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REVIEW ARTICLE

The Fading Art of Microsphere-Derived Measurement of Absolute Myocardial Blood Flow

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Abstract

The measurement of absolute myocardial blood flow (MBF) has played a pivotal role in the development of nuclear cardiology and other perfusion imaging techniques. However, the capacity to perform such experiments may be diminished. This review examines the basic physiology of microsphere measurement of absolute MBF which was developed over 50 years ago, with multiple refinements over time. The use of different types of microspheres is presented in depth. The set-up and performance of a large animal model is detailed with tips and pitfalls explained. It is the purpose of this review to stimulate the next generation of investigators into considering this skill as part of their research tool box.

Keywords: Coronary blood flow, Microspheres, Cardiac imaging
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Absolute myocardial blood flow (MBF) measures are the goal of noninvasive perfusion techniques. Introduced in the 1960s and 70s (1–7), the use of labeled microspheres in animal models has provided a gold-standard to which noninvasive perfusion tracers can be compared. From thallium-201 (8, 9) and ^{99m}Tc sestamibi (10–12) to ^{18}F-Flurpiradaz (13–15), Rubidium-82 (16) first-pass MRI (17–19) and first-pass coronary CT (20–22), microsphere studies have provided important validations. There are relatively few labs that have maintained expertise in the technique and they are becoming fewer. It is the purpose of this review to stimulate the next generation of investigators into developing this skill as a component of their research tool box.

Overview

Physiology

A major principle in nuclear cardiology is that tracer uptake in the heart should be proportional to MBF. But the uptake and retention are less than ideal for all tracers, with only water being freely permeable. The tracers used require an uptake mechanism and they are never 100% extracted from the circulation on the first-pass. Isolated heart Langendorff preparations of coronary flow of both small and large animals have been used extensively to calculate the first-pass extraction fraction of a perfusion tracer at specific coronary blood flow rates (23, 24). This approach can be combined with microsphere deposition and quantified. Consequently, perfusion tracer uptake and retention in the myocardium can be imaged and quantified at known coronary flow rates. Alternatively, one can also solve for tissue MBF in-vivo and assess perfusion tracer properties under physiologic conditions using the labeled-microsphere technique.

Microspheres are made from polymers such as polyvinyl alcohol or ceramic compounds and labeled with radioactivity or color. Due to their size (15 microns), they are 100% trapped in myocardial capillaries and cannot redistribute with time (2, 3). This is true for both high and low flow rates. In order to quantitate the quantity of microspheres deposited, the heart must be removed and sectioned, which necessitates sacrifice of the animal. In addition, the potential microspheres available to the coronary arteries needs to be established (an arterial input function). This is accomplished using an arterial reference sample of known flow-rate that can capture microspheres in the arterial circulation (5). If the reference sample contains n number of microspheres at a known flow rate q, the measurement of X number of microspheres in a

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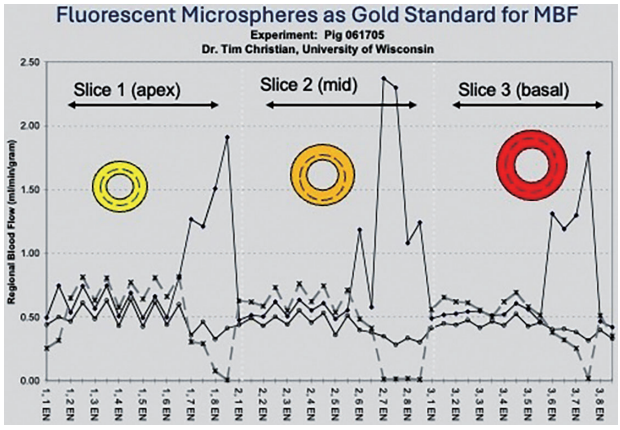

