Supporting Information

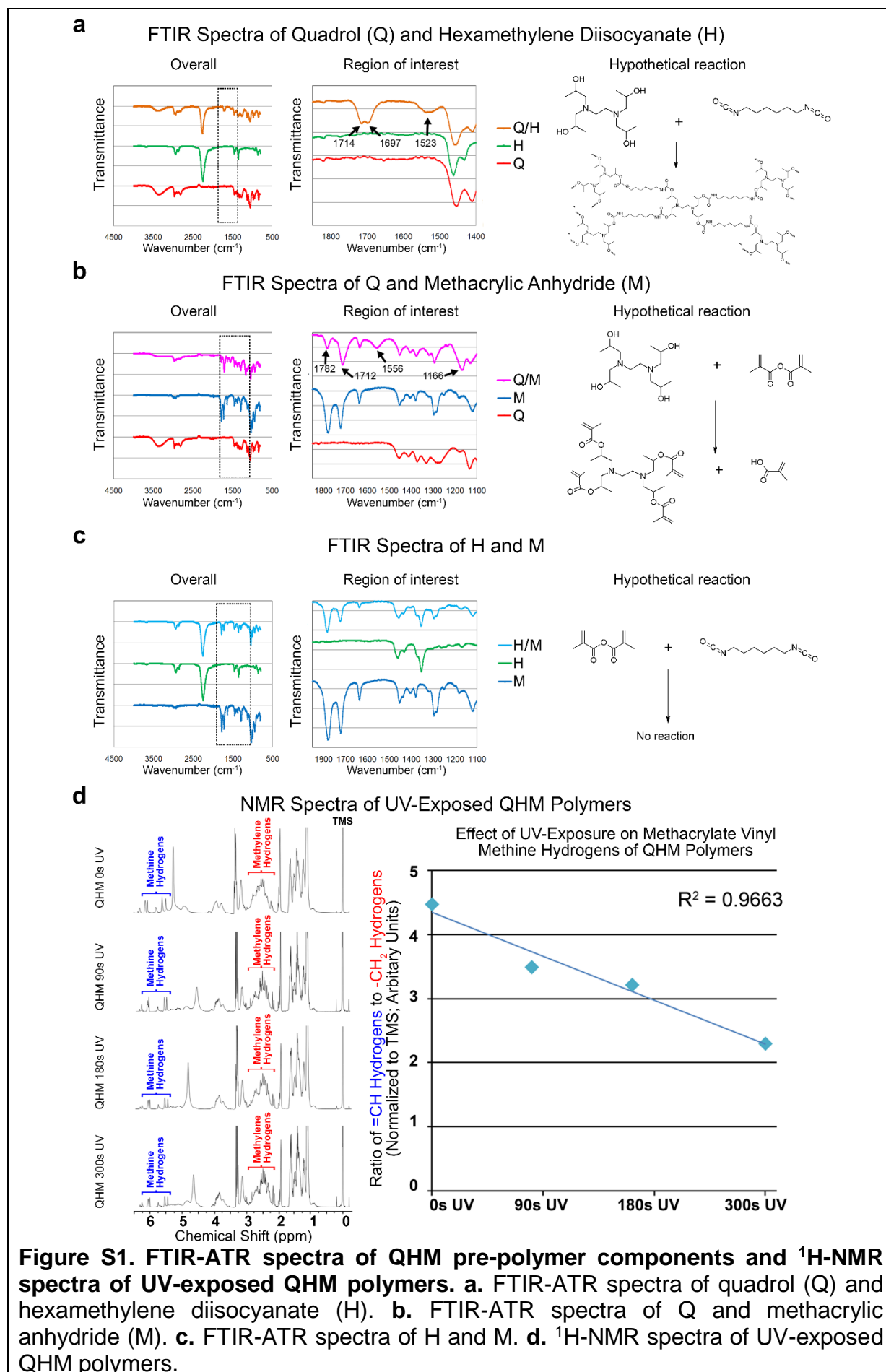## Mechanically-Graded, Bone- and Tendon-Like Polyurethane for Rotator Cuff Repair

