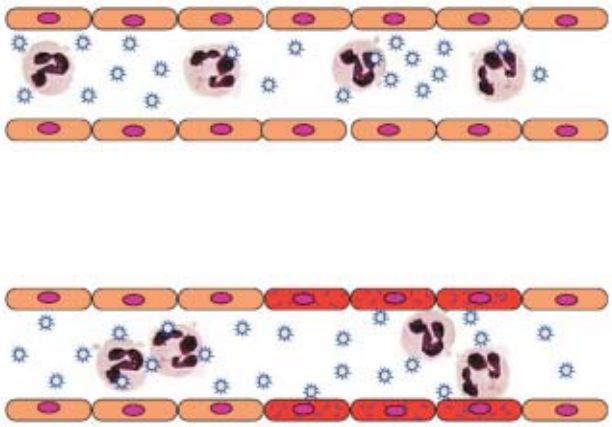

Figure 2. *Demonstration of how albumin-coated perfluorocarbon microbubbles containing drugs attached to their surface selectively adhere to sites of endothelial dysfunction (darker shaded cells in the lower panel). In the absence of endothelial dysfunction (upper panel), note that drug-bearing microbubbles stay in the blood pool and are not adherent to the endothelium.*

Mechanisms for target drug delivery using microbubbles

Two possible strategies for delivering drugs and genes with microbubbles are emerging. The first consists of ultrasound-mediated microbubble destruction, which is based on the cavitation of microbubbles induced by ultrasound application, and the second is the direct delivery of substances bound to microbubbles which target delivery to sites of endothelial dysfunction in the absence of ultrasound. Different drugs and genes can be incorporated onto the surface of ultrasound contrast agents. It has already been demonstrated that perfluorocarbon-filled albumin microbubbles avidly bind proteins and synthetic oligonucleotides.[36] In a similar way, microbubbles can directly take up genetic material, such as plasmids and adenovirus,[36,37] and phospholipid-coated microbubbles may have a high affinity for chemotherapeutic drugs.[38] Furthermore, specific ligands for endothelial cell adhesion molecules, such as P-selectin and leukocyte intercellular adhesion molecule 1 (ICAM-1), can be attached to both lipid- and albumin-coated microbubbles, which increases their deposition to activated endothelium.[39,40]

The mechanisms by which ultrasound facilitates the delivery of drugs and genes result from a complex in-

terplay among the therapeutic agent, the microbubble characteristics, the endothelium, target tissue, and the nature of ultrasound energy. The presence of microbubbles in the insonified field reduces the peak negative pressure needed to enhance drug delivery with ultrasound. This occurs because the microbubbles act as nuclei for cavitation, decreasing the threshold of ultrasound energy necessary to cause this phenomenon.

The results of optical and acoustical studies have suggested the following mechanisms for microbubble destruction by ultrasound: 1- gradual diffusion of gas at low acoustic power, 2- formation of a shell defect with diffusion of gas, 3- immediate expulsion of the microbubble shell at high acoustic power, and 4- dispersion of the microbubble into several smaller bubbles. Cavitation of the microbubbles is characterized by rapid destruction of contrast agents due to a hydrodynamic instability excited during large amplitude oscillations, and is directly dependent on the transmission pressure.[41,42] It has been reported that the application of ultrasound at a high mechanical index in the absence of significant attenuation creates extravasation points in skeletal muscle capillaries.[26,43] High-intensity ultrasound (referred to as a high mechanical index) can rupture capillary vessels in the presence of microbubbles, resulting in increased deposition of whatever is bound to the microbubbles into the surrounding extravascular space. Preliminary studies have indicated that only a small number of capillary ruptures are required to deliver large quantities of the colloidal particles to the muscle. It has also been demonstrated that the ultrasound pulsing interval and microvascular pressure influence the creation of extravasation points at the transport of drugs into the microcirculation. Both were greatest when the pulsing interval was around 5 seconds, indicating that maximal microbubble replenishment within the microcirculation prior to destruction by the ultrasound pulse produced the greatest drug delivery.[43]

