In Vivo Imaging Reveals Effective Injection and Retention of a Collagen Matrix in a Mouse Model of Myocardial Infarction

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Background:

Injectable biomaterials have been shown to improve the regenerative effects of endogenous and exogenous progenitor cells by enhancing their recruitment, retention and survival within ischemic tissues *in vivo*. However, the retention and distribution of such injectable biomaterials are typically not investigated or reported. The ability to be delivered and remain at the injection site becomes more challenging when the target tissue is the heart, a contractile organ. In the present study, our objective was to use different imaging modalities to evaluate the injection and retention properties of a type I collagen-based matrix, which has been shown previously to improve the function of infarcted hearts (with and without co-delivery of therapeutic cells). The collagen matrix was labeled with hexadedecyl-4-[^{18}F]fluorobenzoate (^{18}F-HFB), a tracer for visualization by positron emission tomography (PET), or with Qdot® 525 ITK™ carboxyl quantum dots (q-dots), which are fluorescently tagged. Labeled matrix was delivered to infarcted mouse hearts via ultrasound-guided injections, and imaged by PET (^{18}F-HFB) or by bioluminescence (q-dots) techniques to assess its myocardial retention.

Materials and Methods:

For *in vitro* evaluation, ice cold glutaraldehyde cross-linked rat tail type I collagen matrix mix was labeled with ^{18}F-HFB tracer and incubated at 37^{o}C for 30 minutes to allow the mixture to gel. After rinsing with phosphate buffer saline (PBS), radioactivity was measured in the gel. For *in vivo* studies, 7 days after left anterior descending coronary artery ligation, female C57BL6/J mice (10-wk old) were injected with ^{18}F-NaF (7.5±1.4 MBq) to demarcate the skeleton and with ^{13}N-NH_3 (42.5±4.8 MBq) to delineate the infarcted myocardium during a single PET scan. Mice then received injections of ^{18}F-HFB (3.0±0.9 MBq) labeled matrix (total volume: 50uL), delivered under the guidance of echocardiography to the infarct and peri-infarct areas. PET scans were performed 10 minutes and 2 hours after matrix injection to evaluate its retention and distribution. Co-registration of images was conducted by merging the demarcated skeleton in different scan images. Signal intensity quantification was performed using Inveon Research Workplace (IRW) software. After animal sacrifice, different tissues were collected for biodistribution assessment.

In a separate set of experiments, the collagen matrix was covalently tagged with q-dots. The optimum q-dot concentration was determined by applying serial dilutions of q-dots added to the matrix. After solidification, the matrix was rinsed with PBS for up to 2 hours and q-dot retention was evaluated. For the animal study, q-dot labeled matrix was injected to the infarcted mouse heart, as described above. Animals were sacrificed 10 minutes and 2 hours after matrix injection,

and the hearts were harvested. IVIS® Spectrum was then used to assess the distribution pattern of the injected matrix within the heart *ex vivo*. Furthermore, q-dot fluorescent signal intensity was quantified in digested mouse hearts using a plate reader at 510 nM.

Results:

In vitro, the matrix retained 82.2±1.8% and 81.6±1.9% of the lipophilic ^{18}F-HFB radiotracer at 10 minutes and 2 hours after thermogelation, respectively (n=4), demonstrating labeling stability over time. *In vivo*, PET imaging revealed that the collagen matrix was retained in the infarcted myocardium and peri-infarct regions after injection. In the 2 hour PET scan, the injected matrix was visibly observed to have spread throughout the infarcted ventricle. IRW assessment indicated that 87.6±4.3% of the initial activity of ^{18}F-HFB detected at 10 minutes post-injection persisted 2 hours later. Tracer biodistribution analysis demonstrated significantly higher tracer retention in the myocardium compared to all other tissues analyzed, including lungs, liver, and kidneys (66.2±1.5% of injected dose; n=14, p<0.001).

The optimal q-dot labeling concentration was determined to be 250 nM, which was used for all subsequent experiments. *In vitro*, the q-dot leakage from the matrix was 1.8±0.7% and 3.8±1.6% 10 minutes and 2 hours after solidification of the collagen matrix, respectively. This confirmed the successful linkage of the q-dot label with the collagen matrix. Bioluminescence assessment of the hearts injected with q-dot-labeled collagen matrix confirmed the spreading of the collagen matrix within the infarcted myocardium, 2 hours after injection. Q-dot fluorescent signal quantification indicated that 84.1±7.4% of the injected matrix was retained in the myocardium (n=4).

Discussion:

This study demonstrated that a glutaraldehyde cross-linked collagen matrix injected into the infarcted mouse heart solidifies at body temperature quickly enough to be retained and spread within the contractile myocardium. This study also confirms the accuracy of ultrasound-guided myocardial injections for treatment delivery. The labeling techniques introduced in this study can potentially be applied for assessing *in vivo* thermogelling properties of other injectable matrices. In summary, the even distribution of the collagen matrix upon injection to the infarcted myocardium and its minimal relocation to off-target body tissues make it attractive for the delivery of therapeutics, such as cells or growth factors, for the treatment of myocardial infarction.