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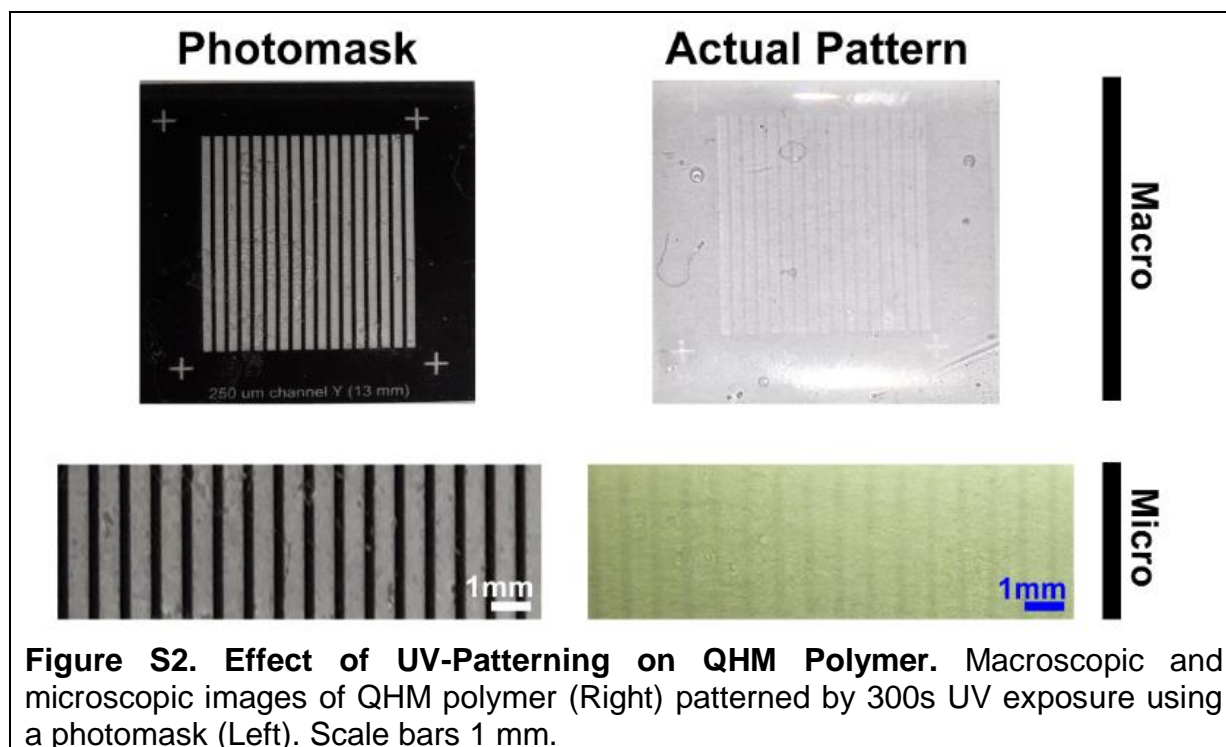
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Supplementary Figures