The formation of pores in the membranes of cells as a result of ultrasound-induced microbubble cavitation has been proposed as a mechanism for facilitating the drug deposition. Taniyama et al.[31] demonstrated the presence of small holes in the surface of endothelial and vascular smooth muscle cells immediately after transfection of a plasmid DNA by ultrasound-mediated microbubble destruction, using electron microscopic scanning. It was then postulated that these transient holes in the cell surface caused by microbubbles and ultrasound resulted in a rapid translocation of plasmid DNA from outside to cytoplasm. Mukherjee et al.[34] demonstrated by electron microscopy of a rat heart performed during application of therapeutic ultrasound, that disruption or pore formation of the membrane of the endothelial cells occurred with acoustic power of 0.8 to 1.0 W/cm^2. However, it was a lower intensity of ultrasound (0.6 W/cm^2) that caused more drug delivery with microbubbles. More recently, voltage clamp techniques were used to obtain real-time measurements of membrane sonoporation in the presence of albumin-coated microbubbles (Optison). Ultrasound in the absence of microbubbles increases the transmembrane current as a direct result of membrane resistance due to pore formation.[44]

Another important therapeutic property of microbubbles is their increased adherence to damaged vascular endothelium. Albumin-coated microbubbles do not adhere to normally functioning endothelium, but their adherence to activated endothelial cells or to extra-cellular matrix of the disrupted vascular wall does occur.[35] Because of this characteristic, the delivery of drugs or genes bound to albumin-coated microbubbles could be selectively concentrated at the site of vascular injury in the presence[45] or absence of ultrasound application.[46] A cartoon depiction of this method of targeting is displayed in Figure 2, where one can see microbubbles carrying a specific drug on their surface adhering selectively to the dysfunctional endothelial cells. Albumin microbubble adherence has been observed selectively following balloon injury and throughout the vasculature during hypertriglyceridemia.

Microbubble use for gene therapy

The clinical use of viral vectors for gene therapy is limited because viral proteins elicit an immune response within the target tissue,[47] and have been shown to cause an intense inflammatory activation in endothelial cells.[48] On the other hand, the nonviral delivery of vehicles, such as plasmids and antisense oligonucleotides, has been associated with a lower transfection efficiency and only transient expression of the gene product.[49]

In 1996, the first published report of targeted DNA delivery was reported using surface ultrasound and intravenously delivered microbubbles carrying antisense

oligonucleotides.[27] In 1997, Bao et al.[50] described the use of ultrasound and albumin-coated microbubbles to enhance the transfection of luciferase reporter plasmid in cultured hamster cells. Since then, many studies have confirmed the efficacy of ultrasound-mediated microbubble destruction for drug and gene delivery, both in vitro and in vivo.[27,31-33] Shohet et al.[33] demonstrated for the first time with an adenovirus vector that the ultrasound-mediated disruption of gas-filled microbubbles could be used to direct transgenic expression to the heart in vivo. They showed that intravenously injected recombinant adenovirus vectors encoding a beta-galactosidase reporter gene were successfully delivered to normal rat myocardium using microbubbles and transthoracic 1.3 MHz diagnostic ultrasound, at a mechanical index of 1.5, delivered at a burst of 3 frames of ultrasound every 4 to 6 cardiac cycles. Of note, transfection was not observed if the adenovirus was administered in the same dose without microbubbles, or if the adenovirus was administered with microbubbles but in the absence of ultrasound. Importantly, using the same model, the authors confirmed that plasmid transgenic expression can be directed to the heart, with an even higher specificity than viral vectors, and that this expression will recur with repeated treatments.[51]

Taniyama et al.[7] have also shown effective transfection of a plasmid DNA with albumin-coated microbubbles (Optison) and ultrasound to endothelial and vascular smooth muscle cells. In vivo studies demonstrated that transfection of wild-type p53 plasmid DNA into balloon-injured blood vessels was effective and resulted in a significant reduction of the ratio of neointimal-to-medial area, as compared with transfection of control vector. In contrast, transfection of p53 plasmid DNA by means of ultrasound without microbubbles failed to inhibit neointimal formation in rat carotid artery.[31] In a recent study, Teupe et al.[52] documented efficient transfer of plasmids encoding either beta-galactosidase or endothelial nitric oxide synthase to the endothelial cells of conductance arteries. Importantly, there was preservation of the functional integrity of the transfected endothelial cell layer after ultrasound treatment.

