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IMAGING VIGNETTE

Imaging the Migration of Therapeutically Delivered Cardiac Stem Cells

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THE INITIAL RESULTS OF HUMAN CLINICAL STUDIES DEMONSTRATED THE SAFETY AND POTENTIAL BENEFIT OF autologous bone marrow cells in improving left ventricular function after acute myocardial infarction (AMI). However, a fundamental problem in developing stem cell (SC)–based therapies has been the inability to monitor the engraftment and spatial migration of SCs in vivo following intracoronary or intramyocardial injection (1). Several integrative approaches have been suggested, such as cardiac magnetic resonance in combination with single-photon emission computed tomography (direct labeling of the cells with iron and nuclear particles), or positron emission tomography (PET) in combination with computed tomography (CT) and bioluminescence (indirect labeling of the cells, such as PET, fluorescence, or bioluminescence reporter gene method) (2). We have previously demonstrated the serial noninvasive imaging of the intramyocardially transplanted SCs, modified for stable expression of PET–reporter gene, in large animal models such as pigs which may have direct relevance for human studies (3). Here we report the first in vitro bioluminescence imaging of the SCs modified for transient expression of Luciferase (Luc) gene for characterizing their biodistribution after percutaneous intracoronary and intramyocardial delivery in pig hearts.

Closed-chest reperfused AMI was created in domestic pigs with 90-min percutaneous occlusion of the left anterior descending coronary artery followed by reperfusion. Three weeks after AMI, 3-dimensional (3D) NH_3-PET-CT was performed to image the myocardial perfusion defect at the anterior wall (Fig. 1A) and the left ventricular function disturbance with cardiac magnetic resonance (Fig. 1B). Porcine mesenchymal stem cells (MSC) were transfected with PET-reporter (MSC-PET-reporter) and injected intramyocardially using 3D NOGA mapping (Biologics Delivery Systems Group of Cordis Corporation, Johnson & Johnson, Diamond Bar, California) guidance in 8 locations (Fig. 1C). The 3D NOGA-guided intramyocardial delivery of SC helps retain the SC in the myocardium with minimal washout and reduced distribution to other organs (2). A corresponding PET-CT image showed confluent PET tracer activities (Fig 1D). In order to enable separation of the injection sites, MSC-PET-reporters were injected

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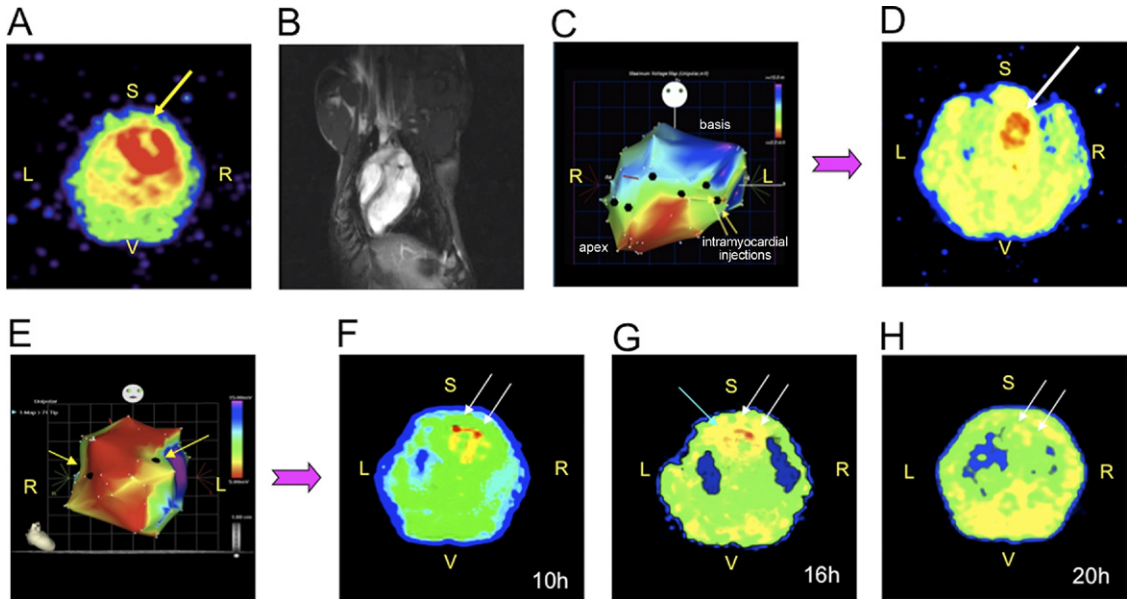

Figure 1. Imaging Modalities in a Porcine Model of Anterior Wall MI and Stem Cell Delivery

