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In vivo monitoring of structural and mechanical changes of tissue scaffolds by multi-modality imaging

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ABSTRACT

Degradable tissue scaffolds are implanted to serve a mechanical role while healing processes occur and putatively assume the physiological load as the scaffold degrades. Mechanical failure during this period can be unpredictable as monitoring of structural degradation and mechanical strength changes at the implant site is not readily achieved in vivo, and non-invasively. To address this need, a multi-modality approach using ultrasound shear wave imaging (USWI) and photoacoustic imaging (PAI) for both mechanical and structural assessment in vivo was demonstrated with degradable poly(ester urethane)urea (PEUU) and polydioxanone (PDO) scaffolds. The fibrous scaffolds were fabricated with wet electrospinning, dyed with indocyanine green (ICG) for optical contrast in PAI, and implanted in the abdominal wall of 36 rats. The scaffolds were monitored monthly using USWI and PAI and were extracted at 0, 4, 8 and 12 wk for mechanical and histological assessment. The change in shear modulus of the constructs in vivo obtained by USWI correlated with the change in average Young's modulus of the constructs ex vivo obtained by compression measurements. The PEUU and PDO scaffolds exhibited distinctly different degradation rates and average PAI signal intensity. The distribution of PAI signal intensity also corresponded well to the remaining scaffolds as seen in explant histology. This evidence using a small animal abdominal wall repair model demonstrates that multi-modality imaging of USWI and PAI may allow tissue engineers to noninvasively evaluate concurrent mechanical stiffness and structural changes of tissue constructs in vivo for a variety of applications.

1. Introduction

Biodegradable polymeric scaffolds have been central to the tissue engineering approach wherein cells infiltrate, proliferate, and elaborate extracellular matrix (ECM) as the scaffold degrades and the newly formed tissue assumes the mechanical role from the degrading scaffold. To design scaffolds which appropriately transfer their mechanical load over time to the ingrowing tissue, temporal analysis is required that verifies the structural and mechanical integrity of remodeling constructs. Current analysis methods are predominantly destructive, requiring animal euthanasia and construct explantation for histological and direct mechanical characterization [1–4]. In addition, different samples are necessarily measured at different times and variance between specimens and animals weakens the analytical power. Ideally, tissue engineers need a system that can non-invasively monitor remodeling in the same specimen over time [5–8]. Non-invasive monitoring that couples structural degradation and mechanical strength changes in tissue constructs would provide an important tool for tissue engineers to evaluate and better design candidate scaffolds.

In our previous studies [7], ultrasound elasticity imaging (UEI) has been applied in vivo to measure the mechanical strength changes of polymeric scaffolds. The polymeric scaffolds were made from three biodegradable elastomers with varying degradation rates and implanted in a rat muscular abdominal wall. Over a 12 wk period the UEI-determined construct stiffness was in good agreement with compressional measurements and histological

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determinations. However, UEI is limited in that: 1) it requires mechanical compression which limits its applications to areas where clear physical access can be achieved, 2) the measured strain inside the constructs needs to be normalized to the overall strain applied to the body, which provides a source of error, and 3) the measured strain inside the constructs can be sensitive to the stress distribution that depends on the applied force and surrounding anatomy [8].

Ultrasound shear wave imaging (USWI) can be a better alternative for mechanical strength measurements because this technique is based on remote palpation and provides absolute elastic modulus reconstructed from the shear wave speed measurements. USWI has been applied to quantify the shear modulus in tissue by generating transient ultrasound (US) radiation force excitation. The propagation speed of the shear wave is related to the underlying tissue shear modulus [9]. The local tissue shear modulus can be determined in vivo from the displacement field of shear waves using an inversion of the Helmholtz equation [10,11].

In addition to knowing the temporal mechanical changes occurring spatially at the site of an implanted scaffold, it is desirable to monitor the scaffold degradation process in a coupled fashion. The relative amount of scaffold remaining, whether scaffold degradation is occurring preferentially in one area and whether tissue ingrowth appears to be matching scaffold removal are examples of desired information. However, in vivo assessment of scaffold structural degradation becomes challenging, particularly at the point when fragmentation occurs and these fragments are imbedded within ingrowing tissue. Photoacoustic imaging (PAI) provides a means by which subtle structural changes or small scaffold fragments may be detectable with proper optical contrast. PAI, which combines optical excitation and US detection, has been applied in biomedical applications mostly for the examination of native tissue [12,13]. In a few studies, photoacoustic microscopy (PAM) techniques have been applied to tissue engineering. The capacity to visualize cell proliferation in a porous polymeric scaffold has been demonstrated in vitro using fibroblasts, embryonic stem cells, and tumor cells [14,15]. Vascular ingrowth into a tissue engineering construct was displayed in vivo by Cai et al. [16]. However, these PAM approaches have imaging depths only up to a few millimeters [8,17] and would provide limited information on changes in scaffolds implanted deep in a tissue. To our best knowledge, there have been no reports applying a PAI technique to monitor the structural changes of implanted polymeric scaffolds in vivo.

The objective of this study was to demonstrate the feasibility of combining USWI and PAI in a multi-modality approach to temporally assess the mechanical and structural properties of polymeric scaffolds implanted in a rat abdominal repair model. Two biodegradable polymers with different stiffness and degradation profiles were utilized: poly(ester urethane urea) (PEUU) for a soft material with moderate degradation rate and polydioxanone (PDO) for a stiff material with faster degradation rate. Scaffolds were stained with indocyanine green (ICG) for optical contrast. The mechanical and structural changes determined by non-invasive imaging were compared to explanted samples subjected to direct mechanical and histological analysis.