Other potential therapeutic applications of microbubble targeted drug delivery

Restenosis after vascular balloon injury or stent deployment has been shown to result from neointimal hyperplasia due to smooth muscle cell migration and proliferation. The c-*myc* protooncogene is responsible for the regulation of gene expression involved in the process of intimal hyperplasia that leads to restenosis. Synthetic antisense oligonucleotides, such as those to the c-*myc* protooncogene, can bind to the messenger ribonucleic acid and inhibit the synthesis of the protooncogenes. Therefore, antisense to c-*myc* protooncogene can prevent its translation into proteins that may be mediators of the pathologic process of restenosis. These synthetic agents, when administered directly into the vessel, have successfully inhibited restenosis after coronary or carotid injury.[53] In 1996 Porter et al.[27] demonstrated that perfluorocarbon-exposed sonicated dextrose albumin (PESDA) microbubbles, unlike room air-containing sonicated dextrose albumin microbubbles, have bioactive albumin on their surface that can avidly bind synthetic antisense oligonucleotides, and then release them in the presence of ultrasound. In the initial study that examined the effectiveness of PESDA and ultrasound in enhancing the delivery of the antisense to c-*myc*, 21 pigs had carotid balloon injury performed with an oversized balloon catheter and were randomized to receive intravenous antisense to c-*myc* bound to PESDA, intravenous antisense alone, or no treatment. The pigs that received antisense bound to PESDA also had transcutaneous 20 kHz ultrasound applied over the carotid wall following injections. The ultrasound-targeted group showed a significantly lower percent area stenosis ($8 \pm 2\%$) than the two control groups ($19 \pm 8\%$ and $28 \pm 3\%$; $p < 0.01$).[27]

Since PESDA microbubbles adhere to sites of endothelial injury even in the absence of ultrasound, the efficacy of this therapy in inhibiting coronary restenosis has been evaluated in animals. Porter et al.[46] measured the uptake of antisense to c-*myc* into coronary arteries using high-phase liquid chromatography in pigs. Intravenous PESDA containing anti c-*myc* was given in the presence or absence of transthoracic 1 MHz ultrasound (0.6 W/cm^2). In this study, the authors demonstrated that anti c-myc could be selectively concentrated within a stretch-injured coronary artery segment when given intravenously bound to PESDA. The decrease in neointimal formation following intravenous injection of anti c-myc with PESDA without ultrasound was similar to that observed with higher doses of the same antisense given directly into the coronary artery using an infiltrator de-

livery system.[54] The basis for this hypothesis stems from previous observations that albumin-coated microbubbles adhere to activated endothelial cells.[35,55,65] Albumin-coated microbubbles have been observed binding to activated leukocytes and monocytes which slowly roll along injured venular endothelial cells.[35] Since leukocyte and monocyte accumulation has also been observed early following arterial balloon injury,[56] it is possible that PESDA microbubbles were concentrated at the injured coronary artery surface by adherence to these activated cells. Other potential mechanisms could be related to complement activation, since both albumin- and lipid-encapsulated microbubbles take up complement proteins,[57] and thus may bind to upregulated complement receptors at the injured surface. Lu et al. have also demonstrated that albumin-coated microbubbles significantly improved transgenic expression in skeletal muscle of mice, even in the absence of ultrasound. However, in this study, the delivery was an intramuscular injection of microbubbles and plasmid into otherwise normal tissue, and not in the setting of endothelial injury.[58]

Another innovative application of microbubbles and ultrasound is in the delivery of proteins that induce growth of endothelial cells, such as vascular endothelial growth factor (VEGF). Mukherjee et al.[34] demonstrated a marked increase in endothelial VEGF uptake using ultrasound alone (eight-fold increase) and using ultrasound and PESDA (ten- to thirteen-fold increase, as compared to control) in the myocardium of rats. In a canine model of chronic myocardial ischemia, intravenous infusion of VEGF combined with ultrasound and an albumin-based contrast agent significantly reduced the infarct area/risk area ratio, and increased myocardial blood flow in the ischemic territory, suggesting a new potential therapeutic approach for angiogenesis.[59]

Optimization of ultrasound parameters for targeted drug delivery and thrombolysis