The chemical reactions among Q, H and M components of QHM pre-polymers (Q/H, Q/M and H/M) as well as the effect of UV exposure (0s, 90s, 180s and 300s UV) on QHM polymers were monitored by FTIR-ATR and ^{1}H-NMR, respectively (**Figure S1**). The FTIR-ATR spectra of Q alone exhibited a broad peak around 3200 – 3500 cm^{-1} which was attributed to stretching vibrations of hydroxyl (O-H) groups and hydrogen bonding (**Figure S1a**).[49] The FTIR-ATR spectra of H alone exhibited a sharp peak around 2200 cm^{-1} which was attributed to stretching vibrations of isocyanate (N=C=O) groups (**Figure S1b**).[48-50] The FTIR-ATR spectra of M alone exhibited two sharp peaks around 1718 cm^{-1} and 1780 cm^{-1} which were attributed to stretching vibrations of anhydride carbonyl (C=O) groups (**Figure S1c**).[48-50] The FTIR-ATR spectra of Q/H were indicative of a reaction between the hydroxyl groups of Q and isocyanate groups of H to form carbamate groups. The spectra exhibited a peak change around 1523 cm^{-1}, which was attributed to bending vibrations of carbamate amine (N-H) groups as well as 2 peak changes around 1697 cm^{-1} and 1714 cm^{-1} which were attributed to stretching vibrations of carbamate carbonyl (C=O) groups (**Figure S1a**).[49] The FTIR-ATR spectra of Q/M were indicative of a reaction between the hydroxyl groups of Q and the anhydride carbonyl groups of M to form ester groups. The spectra exhibited a peak change around 1166 cm^{-1}, which was attributed to stretching vibrations of ester (C-O) groups as well as a peak change around 1556 cm^{-1}, which was attributed to stretching vibrations of carboxylate anion (COO⁻) groups, an intermediate species formed during the reaction of carboxylic acid anhydrides and alcohols (**Figure S1b**).[48-50] Also, there was a relative change in peak intensity between 1712 cm^{-1} and 1782 cm^{-1}, which were attributed to stretching vibrations of ester carbonyl (C=O) groups and stretching vibrations of carboxylic acid carbonyl (C=O) groups although anhydride carbonyl (C=O) groups were also present (**Figure S1b**).[48-50] The FTIR-ATR spectra of H/M did not indicate any chemical reaction between H and M as the spectra did not exhibit any peak changes (**Figure S1c**). Integral intensity ratio analysis of ^{1}H-NMR spectra for 0s, 90s, 180s and 300s UV-exposed QHM polymers indicated a relative decrease in the proton signals at 5 – 6 ppm compared to the proton signals at 2 – 3 ppm, which were attributed to methacrylated vinyl methine hydrogens and methylene hydrogens, respectively (**Figure S1d**).[49] Together, FTIR-ATR spectra indicated a reaction between Q and H as well as Q and M but not H and M while ^{1}H-NMR spectra indicated increased crosslinking with increased UV exposure.