(A) 3-Dimensional (3D) NH_3-positron emission tomography (PET) combined with computed tomography (CT) image of the anterior wall myocardial infarction (MI) (Online Video 1) in a pig (supine position). Tracer uptake **(red)** defect in the apex **(yellow arrow)**. **(B)** End-diastolic cardiac magnetic resonance image with aneurysm of the anterior wall and apex. Left lateral view (left anterior oblique 90°) (Online Video 2). **(C)** 3D NOGA unipolar voltage mapping with NOGA-guided intramyocardial injection points of the mesenchymal stem cells (MSC), modified for stable expression of PET–reporter gene (MSC-PET-reporter) at the border zone of the anterior wall MI **(black points, yellow arrows)** (anteroposterior projection), 3 weeks after MI. **Red** color indicates the loss of electrical activity (infarcted area), **blue** the normal voltage signal (normal myocardium), and **green** and **yellow** the decreased viability (infarct border zone) (Online Video 3). Note that the pig heart is oriented vertically with the apex pointing slightly to the right. **(D)** [18F]-labeled 9-[4-fluoro-3-(hydroxymethyl)butyl]guaninderivatives ([18F]-FHBG)–PET-CT of a pig (supine position, same position as in **panel A**) representing the intramyocardial injection sites (corresponding PET-CT of the heart displayed in **panel C**) with the MSC-PET-reporter **(white arrow)** with high tracer activities, 10 hours after MSC-PET-reporter delivery. **(E)** 3D NOGA unipolar voltage mapping with 2 locations of NOGA-guided intramyocardial injections **(black points, yellow arrows)** of the MSC-PET-reporter at the border zone of the anterior wall MI (anteroposterior projection). Same color coding as in **panel C**. Injections of 2 distant points were necessary to enable the separation of the cell delivery locations in PET-CT images. **(F)** 18F-FHBG-PET-CT image of the same pig (supine position, same position as in **panel A**) 10 hours after 2 NOGA-guided intramyocardial injections of the MSC-PET-reporter **(white arrows),** corresponding PET-CT image of the heart displayed in **panel E**. **(G)** In vivo tracking of the intramyocardially delivered MSC-PET-reporter with serial noninvasive PET-CT images of a pig heart at an additional 16 and 20 hours **(H)**. 18F-FHBG-PET-CT images of the pig in a supine position (same position as in **panel A**). Gradual decrease of the tracer activity in the heart **(white arrows)** and increase in the surrounding tissues. **(H)** Further decrease of number of MSC-PET-reporter at 20 hours **(white arrows)** after delivery, suggesting the migration or death of the cells. L = left side; R = right side; S = sternum; V = vertebra.

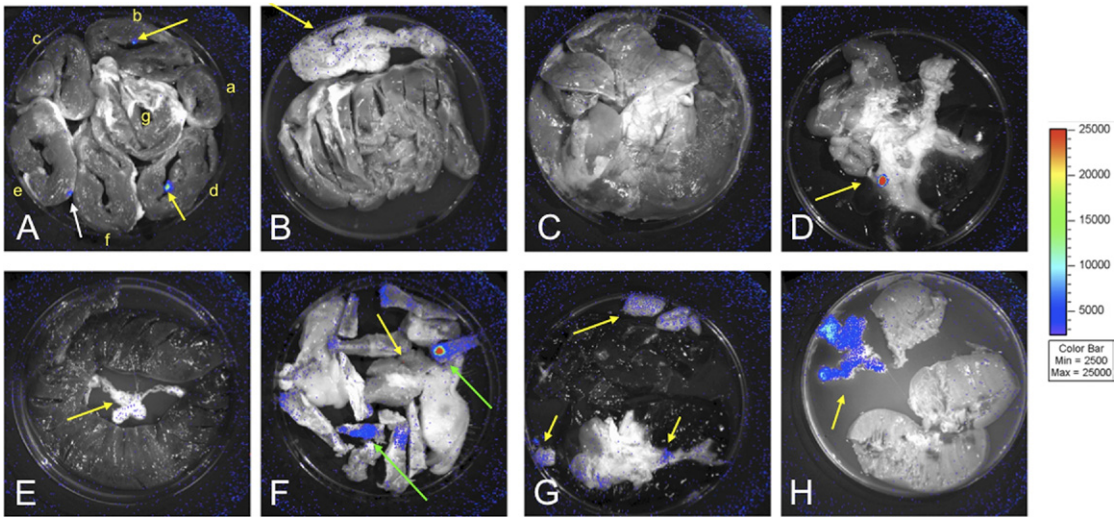

Figure 2. In Vitro Tracking of the Percutaneously Intramyocardially Delivered MSCs, Modified for Transient Expression of MSC-Luc in a Pig Heart and Remote Organs Using Bioluminescence Imaging at 3 Hours After Delivery

(A) Cross sections of the pig myocardium (1 cm thick slices from apex to mitral ring/from **a** to **g**). Hot spots in the border zone of infarction, corresponding with the location of the NOGA-guided endocardial-intramyocardial injections of MSC-Luc **(yellow arrows)**. One hot spot below the pericardial surface of the myocardium **(white arrow)**, suggesting either deep injection or cell migration from the endoluminal surface of the ventricle. **(B)** Low bioluminescent signal in the pericardium **(arrow)**, without visible bioluminescent signal of the endocardial surface of another pig heart. **(C)** No MSC-Luc in the lung. **(D)** No bioluminescent signal in the liver parenchyma, but one high hot spot in the portal lymph node **(arrow)**. **(E)** Weak MSC-Luc-signal in the spleen hilus **(arrow)**. **(F)** High intensity of bioluminescent signal in the bone marrow **(green arrows)** and one hot spot in the skin **(yellow arrow)**. **(G)** Bioluminescent activities in the inguinal and portal lymph nodes **(yellow arrows)**. **(H)** No MSC-Luc detected in the kidney, but bioluminescent activity in the abdominal lymph nodes **(arrow)**. Luc = luciferase gene; other abbreviation as in Figure 1.