The effect of several ultrasound parameters, including transducer frequency and acoustic power, are known to influence microbubble destruction and, thus, the transfection of genes and drugs. Although the optimal ultrasound parameters for maximizing this process are not known, we will briefly discuss some important aspects. Unger et al.[30] have shown that the frequency of ultrasound used to destroy phospholipid-coated microbubbles may regulate how much drug is released in vitro. When analyzing the number of acoustically active particles that persist after exposure to different types of ultrasound in a flow chamber, they demonstrated that a 2.5-MHz transducer resulted in some destruction, but the addition of a lower-frequency transducer (100 kHz) significantly increased the destruction. When the 100-kHz energy was given in a pulsed-wave mode as opposed to a continuous wave, the destruction of microbubbles was even faster. In a similar way, Porter et al.[45] have demonstrated that a continuous wave diagnostic ultrasound frequency of 2 MHz was not able to enhance the carotid uptake of antisense to c-*myc* protooncogene (0.19 ± 0.04 μg/mg), but low-frequency 20 kHz ultrasound significantly increased vascular uptake (0.28 ± 0.04 μg/mg; $p = 0.008$ vs other groups) when compared to antisense bound to PESDA alone (0.21 ± 0.06 μg/mg). The results of this study suggested that a lower frequency could be better suited to target antisense deposition into major vessels. Because there were minimal differences in peak negative pressure generated by 2 MHz and 20 kHz in this study (46 kPa and 13 kPa, respectively), the enhanced uptake was attributed to a lower threshold for cavitation with 20 kHz frequency.

In another study, the efficacy of ultrasound-mediated delivery of VEGF bound to PESDA into the myocardium of rats was evaluated with an ultrasound frequency of 1.0 MHz at various acoustical outputs (0.2, 0.4, 0.6, 0.8 and 1.0 W/cm^2). The authors found a significant increase in VEGF uptake with the combination of ultrasound and PESDA at all power outputs when compared with controls, but there was a dose-dependent increase in the amount of VEGF uptake with increasing power until 0.6 W/cm^2 and a subsequent plateau. Table 2 illustrates some parameters used in previous studies for drug and gene delivery. It seems that at higher frequencies, higher peak negative pressures are necessary to induce cavitation-mediated drug and gene delivery using microbubbles and ultrasound. In a recent study of Chen et al,[32] it was shown that, when using ultrasound at diagnostic frequencies, optimal ultrasound parameters for gene expression by ultrasound-targeted microbubble destruction to the myocardial microcirculation included a low-transmission frequency (1.3 MHz), high mechanical index, and electrocardiogram triggering to allow complete filling of the myocardial capillary bed by microbubbles. The authors

Table 2. Ultrasound parameters and microbubbles used for delivering genes and drugs

Authors	Transfection	Transducer frequency	Delivery mode	Mechanical index	Output	Peak negative pressure	Microbubble	Efficacy
Porter TR et al.[46]	Antisense c-*myc* protooncogene	1 MHz	PW		0.6 W/cm^2		PESDA	+
Zhou Z et al.[60]	VEGA	1 MHz	CW		1.2 W/cm^2		Sonazoid	+
Taniyama Y et al.[31]	Luciferase				2.5 W/cm^2		Optison	+
Teupe C et al.[52]	β-galactosidase/eNOS	2.2-4.4 MHz	CW	1.2			Gas-filled albumin microbubble	+
Porter TR et al.[45]	Antisense c-*myc* protooncogene	2 MHz	CW			13 kPa	PESDA	-
		20 kHz	CW			46 kPa	PESDA	+
Mukherjee D et al.[34]	VEGA	1.0 MHz	CW		0.2 W/cm^2	0.164 MPa	PESDA	9.37 ± 1.98*
		1.0 MHz	CW		0.4 W/cm^2	0.194 MPa	PESDA	18.58 ± 2.46*
		1.0 MHz	CW		0.6 W/cm^2	0.328 MPa	PESDA	23.12 ± 3.95*
		1.0 MHz	CW		0.8 W/cm^2	0.394 MPa	PESDA	25.46 ± 2.78*
		1.0 MHz	CW		1.0 W/cm^2	0.419 MPa	PESDA	26.48 ± 3.98*
Shohet RV et al.[33]	β-galactosidase	1.3 MHz	ECG-triggered	1.5			Perfluorocarbon-filled microbubbles	+
Bao S et al.[50]	Luciferase	2.25 MHz				0.2-0.4 MPa	Albunex	+

* Efficacy is demonstrated as mean ± SD endothelial vascular growth factor uptake by enzyme-linked immunosorbent assay.
CW = continuous wave; ECG = electrocardiogram; eNOS = endothelial nitric oxide synthase; PESDA = perfluorocarbon-exposed sonicated dextrose and albumin; PW = pulsed wave; VEGF = vascular endothelial growth factor.

found that maximal acoustic pressure resulted in higher myocardial gene expression, providing indirect evidence that high peak negative pressures increase the amount of gene delivery from microbubbles.