Figure S1. FTIR-ATR spectra of QHM pre-polymer components and ¹H-NMR spectra of UV-exposed QHM polymers. a. FTIR-ATR spectra of quadrol (Q) and hexamethylene diisocyanate (H). **b.** FTIR-ATR spectra of Q and methacrylic anhydride (M). **c.** FTIR-ATR spectra of H and M. **d.** ¹H-NMR spectra of UV-exposed QHM polymers.
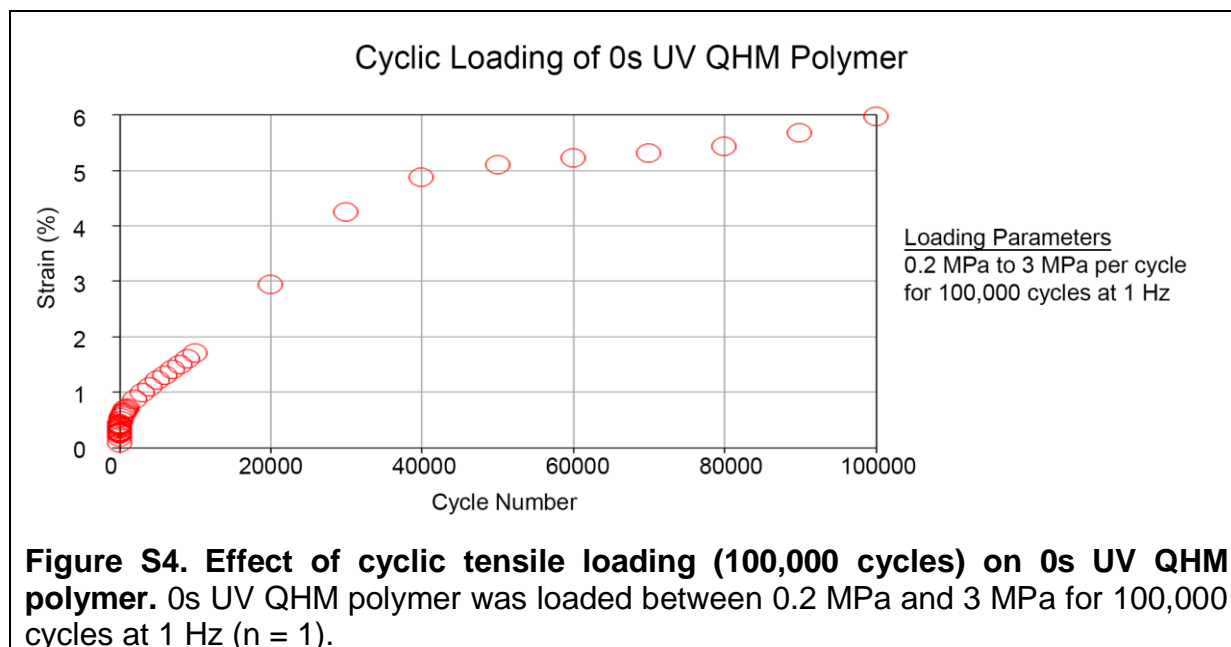
To determine if QHM polymer could be UV-patterned at physiologically-relevant length scales, QHM polymer was UV-exposed for 300s under a photomask. This resulted in alternating regions of 0s and 300s UV-exposed QHM polymer (similar to the photomask pattern), each measuring approximately 250 µm and 500 µm in width, respectively (**Figure S2)**. As such, QHM polymer could potentially be fabricated with bone- and tendon-like properties at physiologically-relevant length scales.

Figure S2. Effect of UV-Patterning on QHM Polymer. Macroscopic and microscopic images of QHM polymer (Right) patterned by 300s UV exposure using a photomask (Left). Scale bars 1 mm.

To determine glass transition temperature of QHM polymers, differential scanning calorimetry (DSC) was performed. DSC heating curves showed that 0s UV, 90s UV, 180s UV and 300s UV QHM polymers possessed a glass transition temperature of 27.21 °C, 33.99 °C, 39.08 °C and 43.55 °C, respectively (**Figure S3**). These data show that increasing glass transition temperature with longer UV exposure.