Figure 1 A report of absolute MBF from a pig experiment of hyperemia and coronary occlusion. Each short-axis slice was divided into 8 radial segments then transected into endocardial and epicardial slices (EN=endocardial, no label=epicardial segment). 12 o'clock corresponds to the anterior wall and the segments go clock wise laterally to inferior to septal and back to anterior. Resting MBF (open circles) is approximately 0.6 ml/min/g with epicardial flow consistently higher than endocardial flow. During LAD coronary occlusion (X-marks), resting flow is <0.1 ml/min/g in the antero-septum at apex mid and basal levels. During intracoronary adenosine infusion down the LAD, There is marked hyperemia in the anterior segments with MBF values of 1.7 to 2.4 ml/min/g (17).

defined myocardial zone must mean the flow to that zone was Q ml/mim/g. In this way, an artificial organ with known flow-rate is created (usually from the descending aorta) and used for comparison to tissue concentrations in the myocardium using the following formula:

$$MBF\left(\frac{\frac{ml}{g}}{\text{min}}\right) = \frac{tissue\ counts}{tissue\ weight\ (g)} \times \frac{reference\ flow\ \left(\frac{ml}{min}\right)}{reference\ counts}$$

Where tissue counts=the intensity of the microsphere signal per g tissue and reference counts is the microsphere signal intensity in the blood sample drawn from the descending aorta. Note that counts in the tissue and blood cancel out leaving a value in ml/min/g. Consequently, absolute MBF can be generated from any myocardial region of the left ventricle (LV) and serve as a gold-standard for noninvasive methods attempting to quantify MBF. Figure 1 is an example of the quantitation of absolute MBF for three short-axis slices of the LV sectioned into endocardial and epicardial segments (note the consistently higher epicardial flow).

Resolution

As with all imaging, there is a trade-off between spatial resolution and signal intensity. For microsphere studies, the resolution is a function of the number of spheres injected and the size of the myocardial segment to be analyzed. 400 spheres per g tissue is accepted as the minimal signal necessary for reliable detection (25). Sectioning tissue such that counts fall below that threshold introduces error into the flow measures. For both large and small animal studies, there are accepted microsphere dosages to ensure adequate counts in the heart. Initially it was feared that injecting too many microspheres would block arterioles and underestimate blood flow values on subsequent injections but this has been studied extensively and found not to be the case (26). There is some aggregation of microspheres in the capillaries but the overall distribution on a per g basis is uniform (27). The retention of microspheres in the vasculature is stable over prolonged periods of time (28).

Methods

Myocardial blood flow set-up

It is important to generate a wide range of flows in any experiment as the issues of signal during low flow and loss of linearity during high flow states between tracer and MBF are usually factors that need to be addressed. In order to accomplish this, the heart needs to be surgically instrumented, which requires an experienced animal technician/surgeon. After anesthesia, intubation and a thoracotomy, the coronary arteries need to be identified and dissected out from the epicardium. In our experiments (12, 18, 19, 22), the left anterior descending artery (LAD) is freed from the perivascular fat and an occlusive device is placed proximally (an ameroid constrictor or simple suture snare). Either the left circumflex or a diagonal artery is then cannulated with a catheter for intra-coronary adenosine infusion. In this manner, both occlusion and hyperemia can be produced during an experiment (Figure 2). Microspheres can be injected into either the left atrium (through the left atrial appendage) or LV apex through a catheter whenever a flow determination is required (27). A catheter is inserted through the femoral artery into the descending aorta to serve as the reference sampling site. The catheter is attached to a Harvard pump or similar apparatus where the flow rate of withdrawal can be specified. The pump is started shortly before injection and continued for two minutes. It is important to watch the pump during withdrawal as the syringe can lose suction and underestimate flow. Since all the microspheres are trapped in the capillary circulation, there is no recirculation of spheres after the first-pass.

Following the completion of the experiment, the heart is removed from the animal and sectioned into segments for analysis. For the great majority of imaging myocardial studies, the segmentation follows a standard 16 or 17 segment model with three short axis slices (apical, mid, and basal) and five radial segments per slice (Figure 1). In this manner, the segments can be registered to the noninvasive images which the study is analyzing.