Ultrasound and microbubbles have also been utilized to dissolve intravascular thrombi in the absence of a fibrinolytic agent.[60-62] Although these studies were uniformly successful in animal studies where the thrombus formation was acute and there was minimal attenuation of the ultrasound beam, it is unclear how effective this modality will be in the clinical area. Again, the frequency and output of ultrasound used to induce dissolution of thrombi ranged from the kilohertz to megahertz range. As with ultrasound-mediated drug delivery, however, the higher peak negative pressures appeared to have the highest success rates.[62]

However, a high peak negative pressure may have detrimental bioeffects. Several investigators have reported on the occurrence of tissue hemorrhage and endothelial cell damage after ultrasound exposure of cultured cells and organs containing air, such as the lungs or the intestine.[63-65] Ay et al.[64] examined the functional and morphological effects of ultrasound and contrast in an isolated rabbit heart preparation, using increasing levels of acoustic energy. Simultaneous exposure to contrast and high-energy ultrasound resulted in a reversible and transient decrease in left ventricular contractile performance, increase in the coronary perfusion pressure, increase in the myocardial lactate release, and presence of intramural hemorrhage in the plane of ultrasound transmission. Additionally, light microscopy revealed the presence of capillary ruptures, erythrocyte extravasation and endothelial cell damage. These effects were directly related to the mechanical index. These studies indicate that although high-energy ultrasound seems to be necessary to induce tissue permeability facilitating local drug delivery, it may also have significant bioeffects in the myocardium. Therefore, the optimal ultrasound parameters to enhance drug delivery with microbubbles remain to be determined.

REFERENCES

1. Mulvagh SL, DeMaria AN, Feinstein, SB, et al. Contrast echocardiography: current and future applications. *J Am Soc Echo-*