2. Materials and methods

2.1. Scaffold fabrication

PEUU was synthesized based on poly(caprolactone) diol (PCL, Mn = 2000, Sigma) and diisocyanatobutane (BDI, Sigma), followed by chain extension with putrescine (Sigma) [18]. PDO was purchased from Sigma–Aldrich (CAS No. 31621-87-1, St. Louis, MO). Wet electrospun PEUU and PDO scaffolds were fabricated by a combination of electrospinning and electrospraying previously reported [19,20]. Polymer solution from a mounted syringe pump was fed at 1.5 mL/h through a stainless steel capillary (1.2 mm inner diameter) and the capillary was perpendicularly located 15 cm from targeted a stainless steel mandrel (19 mm diameter) rotating at 250 rpm. At the same time, Dulbecco's Phosphate Buffered Saline (DPBS) solution was fed at 12 mL/h through a capillary suspended 5 cm over the mandrel during the electro-spinning. The mandrel was mounted on a stage that reciprocally translated 8 cm along the direction of the mandrel axis at a rate of 0.15 cm/s. Three high-voltage generators were used to charge the polymer feeding capillary to 10 kV, the DPBS feeding capillary to 8 kV and the mandrel (ground) to −4 kV. After 7 h, the fibrous sheet (sample thickness: 0.8–1.0 mm) was removed from the mandrel and stored in DPBS at room temperature overnight. Subsequently, the PEUU and PDO scaffolds were immersed in ICG (PSA1355, H.W. SANDS CORP, FL, USA) solution (0.2 g/L in acetone: H_2O = 98:2) for 24 h to achieve ICG staining and washed with DPBS (at least 10 times) to remove unbound ICG). Scaffolds were then stored in DPBS. Square wet electrospun patches of 1 cm by 1 cm were cut and then were sterilized under ultraviolet (UV) irradiation prior to implantation.

Elution of ICG dye from the scaffold was examined in vitro. The scaffolds dyed with ICG were kept in DPBS solution under a continuous rocking condition at 37 °C. The concentrations of ICG in the solution were measured at 1, 2, 4, 8 and 12 weeks using an UV spectrum with an 800 nm absorption peak. The remained ICG amounts in the scaffolds were measured after the ICG dye was extracted using a 70% ethyl alcohol solution.

2.2. Animal preparation

Female Lewis 12-week old rats (200 g) were used under a protocol consistent with the National Institutes of Health (NIH) guidelines for animal care and approved by the University of Pittsburgh Institutional Animal Care and Use Committee. The surgery was performed in a sterile environment using a procedure based on a previous study [7]. An incision was made along the midline of the abdomen, and subcutaneous pockets were created in each side of the abdominal wall. A full thickness square defect (1 × 1 cm) involving all layers of the abdominal wall, including external oblique, internal oblique and transverse abdominis muscle and peritoneum was created in each pocket. Two same type of scaffold were implanted for each rat. Total of 36 rats were evenly divided into two groups where 18 rats were implanted with PEUU and 18 with PDO. The rats were sacrificed in four batches; at week 0 (n = 3 for each group), at week 4 (n = 5 for each group), week 8 (n = 4 for PEUU and n = 5 for PDO group), and week 12 (n = 5 for each group). Note that there is one less rat at week 8 for PEUU group due to unexpected death for unknown reason between week 4 and 8. After sacrifice, all scaffolds were harvested, for either compression testing or histological assessments as summarized in Table 1.

2.3. Ultrasound shear wave imaging

The experimental setup for USWI is shown in Fig. 1. USWI pulse/imaging sequences were implemented on a commercial US scanner (US scanner I) (V-1, Verasonics Inc., Redmond, WA, USA) connected with a clinical transducer (L7-4, ATL 5 MHz central frequency). Using 128 transducer elements, four push pulses of 180 μs long were sequentially induced at four depths 23.7, 28.6, 33.6 and 38.5 mm (white dots in Fig. 2A and B) to create a planar shear wave. Immediately after, a very high frame rate imaging of 8000 Hz was performed by transmitting unfocused plane-waves with 64 transducer elements of the probe to record the propagating shear wave [11]. All backscattered radio frequency (RF) frames were stored in each channel of 64, then transferred to a computer.

In vitro USWI was performed using a scaffold embedded in a tissue mimicking phantom block. A piece of wet PEUU scaffold (1 × 1 × 0.6 cm) was placed 1 cm below the surface of a plastic container (15 × 10 × 6 cm) filled with 10% PVA solution (Fig. 2A), mixed with US scatterers, cellulose powder 5% by weight of the PVA solution (Sigmacell, 20μ, Sigma–Aldrich, St. Louis, MO). After two cycles of freezing at −20 °C for 12 h and thawing in room temperature for 12 h, the container was removed from the phantom block. The phantom block was placed in a water tank and localized US push pulses were generated by a Philips L7-4 clinical transducer. The same transducer then subsequently acquired RF frames during 4 ms while the created shear wave was propagating through the phantom block. Axial displacements of the shear wave were calculated from the RF frames using the Loupas'

Table 1
Study design.

Type of scaffold	Weeks	Animals[a] sacrificed	Samples studied for USWI, PAI, and UEI	Samples for compression tests	Samples for histology
PEUU	0	3	10 (of 36)	6	0
	4	5	10 (of 30)	5	5
	8	4	10 (of 18)	4	4
	12	5	10 (of 10)	5	5
PDO	0	3	10 (of 36)	6	0
	4	5	10 (of 30)	5	5
	8	5	10 (of 20)	5	5
	12	5	10 (of 10)	5	5

[a] All animals were scanned before sacrifice.