Combined Optical and Magnetic Resonance Imaging Enables Noninvasive Trafficking of Tissue Regeneration

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Abstract: Biodegradable scaffolds could revolutionize tissue engineering and regenerative medicine; however, *in vivo* matrix degradation and tissue ingrowth processes are not fully understood. Currently a large number of samples and animals are required to track biodegradation of implanted scaffolds, and such nonconsecutive single-time-point information from various batches result in inaccurate conclusions. To overcome this limitation, we developed functional biodegradable scaffolds by employing invisible near-infrared (NIR) fluorescence and followed their degradation behaviors in vitro and in vivo. Using optical fluorescence imaging, the scaffold degradation could be quantified in real-time, while tissue ingrowth was tracked by measuring vascularization using magnetic resonance imaging in the same animal over a month. Moreover, we optimized the *in vitro* process of enzyme-based biodegradation to predict implanted scaffold behaviors *in vivo*, which was closely related to the site of inoculation. This combined multimodal imaging will benefit tissue engineers by saving time, reducing animal numbers, and offering more accurate conclusions.

Methods: *Engineering NIR fluorescent scaffolds.* The biodegradable scaffold using porcine small intestinal submucosa (SIS) was prepared as previously reported. An 800 nm NIR fluorescence emitting ZW800-1 was conjugated on the scaffold using conventional NHS ester chemistry in phosphate buffer at pH 8.0.

Scaffold implantation and optical imaging. NIR scaffolds pre-wetted with Matrigel were implanted into the subcutaneous pocket of axilla and thigh region of nude mice, respectively. An unconjugated scaffold (control) was also transplanted in the middle of 2 NIR scaffold sites to retain space among scaffolds. Noninvasive optical imaging was performed from the same animal every 3 days using the FLARE™ imaging system (www.theflarefoundation.org). One animal at each time point was sacrificed to quantify the biodistribution of the degraded scaffolds, and the implanted constructs were harvested to use for histological analysis after H&E staining.

Magnetic resonance imaging (MRI). To confirm tissue formation in the scaffold, 1T micro MRI system (Aspect, Israel) was used. 50 µL of Magnevist diluted 10-fold in saline was injected in the tail vein to track tissue formation by vascularization. The imaging protocol consisted of T1-weighted spin echo sequence with TR/TE = 412.6/10.9 ms, matrix size = 256 x 256, slice thickness = 2.0 mm, gap = 0.0 mm, field of view = 60 mm.

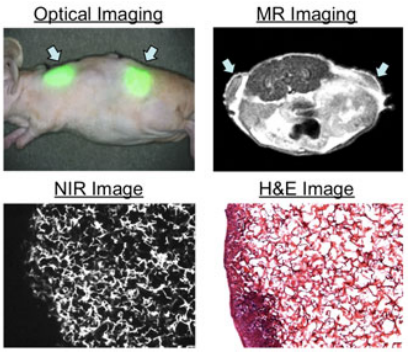

Figure 1. Optical and MR imaging of a scaffold-implanted nude mouse (top). NIR fluorescence and H&E images of resected scaffold at 3 day post-implant (bottom).

Results & Discussion: To investigate the effect of local enzyme amount-mediated biodegradation in the body, NIR scaffolds were implanted into the subcutaneous pocket of axilla and thigh region separately. A significant decrease in the NIR fluorescence signal was observed from the scaffolds over a month of observation period. The degree of signal reduction was remarkable in the axilla site compared to the thigh region. The pattern of signal reduction in both regions was similar to those from the *in vitro* test incubated with different collagenase concentrations. MRI was used to confirm the tissue infiltration process into the scaffold by measuring vascular formation in the same animal. T1-weighted MRI without contrast agent reveals little to no evidence of vasculature within the implanted scaffold as evidenced by distinct fat and tissue separation between native and implanted region. By administrating Magnevist, the images show bright signal around boundaries of the implanted scaffold, which suggests onset of vascularization within and around scaffold borders. When the implant site was fully vascularized at 28 day post-implantation, the MR images of the axilla site showed bright, homogenous signal throughout the implanted scaffold. Neovascular formation in the thigh region was slower than axilla corresponds to slow degradation of the implanted scaffold.

Conclusions: When combined with MRI, optical imaging using the NIR wavelength can be one of the solutions for monitoring scaffold degradation continuously along with tissue development. By tagging an invisible fluorophore covalently to the biodegradable scaffold, the degradation behavior of various biomaterials could be monitored in real time. Since light scattering and absorption in human tissue are the major obstacles in optical fluorescence imaging, NIR wavelength can efficiently minimize tissue autofluorescence, resulting in less background interference and enhanced signal-to-background ratio.