cardiogr 2000;13:331-42.
2. Kaul S, Senior R, Dittrich H, et al. Detection of coronary artery disease with myocardial contrast echocardiography: comparison with 99mTc-sestamibi single-photon emission computed tomography. *Circulation* 1997;96:785-92.
3. Porter TR, Li S, Kricsfeld D, Armbruster RW. Detection of myocardial perfusion in multiple echocardiographic windows with one intravenous injection of microbubbles using transient response second harmonic imaging. *J Am Coll Cardiol* 1997;29: 791-9.
4. Porter TR, Xie F, Silver M, et al. Real-time perfusion imaging with low mechanical index pulse inversion Doppler imaging. *J Am Coll Cardiol* 2001;37:748-53.
5. Shimoni S, Zoghbi WA, Xie F, et al. Real-time assessment of myocardial perfusion and wall motion during bicycle and treadmill exercise echocardiography: comparison with single-photon emission computed tomography. *J Am Coll Cardiol* 2001;37:741-7.
6. Wei K, Ragosta M, Thorpe J, et al. Noninvasive quantification of coronary blood flow reserve in humans using myocardial contrast echocardiography. *Circulation* 2001;103:2560-5.
7. Peltier M, Vancraeynest D, Pasquet A, et al. Assessment of the physiologic significance of coronary disease with dipyridamole real-time myocardial contrast echocardiography: comparison with technetium-99m sestamibi single-photon emission computed tomography and quantitative coronary angiography. *J Am Coll Cardiol* 2004;43:257-64.
8. Villanueva FS, Glasheen WP, Sklenar J, Kaul S. Assessment of risk area during coronary occlusion and infarct size after reperfusion with myocardial contrast echocardiography using left and right atrial injections of contrast. *Circulation* 1993;88:596-604.
9. Coggins MP, Sklenar J, Le DE, et al. Noninvasive prediction of ultimate infarct size at the time of acute coronary occlusion based on the extent and magnitude of collateral-derived myocardial blood flow. *Circulation* 2001;104:2471-7.
10. Kloner RA, Ganote CE, Jennings RB. The "no-reflow" phenomenon after temporary coronary occlusion in the dog. *J Clin Invest* 1974; 54:1496-508.
11. Ito H, Tomooka T, Sakai N, et al. Lack of myocardial perfusion immediately after successful thrombolysis: a predictor of poor recovery of left ventricular function in anterior myocardial infarction. *Circulation* 1992;85:1699-705.
12. Ito H, Okamura A, Iwakura K, et al. Myocardial perfusion patterns related to thrombolysis in myocardial infarction perfusion grades after coronary angioplasty in patients with acute anterior wall myocardial infarction. *Circulation* 1996;93:1993-9.
13. Sabia PJ, Powers ER, Jayaweera AR, et al. Functional significance of collateral blood flow in patients with recent acute myocardial infarction: a study using myocardial contrast echocardiography. *Circulation* 1992;85:2080-9.
14. Ito H, Iwakura K, Oh H, et al. Temporal changes in myocardial perfusion patterns in patients with reperfused anterior wall myocardial infarction: their relation to myocardial viability. *Circulation* 1995;91:656-62.
15. Sakuma T, Otsuka M, Okimoto T, et al. Optimal time for predicting myocardial viability after successful primary angioplasty in acute myocardial infarction: a study using myocardial contrast echocardiography. *Am J Cardiol* 2001;87:687-92.
16. Brochet E, Czitrom D, Karila-Cohen D, et al. Early changes in myocardial perfusion patterns after myocardial infarction: relation with contractile reserve and functional recovery. *J Am Coll Cardiol* 1998;32:2011-7.
17. Bolognese L, Antoniucci D, Rovai D, et al. Myocardial contrast echocardiography versus dobutamine echocardiography for predicting functional recovery after acute myocardial infarction treated with primary coronary angioplasty. *J Am Coll Cardiol* 1996;28:1677-83.
18. Swinburn JM, Lahiri A, Senior R. Intravenous myocardial contrast echocardiography predicts recovery of dysynergic myocardium early after acute myocardial infarction. *J Am Coll Cardiol* 2001; 38:19-25.
19. Senior R, Swinburn JM. Incremental value of myocardial contrast echocardiography for the prediction of recovery of function in dobutamine nonresponsive myocardium early after acute myocardial infarction. *Am J Cardiol* 2003;91:397-402.
20. Afridi I, Grayburn PA, Panza JA, et al. Myocardial viability during dobutamine echocardiography predicts survival in patients with coronary artery disease and severe left ventricular systolic dysfunction. *J Am Coll Cardiol* 1998;32:921-6.
21. Nagueh SF, Vaduganathan P, Ali N, et al. Identification of hibernating myocardium: comparative accuracy of myocardial contrast echocardiography, rest-redistribution thallium-201 tomography and dobutamine echocardiography. *J Am Coll Cardiol* 1997;29:985-93.
22. deFilippi CR, Willett DL, Irani WN, et al. Comparison of myocardial contrast echocardiography and low-dose dobutamine stress echocardiography in predicting recovery of left ventricular function after coronary revascularization in chronic ischemic heart disease. *Circulation* 1995;92:2863-8.
23. Nagueh SF, Zoghbi WA. Stress echocardiography for the assessment of myocardial ischemia and viability. *Curr Probl Cardiol* 1996;21:445-520.
24. Shimoni S, Frangogiannis NG, Aggeli CJ, et al. Identification of hibernating myocardium with quantitative intravenous myocardial contrast echocardiography: comparison with dobutamine echocardiography and thallium-201 scintigraphy. *Circulation* 2003;107:538-44.
25. Skyba DM, Price RJ, Linka AZ, et al. Direct in vivo visualization of intravascular destruction of microbubbles by ultrasound and its local effects on tissue. *Circulation* 1998;98:290-3.
26. Price RJ, Skyba DM, Kaul S, Skalak TC. Delivery of colloidal particles and red blood cells to tissue through microvessel ruptures created by targeted microbubble destruction with ultrasound. *Circulation* 1998;98:1264-7.
27. Porter TR, Iversen PL, Li S, Xie F. Interaction of diagnostic ultrasound with synthetic oligonucleotide-labeled perfluorocarbon-