Figure S3. Effect of UV exposure on glass transition temperature of QHM polymers. a. Differential scanning calorimetry (DSC) thermogram of QHM polymers. **b.** Glass-liquid transition temperature (Tg) of QHM polymers.
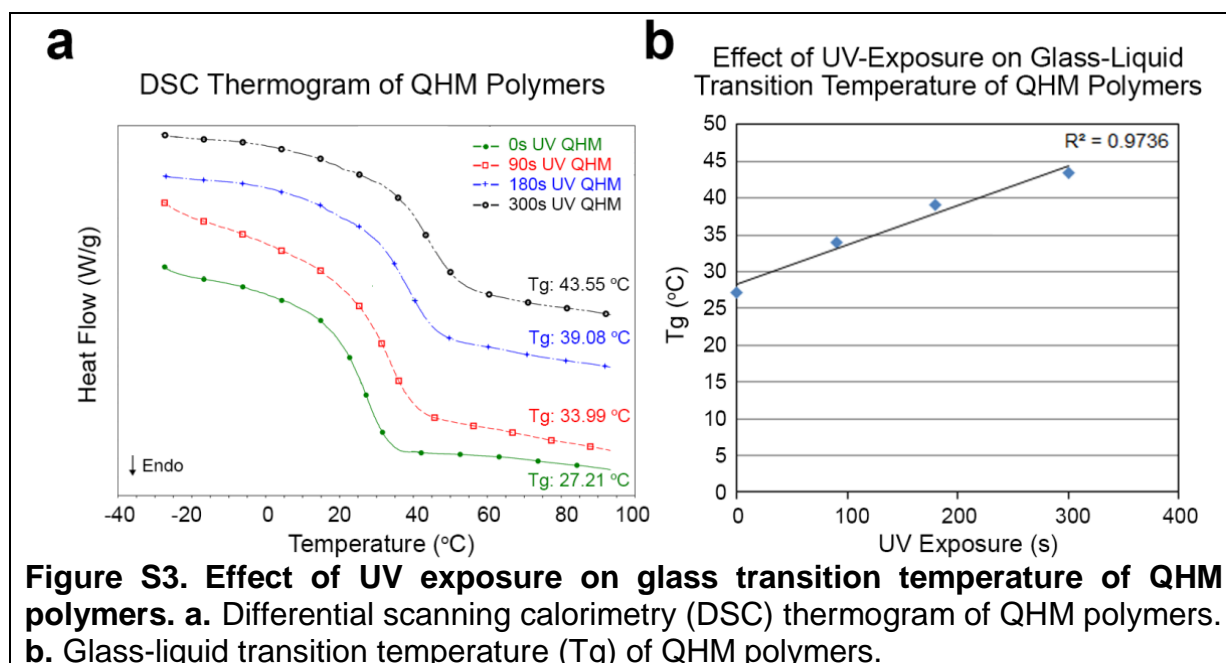
To analyze long-term material fatigue, a single sample of 0s UV QHM polymer was subjected to tensile loading between 0.2 – 3 MPa at 1 Hz for 100,000 cycles. 0s UV QHM polymer exhibited primary stage creep between 0 and 4,000 cycles and secondary stage creep between 4,000 to 100,000 cycles with a steady-state creep rate of 5.1×10^{-5} % s^{-1} (**Figure S4**). Following testing, 0s UV QHM polymer recovered its original length (**Data not shown**). Together, these data indicate that 0s UV QHM polymer possesses robust tensile properties.

