Evaluation of Myocardial Tissue Fluid Flow by Fluorescence Cardioscopy in Patients With Coronary Artery Disease

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Summary

Myocardial tissue fluid flow (MTFF) directly represents the oxygen supply to the cardiomyocytes. Therefore, imaging of MTFF is carried out by fluorescence cardioscopy (FC).

Sixty-six patients with coronary artery disease underwent FC using fluorescein as an indicator of MTFF because this dye exhibits fluorescence in tissue fluid but not in the blood. Three mL of 10% fluorescein was injected intravenously and fluorescence images of the left ventricular endocardial surface were obtained by FC at 30 seconds and 1, 3 and 6 minutes later to evaluate the MTFF.

The CF images were classified as follows: diffuse with high intensity indicating normal MTFF; diffuse but with low intensity indicating decreased MTFF, no fluorescence indicating absent MTFF, and patchy fluorescence indicating patchy preservation of MTFF. MTFF was normal in all 18 patients with chest pain syndrome, patchy fluorescence was decreased or absent in 16 of 20 patients with angina and/or old myocardial infarction due to organic coronary artery disease, and was patchy in 21 of 28 patients with vasospastic angina. Ten of these 20 patients underwent coronary stenting with successful angiographic results in all. However, MTFF disturbance frequently remained.

FC is clinically feasible for evaluation of MTFF disturbance, for evaluation of even emergency coronary interventions, and for guidance of transendocardial angiogenic and myogenic therapies in patients with coronary artery disease. (Int Heart J 2010; 51: 153-158)

Key words: Fluorescence cardioscopy, Fluorescein, Myocardial tissue fluid flow, Myocardial ischemia, Coronary stenting

It is well known that oxygen is transported by diffusion from the coronary arterial trees into the interstitial space and flows with tissue fluid towards the cardiomyocytes before it is finally utilized by the cardiomyocytes. Therefore, myocardial tissue fluid flow (MTFF) directly represents oxygen supply to the cardiomyocytes. If MTFF is visualized (imaged), oxygen supply and therefore ischemia can be evaluated clinically.

Several modalities are clinically available to image coronary blood flow. Coronary angiography can image blood flow in small coronary vessels by myocardial staining with contrast media. Contrast echocardiography can roughly image blood flow in relatively large coronary vessels.10 Radionucleid imaging can indirectly image myocardial blood flow, though not MTFF itself.2 Magnetic resonance imaging (MRI) can detect movement of hydrogen nuclei, therefore, it can not discriminate water either inside or outside the blood vessels, and accordingly can not image the MTFF separately.3,4 Enhanced MRI, using a marker such as gadolinium diethylene-triamine penta-acetic acid can detect reversible and irreversible cardiomyocyte necrosis, and their locations, but not MTFF itself.4,5 Computed tomography using contrast media can image coronary blood flow but can not selectively image MTFF.5,6 Conventional cardioscopy can detect blood flow in the shallow layers of the subendocardial myocardium by the changes of color, however, not MTFF.7,9 Thus, there are no suitable clinically available modalities that can be used during cardiac interventions for selective, real-time, and two-dimensional imaging of MTFF and accordingly myocardial blood flow.

Fluorescein is a fluorescence dye that is clinically used to evaluate retinal vessels.10 When injected into the vessels, it diffuses rapidly through the microvessels of the arterial side into the extravascular interstitial spaces supplied by the fluid, and finally drains into the venous system. When this dye is present within the blood vessels it can not exhibit fluorescence as it gets masked by the blood cells. However, when drained into the interstitial spaces, it exhibits strong fluorescence, directly representing MTFF and accordingly the myocardial oxygen supply. Therefore, if the fluorescence of this dye in the myocardium is visualized in vivo, real-time evaluation of MTFF and the corresponding oxygen supply enable us to directly evaluate whether or not ischemia exists.

The authors of the present study developed a fluores-
cence cardiосcopy (FC) system which enables observation of the ventricular wall from inside, and using fluorescein as an indicator, the left ventricular subendocardial MTFF was evaluated in patients with coronary artery disease, in anticipation for the evaluation of MTFF (and accordingly myocardial blood flow) and to provide guidance in transendocardial angiogenic and myocardial therapies.