exposed sonicated dextrose albumin microbubbles. *J Ultrasound Med* 1996;15:577-84.
28. Main ML, Grayburn PA. Clinical applications of transpulmonary contrast echocardiography. *Am Heart J* 1999;137:144-53.
29. Wei K, Skyba DM, Firschke C, et al. Interactions between microbubbles and ultrasound: in vitro and in vivo observations. *J Am Coll Cardiol* 1997;29:1081-8.
30. Unger EC, McCreery TP, Sweitzer RH, et al. Acoustically active lipospheres containing paclitaxel: a new therapeutic ultrasound contrast agent. *Invest Radiol* 1998;33:886-92.
31. Taniyama Y, Tachibana K, Hiraoka K, et al. Local delivery of plasmid DNA into rat carotid artery using ultrasound. *Circulation* 2002;105:1233-9.
32. Chen S, Shohet RV, Bekeredjian R, et al. Optimization of ultrasound parameters for cardiac gene delivery of adenoviral or plasmid deoxyribonucleic acid by ultrasound-targeted microbubble destruction. *J Am Coll Cardiol* 2003;42:301-8.
33. Shohet RV, Chen S, Zhou YT, et al. Echocardiographic destruction of albumin microbubbles directs gene delivery to the myocardium. *Circulation* 2000;101:2554-6.
34. Mukherjee D, Wong J, Griffin B, et al. Ten-fold augmentation of endothelial uptake of vascular endothelial growth factor with ultrasound after systemic administration. *J Am Coll Cardiol* 2000;35:1678-86.
35. Villanueva FS, Jankowski RJ, Manaugh C, Wagner WR. Albumin microbubble adherence to human coronary endothelium: implications for assessment of endothelial function using myocardial contrast echocardiography. *J Am Coll Cardiol* 1997;30:689-93.
36. Miller MW. Gene transfection and drug delivery. *Ultrasound Med Biol* 2000;26 Suppl 1:S59-S62.
37. Unger EC, McCreery TP, Sweitzer RH, et al. Acoustically active lipospheres containing paclitaxel: a new therapeutic ultrasound contrast agent. *Invest Radiol* 1998;33:886-92.
38. Fritz TA, Unger EC, Sutherland G, Sahn D. Phase I clinical trials of MRX-115: a new ultrasound contrast agent. *Invest Radiol* 1997;32:735-40.
39. Lindner JR, Song J, Christiansen J, et al. Ultrasound assessment of inflammation and renal tissue injury with microbubbles targeted to P-selectin. *Circulation* 2001;104:2107-12.
40. Weller GE, Lu E, Csikari MM, et al. Ultrasound imaging of acute cardiac transplant rejection with microbubbles targeted to intercellular adhesion molecule-1. *Circulation* 2003;108:218-24.
41. Chomas JE, Dayton P, Allen J, et al. Mechanisms of contrast agent destruction. *IEEE Trans Ultrason Ferroelectr Freq Control* 2001;48:232-48.
42. Chomas JE, Dayton P, May D, Ferrara K. Threshold of fragmentation for ultrasonic contrast agents. *J Biomed Opt* 2001; 6:141-50.
43. Song J, Chappell JC, Qi M, et al. Influence of injection site, microvascular pressure and ultrasound variables on microbubble-mediated delivery of microspheres to muscle. *J Am Coll Cardiol* 2002;39:726-31.
44. Deng CX, Sieling F, Pan H, Cui J. Ultrasound-induced cell membrane porosity. *Ultrasound Med Biol* 2004;30:519-26.
45. Porter TR, Hiser WL, Kricsfeld D, et al. Inhibition of carotid artery neointimal formation with intravenous microbubbles. *Ultrasound Med Biol* 2001;27:259-65.
46. Porter TR, Knnap D, Venneri L, et al. Increased suppression of intracoronary c-myc protein synthesis within the stent or balloon injury site using an intravenous microbubble delivery system containing antisense to c-myc: comparison with direct intracoronary injection. *J Am Coll Cardiol* 2003;41:431A.
47. Verma IM, Somia N. Gene therapy: promises, problems and prospects. *Nature* 1997;389:239-42.
48. Newman KD, Dunn PF, Owens JW, et al. Adenovirus-mediated gene transfer into normal rabbit arteries results in prolonged vascular cell activation, inflammation, and neointimal hyperplasia. *J Clin Invest* 1995;96:2955-65.
49. Felgner PL. Nonviral strategies for gene therapy. *Sci Am* 1997; 276:102-6.
50. Bao S, Thrall BD, Miller DL. Transfection of a reporter plasmid into cultured cells by sonoporation in vitro. *Ultrasound Med Biol* 1997;23:953-9.
51. Bekeredjian R, Chen S, Frenkel PA, et al. Ultrasound-targeted microbubble destruction can repeatedly direct highly specific plasmid expression to the heart. *Circulation* 2003;108:1022-6.
52. Teupe C, Richter S, Fisslthaler B, et al. Vascular gene transfer of phosphomimetic endothelial nitric oxide synthase (S1177D) using ultrasound-enhanced destruction of plasmid-loaded microbubbles improves vasoreactivity. *Circulation* 2002;105:1104-9.
53. Shi Y, Fard A, Galeo A, et al. Transcatheter delivery of c-myc antisense oligomers reduces neointimal formation in a porcine model of coronary artery balloon injury. *Circulation* 1994;90: 944-51.
54. Kipshidze NN, Kim HS, Iversen P, et al. Intramural coronary delivery of advanced antisense oligonucleotides reduces neointimal formation in the porcine stent restenosis model. *J Am Coll Cardiol* 2002;39:1686-91.
55. Keller MW, Geddes L, Spotnitz W, et al. Microcirculatory dysfunction following perfusion with hyperkalemic, hypothermic, cardioplegic solutions and blood reperfusion: effects of adenosine. *Circulation* 1991;84:2485-94.
56. Xing D, Miller A, Novak L, et al. Estradiol and progestins differentially modulate leukocyte infiltration after vascular injury. *Circulation* 2004;109:234-41.
57. Fisher NG, Christiansen JP, Klibanov A, et al. Influence of microbubble surface charge on capillary transit and myocardial contrast enhancement. *J Am Coll Cardiol* 2002;40:811-9.
58. Lu QL, Liang HD, Partridge T, Blomley MJ. Microbubble ultrasound improves the efficiency of gene transduction in skeletal muscle in vivo with reduced tissue damage. *Gene Ther* 2003;10:396-405.
59. Zhou Z, Mukherjee D, Wang K, et al. Induction of angiogenesis in a canine model of chronic myocardial ischemia with intravenous infusion of vascular endothelial growth factor (VRGF) combined with ultrasound energy and echo contrast agent. *J Am Coll*