Figure S4. Effect of cyclic tensile loading (100,000 cycles) on 0s UV QHM polymer. 0s UV QHM polymer was loaded between 0.2 MPa and 3 MPa for 100,000 cycles at 1 Hz (n = 1).
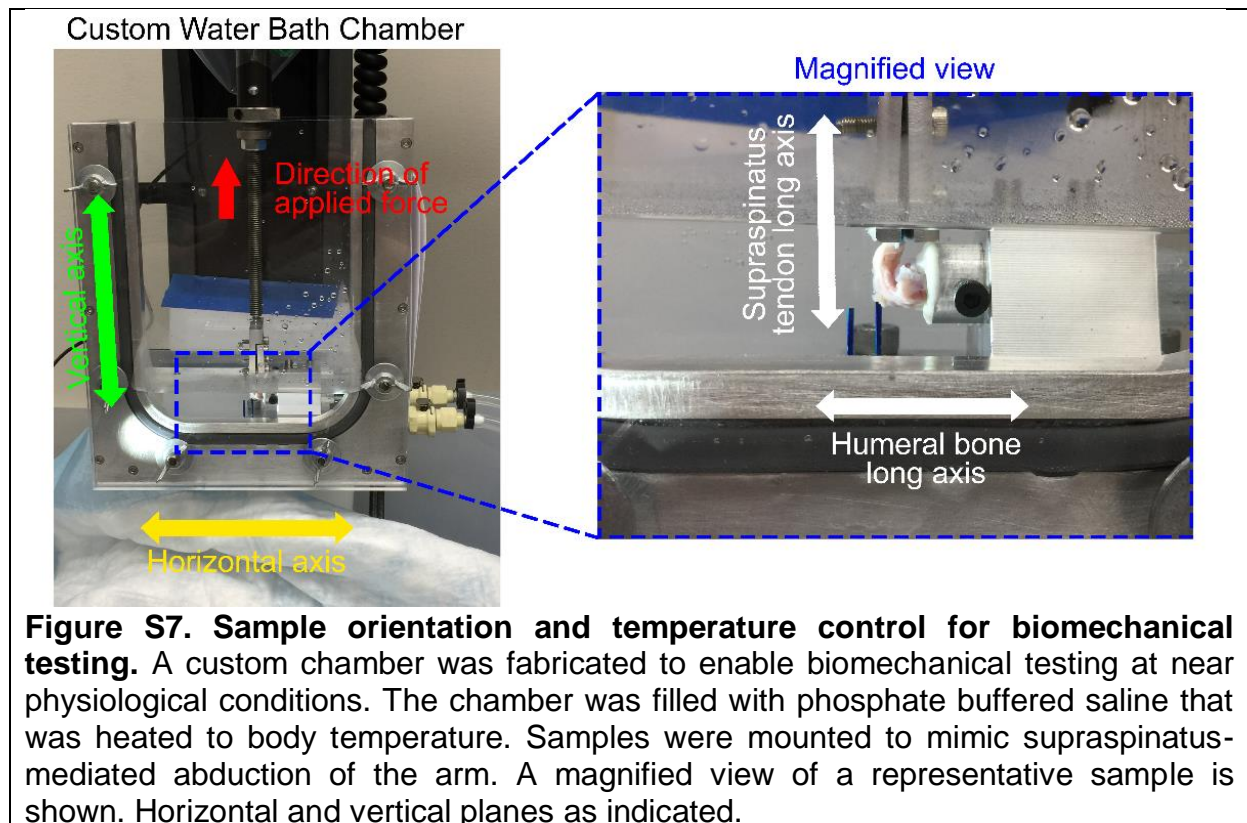
To determine the effect of material properties on stress concentrations, finite element analysis (FEA) was performed on uniform, gradually-graded and steeply-graded quarter models with varying Poisson's ratios. When 10 MPa of uniform tensile stress was applied, an increase in von Mises stress was observed at the interface (**Figure S5**). Peak stress was localized to a small region at the intersection of the interface and free edge. Although not shown in the color plots, this peak stress was 165 MPa for steeply-graded models with a Poisson's ratio of 0.40 (top half) and 0.30 (bottom half) whereas peak stress was 242 MPa for steeply-graded models with a Poisson's ratio of 0.49 (top half) and 0.30 (bottom half). In addition, high stress regions were primarily located in the bottom half (stiffer region) near the interface and a centrally located stress decrease was observed in the model's top half (compliant region) near the interface (**Figure S5**). This stress redistribution was most prominent in steeply-graded models and intermediate in gradually-graded models (**Figure S5**). Thus, steeply-graded models exhibited the largest stress increase compared to gradually-graded or uniform models, the magnitude of which varied with Poisson's ratio.

Figure S5. Effect of varying Poisson's ratio on the formation of stress concentrations in uniform, gradually-graded and steeply-graded materials subjected to 10 MPa tensile stress. Finite element quarter models include uniform (left), gradually-graded (center) and steeply-graded (right) materials. The top row indicates materials with a Poisson's ratio of 0.40 for the top half and 0.30 for the bottom half of the model. The bottom row indicates materials with a Poisson's ratio of 0.49 for the top half and 0.30 for the bottom half of the model. Uniform tensile stress of 10 MPa was applied to top face of each model. Peak values of concentrated stress not presented in color plot.