**METHODS**

**Fluorescence cardiосcopy (FC) system:** The fluorescence cardiосcopy (FC) system was composed of a fluorescence excitation unit, fiberscope guiding balloon catheter, fluorescence emission unit, intensified charged coupled device (ICCD) camera, camera controller, DVD recorder, and television monitor.

The fluorescence excitation unit (CLV-A, Olympus Corporation, Tokyo) comprised a xenon lamp and a filter disc with a 470 nm band pass filter (BP) for fluorescence excitation and a disc without a BP filter exchangeable by rotation for conventional cardiосcopy using white light.

The fiberscope (AF 14, Olympus) was composed of a 5-F fiberscope containing 3000 glass fibers for image guidance and 300 glass fibers for light guidance.

The fiberscope could pass through the 9-F guiding balloon catheter (Clinical Supply Co, Gifu, Japan). The balloon was inflated with CO2. The catheter had a Y connector at the proximal end; one channel for fiberscope insertion and another for saline flush.

The fluorescence emission unit (DD-2, Olympus) was composed of a dichroic membrane which cut the wavelength of light below 515 nm, a band absorption filter (BA) of 515 nm which allowed wavelengths of light more than 515 nm, an ICCD camera (C3505, Hamamatsu Photonics Co, Hamamatsu, Japan), and a camera controller (C3510, Hamamatsu Photonics Co.). This combination of light wavelength for BP and BA filters was optimal for detecting the fluorescence of fluorescein in a preliminary study.

The fiberscope and guiding balloon catheter have been approved for clinical use by the Japanese Ministry of Health, Labor and Welfare.

To observe the endocardial surface of the left ventricle, the light and image guides were connected to the excitation and emission units, respectively.

After setting up the BP and BA filters, the light was irradiated onto the target through the BP filter and light guide. The fluorescence thus evoked was picked up by the ICCD camera through the DM and BA filters for successive two-dimensional imaging. The camera controller could change the camera sensitivity from 0 to 10 steps. However, the fluorescence intensity taken at step 5 alone was utilized to compare the fluorescence intensity of the obtained images among patients.

In a preliminary experiment in beagles, 0.1 mL of 10% fluorescein (Fluoreside⃝, Japan Alcon Co, Tokyo) was injected into the left coronary artery and the heart was removed. The blood inside the coronary vessels was washed out by an intracoronary infusion of saline solution and then the endocardial surface was observed by FC. The surface exhibited fluorescence, indicating that fluorescence was elicited by fluorescein in the tissues outside the blood vessels rather than within the blood vessels. The FC system could not detect any fluorescence in the endocardial surface in beagles not administered fluorescein. In another experiment in beagles, 0.05 mL of 10% fluorescein was injected by a thin needle into the excised left ventricular wall and fluorescence was observed by FC. The present FC system revealed that fluorescence of the dye injected within 1 mm in depth could be detected.

In addition, whole human blood containing 0.5% of fluorescein did not exhibit fluorescence but the plasma which contains the same concentration of fluorescein exhibited fluorescence, indicating that blood cells masked the fluorescence of the dye.

**Evaluation of subendocardial myocardial tissue fluid flow (MTFF) by FC in patients with coronary artery disease:** Clinical studies were carried out at Toho University Sakura Hospital and were approved by the Institutional Review Board. Sixty-six patients with coronary artery diseases classified by the criteria described previously underwent FC. There were 47 males and 19 females; 61.1 ± 2.0 (mean ± SE) years old; 18 with chest pain syndrome (CPS), 28 with vasospastic angina (VSA), 11 with stable angina due to significant (75% or more in % diameter stenosis) organic coronary artery stenosis (AP), and 9 with old myocardial infarction (OMI) complicated with AP. All of the patients provided informed consent for the procedures.

After left ventriculography, a guiding balloon catheter was introduced into the left ventricle and the balloon was inflated with CO2. Next, a fiberscope was introduced into the guiding catheter to place the fiberscope tip at the distal most end of the guiding catheter. Thereafter, the balloon was gently pushed against the endocardial surface. Since the balloon protruded 5 mm ahead of the catheter tip, the distance between the fiberscope tip and the endocardial luminal surface was maintained almost at 5 mm. Heparinized saline solution (10 IU/mL) was then infused at a rate of 10 mL/s by a power injector for 5 seconds to displace the blood between the endocardial surface and the fiberscope.