Cardiol 2003;39:396A.

60. Birnbaum Y, Luo H, Nagai T, et al. Noninvasive in vivo clot dissolution without a thrombolytic drug: recanalization of thrombosed iliofemoral arteries by transcutaneous ultrasound combined with intravenous infusion of microbubbles. *Circulation* 1998;97:130-4.
61. Culp WC, Porter TR, Xie F, et al. Microbubble -potentiated ultrasound as a method of declotting thrombosed dialysis grafts: experimental study in dogs. *Cardiovasc Intervent Radiol* 2001; 24:407-12.
62. Culp WC, Porter TR, McCowan TC, et al. Microubble-augementd ultrasound declotting of thrombosed arteriovenous dialysis grafts in dogs. *J Vasc Interv Radiol* 2003;14:343-7.
63. ter Haar GR. Ultrasonic contrast agents: safety considerations reviewed. *Eur J Radiol* 2002;41:217-21.
64. Ay T, Havaux X, Van Camp G, et al. Destruction of contrast microbubbles by ultrasound: effects on myocardial function, coronary perfusion pressure, and microvascular integrity. *Circulation* 2001;104:461-6.
65. Holland CK, Deng CX, Apfel RE, et al. Direct evidence of cavitation in vivo from diagnostic ultrasound. *Ultrasound Med Biol* 1996;22:917-25.

微泡 (Microbubbles) 在診斷及治療上的應用

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在進行催迫性心臟超音波檢查時，利用靜脈注射微泡 (microbubbles) 配合心肌顯影超音波 (myocardial contrast echocardiography) 檢查，能夠有效的偵測出心肌灌注異常的部位。近年來，憑藉即時呈現低功率頻序譜 (low mechanical index pulse sequence schemes) 技術的發展，甚至能夠讓臨床醫師在進行藥物催迫性超音波檢查時，同步的偵測出心肌收縮及灌注的異常。對於急性心肌梗塞及慢性冠狀動脈心臟病，使用靜脈注射微泡配合心肌顯影超音波檢查，也有助於判斷收縮異常部位的心肌存活度 (viability)。微泡由於體積極小，因此可以自由的經由血管循行全身。而若某處血管的內皮細胞功能異常時，微泡即會附著於該處。利用微泡的此種特性，或可將藥物或基因結合於微泡表面，而選擇性的讓有內皮細胞異常的欲治療部位之藥物或基因濃度提高。利用超音波能量擊破微泡亦可能有助於局部藥物釋放及血管內血栓溶解。

關鍵詞：心肌顯影超音波、藥物釋放、血栓溶解。