To determine stress within QHM polymers, a photoelastic tensile-color interference chart was constructed (**Figure S6**). 0s UV QHM polymer transitioned from clear at 0 N to dark brown at 100 N to light blue and yellow at 200 N to orange, purple and blue at 300 N and green, yellow and purple at 400 N. 120s UV QHM polymer transitioned from clear at 0 N to dark brown at 100 N to light blue at 200 N to yellow at 300 N and purple and blue at 400 N. 300s UV QHM polymer transitioned from clear at 0 N to light brown at 100 N to blue at 200 N to yellow and light blue at 300 N and yellow and orange at 400 N. When normalized by the cross sectional area of QHM polymer, this color interference chart enabled photoelastic tensile stress analysis.

Figure S6. Photoelastic tensile-color interference chart. Top row, 0s UV QHM polymer. Middle row, 120s UV QHM polymer. Bottom row, 300s UV QHM polymer (n = 3).
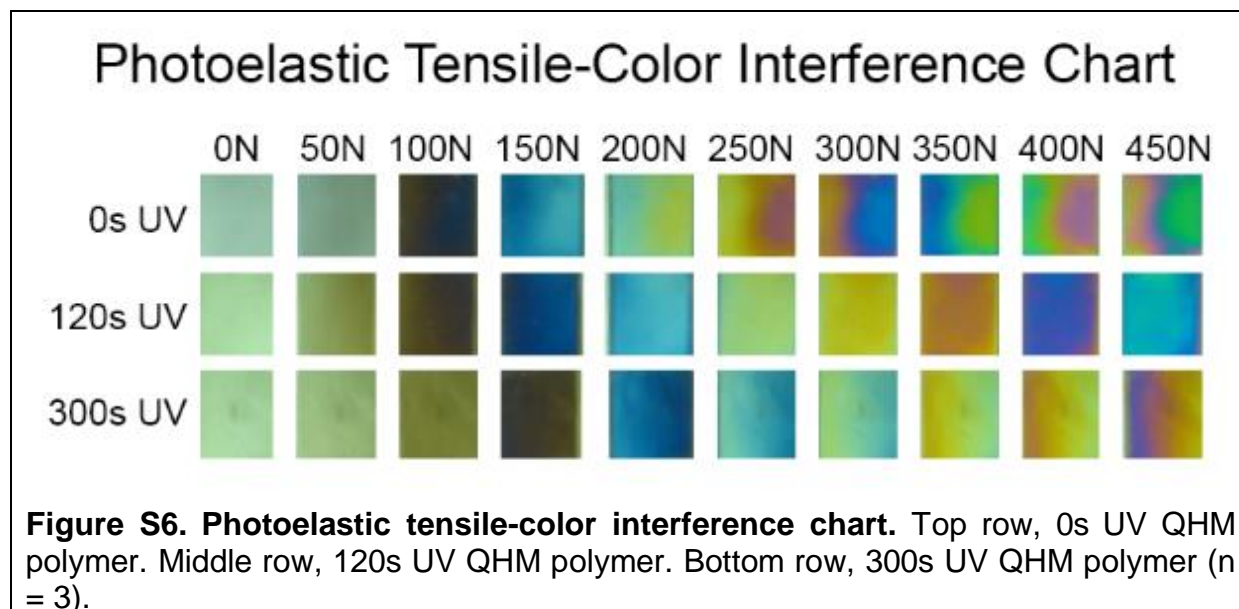
To facilitate biomechanical testing, supraspinatus tendon-humeral samples were mounted in a custom-fabricated water bath (**Figure S7**). The supraspinatus tendon was clamped and oriented vertically. The supraspinatus tendon-humeral sample was potted in a hollow aluminum cylinder filled with polymethylmethacrylate and fixed with the long axis of humeral bone oriented in the horizontal plane. This positioned samples in a manner that mimicked supraspinatus-mediated shoulder abduction. Prior to biomechanical testing, the water bath was filled with phosphate buffered saline heated to 37 °C. Thus, sample orientation and temperature control enabled biomechanical testing of samples at near physiological conditions.