**Figure 1.** Quantitative measurement of fluorescence intensity. A: A gray scale image of MTFF obtained using an AquaCosmos image analyzer. Arrow: a window for measurement of fluorescence intensity. B: An example demonstrating fluorescence intensity within the window (arrow). Abscissa: fluorescence intensity. Ordinate: time in seconds from the beginning of measurement. From 1 to 4: fluorescence intensity of successive 4 frames. The intensity changed due to cardiac motion.
and to observe the endocardial surface by cardioscopy using white light without the BP and BA filters, namely “conventional cardioscopy”. Next, the BP and BA filters were set in place and 3 mL of 10% fluorescein was injected into the right femoral vein to evaluate regional MTFF as a whole by FC. The FC images were directly obtained by repeated infusion of saline solution at 30 seconds, and 1, 3, and 6 minutes after completing the fluorescein injection. The wall segments observed were as follows: the apical or inferior segment in patients with CPS; the wall segment which was irrigated by the artery in which vasospasm was induced in patients with VSA; the wall segment irrigated by the stenotic artery in patients with AP; and hypo- or akinetic wall segment in patients with OMI.

FC images were displayed on a television monitor simultaneously with fluoroscopic images and the electrocardiogram. Details of the cardioscopic procedures are reported elsewhere. 

Measurement of fluorescence intensity: The fluorescence intensity was quantitatively measured by an image analyzer, AquaCosmos (C7746, Hamamatsu Photonics Co.). CF images were converted into a gray scale image. Next, a window was set on the portion exhibiting the highest fluorescence intensity at end-diastole, and the intensity within the window was measured. The intensity of fluorescence was arbitrarily defined as high, low, or no fluorescence when the intensity scale was more than 150, less than 150 and more than 50, and less than 50, respectively (Figure 1).

Diffuse and high fluorescence intensity were defined as the normal MTFF image pattern. Diffuse fluorescence with low fluorescence intensity was defined as decreased MTFF, patchy fluorescence was identified as patchy preservation of MTFF, and no fluorescence indicated an absence of MTFF.

Evaluation of the effects of coronary stenting on MTFF: Ten of 66 patients having significant stenosis of one or more coronary arteries (4 with AP and 6 with OMI complicated with AP) underwent deployment of a bare-metal stent (Multilink) into the targeted stenotic segment. FC of the wall segment irrigated by the stented artery was performed before and immediately after stent deployment. Diameter stenosis of the coronary artery was measured by TCS Symphony 2.02 (McKesson Co, North Charleston, USA).

Statistical analysis: The data obtained were tested by the χ² test. A P < 0.05 was considered significant.

Results

Changes in MTFF in patients with coronary artery disease: Figure 2 shows the time-course changes in FC images of the left ventricular apical segment in a patient with CPS having normal coronary arterial trees and in whom coronary vasospasm was not inducible by the selective injection of acetylcholine. On conventional cardioscopy, the endocardial surface exhibited a brown color, indicating normal blood flow. Prior to the fluorescein injection, the endocardial surface did not exhibit any fluorescence.

After injecting the dye, the endocardial surface exhibited diffuse and high-intensity fluorescence. The peak fluorescence intensity was attained at 30 seconds after the intravenous dye injection, though it gradually decreased.

Figure 3 shows the time-course changes in FC images in a patient with OMI complicated with AP. The FC images were patchy and the peak fluorescence intensity was attained at 3 minutes, indicating patchy preservation of MTFF and its delayed filling.

Figure 4 shows the time-course changes in FC images in a patient with OMI complicated with AP due to 3-vessel disease. The endocardial surface exhibited a light brown to

![Figure 2](image1.png)  
**Figure 2.** Time-course changes in FC images of the left ventricle in a patient with chest pain syndrome. Sixty-three year old male. CPS. A: Left ventriculogram at end-diastole and at right oblique projection. Arrow: anteroapical segment that was observed by FC. B: Endocardial surface which was observed by conventional cardioscopy. The segment was brown in color, indicating normal blood flow. C to F: FC images of the same segment at 30 seconds and 1, 3, and 6 minutes after the injection of fluorescein, respectively. Diffuseness and high intensity of fluorescence indicates normal MTFF.