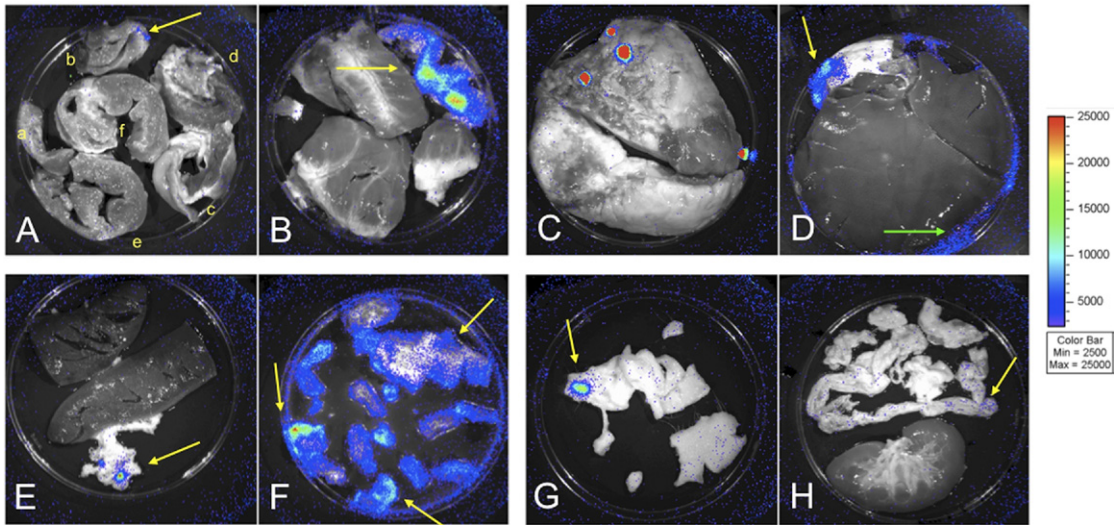

Figure 3. In Vitro Tracking of the Intracoronary Delivered MSCs, Modified for Transient Expression of MSC-Luc in a Pig Heart and Remote Organs Using Bioluminescence Imaging at 3 Hours After Delivery

The bioluminescent signal intensity is much higher in the remote organs, as compared to the intramyocardial delivery mode. **(A)** Cross sections of the pig myocardium (1 cm thick slices from apex to mitral ring/from **a** to **f**). Weak bioluminescent signal in the border zone of infarction in one section **(arrow)**. **(B)** High bioluminescent activity in the pericardium **(arrow)**, with no cells visible in the myocardium of another pig heart. **(C)** Hot spots of the left lung. **(D)** No bioluminescent signal in the liver parenchyma, but high activity in the blood pool of the liver **(green arrow)** and in the portal lymph node **(yellow arrow)**. **(E)** Presence of MSC-Luc in the spleen hilus **(arrow)**, but not in the spleen. **(F)** Very high intensity of bioluminescent signal in the spongiotic tissue of the sternum (e.g. bone marrow) **(arrows)**, as early as 3 hours after MSC-Luc intracoronary delivery. **(G)** Hot spot in the skin **(arrow)**. **(H)** No MSC-Luc detected in the kidney, but some bioluminescent activity in abdominal lymph nodes **(arrow)**. Abbreviations as in Figures 1 and 2.

in 2 distinct locations (Fig. 1E) and tracked with serial PET images (Figs. 1F, 1G, and 1H). To overcome the low spatial resolution of the PET images and fast decay of the PET tracer, and to verify the biodistribution of the cells in remote organs, pig MSCs were transfected with Luc (MSC-Luc), and delivered intramyocardially or intracoronary. In vitro bioluminescence imaging of MSC-Luc (Figs. 2 and 3) indicated a higher degree of cell distribution in remote organs after intracoronary cell delivery and the retention of the cells mostly in the lymph nodes, bone marrow, and blood pool. The underlying mechanism of migration to remote organs, whether active or passive, remains unclear.

The in vivo and in vitro tracking of the cardially delivered SCs provides the ability to explore the fate and distribution of SCs. Future studies are required for characterizing molecular technologies that enhance SC engraftment in the heart while avoiding the unnecessary migration to remote organs. Furthermore, the development of safe and efficient self-activating and -inactivating vectors is currently under way for the pre-clinical and clinical use of reporter gene imaging.

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APPENDIX

For supplementary videos and their legends, please see the online version of this article.