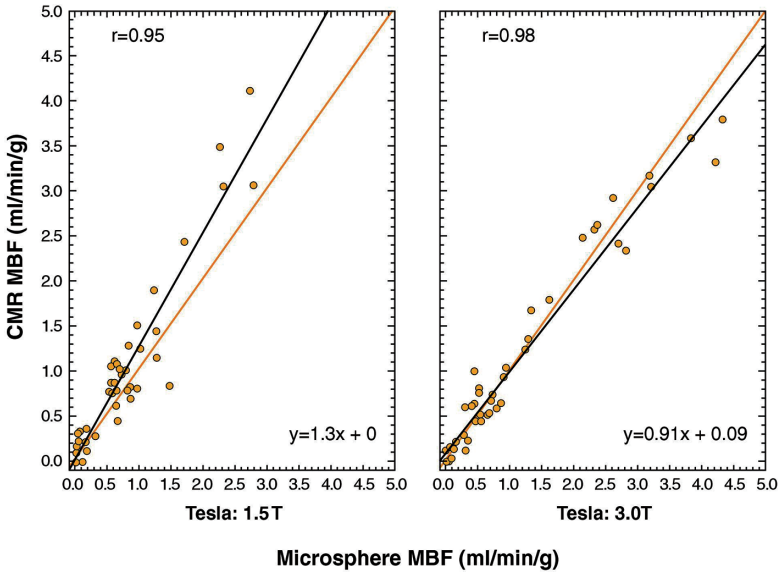

Figure 2 The overall estimates of absolute MBF by first-pass MRI T 1.5 T (N=9) and 3.0 T (N=8), y-axis correlated with absolute MBF by fluorescent microspheres (x-axis) from the pig experiments shown in Figure 2. These correlations with fluorescent labeled microspheres are typical outcome figures in such experiments. Note the wide range in MBF (up to 4.0 ml/min/g). This is important to establish linearity on lack of it at higher flow rates for any tracer (19).

Table 1 Radioisotopes for potential tagging to microspheres

Isotope	Primary gamma energy (KeV)	½ life (days)
Cobalt 57	122	270
Cerium 141	145	33
Chromium 51	320	28
Sn 113 (tin)	393	115
Ruthenium 103	497	39
Strontium 85	514	64
Nubium 95	765	35

A note about statistics

The segments are not independent data points. They are linked by experiment. Conditions can vary between experiments and impact MBF results as a whole. Caution should be used when very large correlations are employed. Some means of compressing the segments into mean coronary territory MBF can help address this issue as well as other statistical approaches (29). A typical linear correlation between noninvasive estimates of absolute MBF and microsphere-derived absolute MBF is shown in Figure 2.

Microsphere choices

Microspheres alone are not easily detectable in the myocardium and so they require a tag for identification. The two major categories of tags are radioisotopes and fluorescent dye or color. Experiments in MBF are often serial in nature, i.e. MBF at rest, during hyperemia and/or during ischemia. The use of multiple radioisotopes or colors for each phase of the experiment allows for measurement of different MBF states even though the information is collected post-mortem. Because isotope photopeak and color spectra can be separated by energy, different flow rates are generated for a single animal (Figure 1). There are strengths and weaknesses for both methods.

Radio-isotope labeled microspheres

Radio-labeled microspheres take advantage of the characteristic gamma energy decay given off for a given isotope (Table 1). These isotopes generally have long half-lives so that they can be stored for a significant period of time prior to use. ^{141}Ce, ^{85}Sr, ^{57}Co, and ^{95}Nb are isotopes among others that have been used to tag microspheres (3). Because the energy photo-peaks vary by isotope, multiple measures of MBF can be made in the same animal (28, 30). Using 5 different isotopes (12), we were able to quantify serial changes in blood flow weekly over 5 weeks in a single animal with progressive occlusion of the LAD artery (Figure 3).