![Figure 3](image2.png)  
**Figure 3.** Time-course changes in FC images of the left ventricle in a patient with old myocardial infarction. Sixty-one year old male. OMI of anterior wall of the left ventricle due to two-vessel disease. A: Left ventriculogram at end-diastole at right anterior oblique projection. Arrow: anteroapical segment which was observed by FC. B: Endocardial surface observed by conventional cardioscopy, showing yellowish brown color, indicating fibroelastosis. C to F: FC images of the same portion at 30 seconds and 1, 3, and 6 minutes after the injection of fluorescein, respectively. Delayed and patchy CF images, indicating patchy preservation of MTFF.
white color by conventional cardioscopy, indicating severe ischemia.\(^7,9\) The wall segment exhibited diffuse fluorescence after dye injection, but the fluorescent intensity was low, indicating decreased MTFF.

**Time to peak fluorescence intensity (filling time):** As the time-lag from the cessation of fluorescein injection to reach peak fluorescence intensity correlates with the time required for MTFF filling, it was termed “filling time”. This was compared among the patient groups. As the filling time in the patients with CPS was within 30 seconds, it was considered as the normal filling time value, while a filling time longer than 30 seconds was termed “filling time delay”. Filling time delay was observed in 75% of patients with VSA and in 80% of patients with organic coronary artery disease, namely AP and OMI (Figure 5).

**FC image patterns:** In all the patients studied, before the fluorescein injection, the endocardial surface did not exhibit any fluorescence using the present FC system. Following the fluorescein injection, the endocardial surface exhibited diffuse fluorescence and the peak fluorescent intensity exceeded 150 in all patients with CPS, indicating a normal MTFF. However, diffuse fluorescence with low-fluorescence intensity indicating decreased MTFF and no fluorescence indicating absent MTFF were observed in those patients with organic coronary artery disease. Although it is a functional coronary artery disease, the patients with VSA exhibited patchy fluorescence, indicating patchy preservation of MTFF (Figure 5).

**Effects of coronary stenting on MTFF:** Figure 6 shows a demonstrable example of MTFF recovery induced by coronary stenting in a patient with OMI complicated with AP. A stenotic proximal segment of the left anterior descending artery was dilated by deployment of a bare metal stent. A: Apical segment of the left ventricle that was observed by conventional cardioscopy before stent deployment. The endocardial surface was white in color due to severe ischemia.\(^7,9\) B to D: FC images at 30 seconds and 1 and 3 minutes after the injection of fluorescein before stent deployment, respectively. A: The same apical segment observed by conventional cardioscopy 10 minutes after stent deployment. The color of the apical segment turned pink due to restoration of blood flow.\(^7,9\) a to d: FC images of the same segment after stent deployment and at 30 seconds and 1 and 3 minutes after fluorescein injection, respectively. Increased fluorescence intensity was evident indicating improved MTFF.
Before coronary stenting, absent, decreased, or patchy fluorescence was observed in all 10 patients. This abnormal fluorescent staining remained in 5 patients even after successful stent deployment assessed by angiography, indicating failure to restore normal MTFF (Figure 7).

**Complications:** Following the injection of fluorescein, 7 of 66 patients complained of itching of the skin, though without any eruption. One mL of 1% dl-chlorpheniramine maleate solution (Chlodamin®, Kowa Co, Tokyo), an antihistaminic agent, was injected intravenously, with prompt disappearance of the complaint. No complications attributable to fluorescein were noted in the later phase.

**DISCUSSION**

It is well known that oxygen enters from the capillaries of the coronary arterial side into the interstitial space by diffusion and is conveyed by the tissue fluid towards the cardiomyocytes, where it is finally utilized. Therefore, MTFF directly represents the modality of oxygen supply to the cardiomyocytes. Fluorescein also rapidly diffuses from the blood vessels into the tissue fluid of the interstitial space by diffusion, indicating that it can represent MTFF and accordingly oxygen supply. Therefore, this dye was used as an indicator of MTFF in this study.

The myocardium may itself elicit autofluorescence. However, in this study, the endocardial surface did not exhibit any autofluorescence prior to fluorescein injection. This indicates that the fluorescence that appeared in response to fluorescein injection solely represented the fluorescence elicited by fluorescein and therefore MTFF.

A filling time of 30 seconds and diffuse and high fluorescence intensity observed in patients with CPS was considered as a normal MTFF pattern in this study. Compared to TMFF in CPS, filling time delay which indicated a slow speed of MTFF, patchy fluorescence indicating a regional difference in MTFF, and low fluorescence intensity or no fluorescence which indicated decreased or no MTFF were frequently observed in patients with AP and OMI. The results suggest the feasibility of the present FC system to assess MTFF in patients with coronary artery disease. In addition, recovery of filling time and fluorescent intensity were observed in several patients following coronary intervention, indicating restored MTFF and consequently myocardial blood flow.

In this study, despite the apparently normal coronary arterial trees observed by angiography, a high incidence of MTFF disturbance was observed in patients with VSA, a functional disease. The frequent and severe ischemia caused by vasospasm probably lead to regional endocardial and/or myocardial fibrosis, or to myocardial stunning, resulting in a decrease in the vascular bed. This could have led to the high incidence of MTFF disturbance in this category of coronary artery disease.

Patchy MTFF images were observed in a few patients with complete occlusion of the irrigating epicardial coronary artery. Blood supply from the epicardial or intramycocardial collateral vessels most likely contributed to the image patterns. However, as fluorescein was administered intravenously, it was not determined whether the collateral vessels contributed to this image pattern or not. Selective intracoronary administration of the dye may present important information on collateral circulation.

However, unexpectedly, successful coronary stenting, evaluated by angiography, did not necessarily result in normalization of MTFF. One of the several mechanisms listed could be responsible for this unexpected phenomenon: pre-existent small vessel obstruction not demonstrated by angiography, distal embolism caused by coronary stenting, endothelial dysfunction of the irrigating small coronary vessels, and myocardial fibrosis.

Although not performed in this study, a histological examination of the observed portions by cardioscope-guided endomyocardial biopsy could provide valuable insight into the nature of such MTFF disturbance that remained after coronary stenting.

MTFF is dependent on the blood flow of the irrigating coronary artery. However, whether or not the former correlates with the latter remains to be elucidated.

In a preliminary study in beagles, fluorescence due to the fluorescein located within 1 mm in depth from the endocardial surface was detected by the present FC system. Therefore, the fluorescence images obtained in the patients studied represented the MTFF within 1 mm from the endocardial surface. As the imaging of MTFF using the present FC system was limited to such a thin myocardial layer, whether MTFF in the deeper myocardial layers shows the same changes or not remained unclear. Nevertheless, this imaging modality can be clinically used to evaluate MTFF which represents oxygen supply in the subendocardial myocardium that is most susceptible to ischemia.

Transendocardial angiogenic and myogenic therapies are currently being applied. TI scintigraphy can discriminate viable and nonviable myocardium, and perfusion CT and MRI can discriminate myocardial tissue fluid. They are clinically used for the evaluation of myocardial circulation before and after coronary interventions in patients other than those with acute coronary syndrome. However, there may be many limitations if they are applied to patients with acute coronary syndrome who undergo emergency coronary interventions. FC can be performed during emergency coronary interventions because it is performed during catheterization.

![Figure 7](image-url)
CT and MRI apparatuses are large in size, require noise elimination, require a considerably long time for imaging compared to FC, and can not perform pinpoint guidance of transendocardial therapies. They are therefore difficult to use in the catheterization laboratory. In contrast, the FC system is small in size, can be used without influence of X-ray and metals, requires only a few minutes for imaging, can be repeated with an interval of around 10 minutes, and can inject angiogenic factors and cells into the required myocardial segment with FC guidance when a needle is incorporated in the cardioscope.\(^1\)

Based on these clinical merits, we conclude that FC may greatly contribute to regeneration cardiology.

**Study limitations:** (1) Contribution of collateral circulation was not assessable by intravenous injection of fluorescein. However, by selectively injecting into the individual coronary artery, the contribution of the collateral vessels can be evaluated by FC. (2) Imaging of MTFF was limited within 1 mm in depth from the endocardial surface. Therefore, imaging of TMFF changes in deeper myocardial layers is beyond the scope of the present FC system. Application of near-infrared fluorescence may enable imaging of MTFF in deeper layers by the same CF system.

**Conclusions:** MTFF was evaluated by FC, using fluorescein as an indicator. MTFF disturbance was frequently observed both in patients with AP and OMI, as well as in those having VSA, a functional disease. In patients who had undergone angiographically successful coronary stenting, MTFF disturbance frequently remained when assessed by FC.

Transendocardial angiogenic and myogenic therapies are currently being used. This imaging modality can be used as a real-time and pinpoint guiding tool in these promising therapies.

**References**