Following completion of an experiment and myocardial sectioning, each segment is placed in a test-tube in preparation for radioactivity measurement. This is accomplished with a gamma-well counter. There are a number of parameters to be set for the gamma well counting. These include the photopeak energy, the peak and minimal energy to be determine what photons are counted, and the length of time the sample is in the well counter. It usually takes several hours to run an entire heart through the well counter. For some isotopes, a “cross-

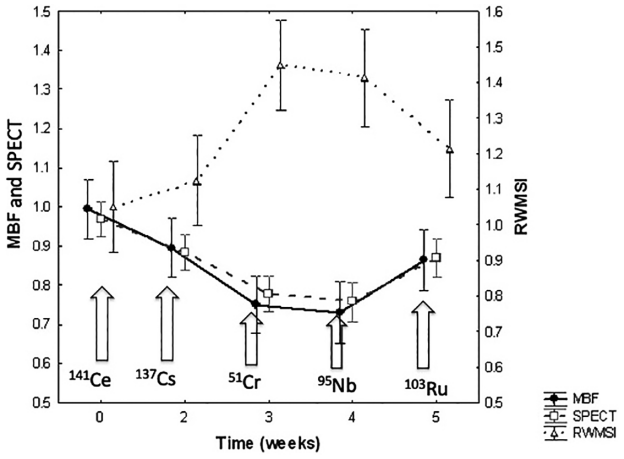

Figure 3 A 5 week experiment of progressive mechanical occlusion of the LAD artery. Five determinations of absolute MBF using different radio-labeled microspheres. There is progressive decrease in LAD MBF (closed circles) with decreasing SPECT activity (open circles), in the anterior wall as well as concomitant development of a regional wall motion abnormality (triangles), until collateral MBF begins to form in this pig mode near week 5 (12).

talk" adjustment has to be made from spill-down in energy from higher energy isotopes into the photo-peak of weaker ones as well as differences in decay half-time for long duration experiments (1, 31). The choice of isotope thus is important when multiple isotopes will be contained in one sample of myocardium.

Advantages and disadvantages of radiolabeled spheres

The primary advantage for radio-labeled spheres is that no tissue processing needs to be done to measure MBF. The samples need only be placed in correct order into the gamma well counter for activity determination, thus completing the study. The investigator can control when and how the samples are counted and there is no additional cost. The only requirement is an on-site gamma well counter.

The disadvantages are multiple. Radioactive spheres cost more initially than colored spheres and the storage time is limited by the decay half-life. The issues of isotope leaching and cross talk in the counter are also sources of error. With the use of ionizing radiation, the experiment now comes under the review of the institutional radiation safety committee and has implications if a clinical scanner such as a single photon emission computed tomography camera is required for the experiment. A spill of an isotope with a 30-day half-life can close the scanner for a prolonged time. If a radioactive tracer such as ^{99m}Tc is given during the experiment, the microsphere counting needs to be delayed for 4 half-lives to avoid contamination. The body of the animal must be stored in a facility until there is sufficient radio-active decay of the microspheres, which adds additional expense. Avoiding long half-life isotopes such as ^{57}Co can reduce this time.

Colored/fluorescent microspheres

The development of a non-radioactive label for microspheres has opened up microsphere experiments to a much broader range of laboratories (32–35). Colored or fluorescent dyes are adhered to polymer-derived microspheres and appear to be more stable over time in biological tissue than radio-labeled spheres (32). Multiple colors (up to 13) can be injected and the dyes are separated out by spectrophotometry with minimal cross-talk. Both the spheres and the analysis are commercially available. The accuracy for measuring MBF has been shown to be at least as good as radio-active spheres (32).

Advantages/disadvantages of fluorescent microspheres

The advantages are multiple. There is no storage issue of the spheres, no decay. They do not require shielding or refrigeration and can be used any time. They are initially cheaper to purchase (but probably more expensive overall). There is no potential contamination of equipment. There is no signal decay over time. They eliminate the need for an on-site gamma well counter, thus expanding the number of centers where this research can be done. There is no need to store the deceased animal.

The major disadvantage is overall cost and loss of control. The samples need to be accurately labeled and sent out to a commercial firm for analysis. The samples are hydrolyzed with potassium hydroxate to separate out the spheres and the analysis is much more time consuming. Consequently, the cost per experiment, while not overwhelming, can be significant. The quality of processing must be taken on faith.

Conclusion

Microsphere-based studies do not require advanced training in basic science. Microsphere determination of absolute MBF should be thought of as a tool that may be part of a nuclear cardiologist's skill set. Having a mentor and a skilled veterinarian technician are essential. But it is a skill obtainable and the studies can be funded from fairly small grants. It is important to keep this avenue of research viable.

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Conflicts of interest

None.

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