Figure S7. Sample orientation and temperature control for biomechanical testing. A custom chamber was fabricated to enable biomechanical testing at near physiological conditions. The chamber was filled with phosphate buffered saline that was heated to body temperature. Samples were mounted to mimic supraspinatus-mediated abduction of the arm. A magnified view of a representative sample is shown. Horizontal and vertical planes as indicated.

Supplementary Tables
Table S1. ^{1}H-NMR peak assignments for QHM components and QHM pre-polymer.

Component	Peak (ppm)	Proton assignment(s)	Reference(s)
Q	1.04	Methyl (CH_3)	Silverstein et al.[49]
	2.00-2.95	Methylene (CH_2)	Silverstein et al.[49]
	3.81	Methine (CH)	Silverstein et al.[49]
	5.00	Hydroxyl (OH)	Silverstein et al.[49]
H	1.42, 1.62 and 3.32	Methylene (CH_2)	Mercado-Pagan et al.[48] and Silverstein et al.[49]
M	1.63, 2.00	Terminal vinyl ($=CH_2$)	Mercado-Pagan et al.[48] and Silverstein et al.[49]
	5.83, 6.24	Methyl (CH_3)	Mercado-Pagan et al.[48] and Silverstein et al.[49]
QHM pre-polymer	1.04	Methyl (CH_3)	Silverstein et al.[49]
	1.63, 2.00	Terminal vinyl ($=CH_2$)	Mercado-Pagan et al.[48] and Silverstein et al.[49]
	1.42, 1.62, 2.00-2.95 and 3.32	Methylene (CH_2)	Mercado-Pagan et al.[48] and Silverstein et al.[49]
	3.81	Methine (CH)	Silverstein et al.[49]
	5.00	Hydroxyl (OH)	Silverstein et al.[49]

Table S2. FTIR-ATR Peak Assignments for QHM pre-polymer.

Functional group	Peak (cm^{-1})	Assigned to	Reference(s)
QH	1523 cm^{-1}	Bending vibrations of carbamate amine (N-H)	Silverstein et al.[49]
	1697 cm^{-1}	Stretching vibrations of carbamate carbonyl (C=O)	Silverstein et al.[49]
QM	1556 cm^{-1}	Stretching vibrations of intermediate carboxylate anions (COO^-)	Kim et al.[50], Mercado-Pagan et al.[48] and Silverstein et al.[49]
	Peak intensity changes between 1714 cm^{-1} and 1782 cm^{-1}	Stretching vibrations of ester carbonyl (C=O) groups and stretching vibrations of carboxylic acid carbonyl (C=O) groups although anhydride and carbamate carbonyl (C=O) groups were also present	Kim et al.[50], Mercado-Pagan et al.[48] and Silverstein et al.[49]

Table S3. Tensile properties (mean ± SEM) of QHM and QH polymers.

Polymer	Tensile strength (MPa)		Tensile modulus (GPa)	Tensile strain at yield or failure (%)
	Yield	Failure		
0s UV QH polymer	-	70 ± 4.1	2.6 ± 0.13	4.8 ± 0.68
90s UV QH polymer	-	71 ± 0.7	2.7 ± 0.05	4.0 ± 0.15
180s UV QH polymer	-	71 ± 2.4	2.7 ± 0.06	3.9 ± 0.30
300s UV QH polymer	-	69 ± 1.2	2.6 ± 0.08	4.2 ± 0.27
0s UV QHM polymer	12 ± 0.7	20 ± 1.5	0.6 ± 0.03	6.5 ± 0.21
90s UV QHM polymer	-	38 ± 3.0	1.7 ± 0.10	4.4 ± 0.17
180s UV QHM polymer	-	65 ± 2.4	2.5 ± 0.07	4.2 ± 0.16
300s UV QHM polymer	-	74 ± 1.5	2.7 ± 0.05	4.4 ± 0.10

Table S4. *P* values comparing tensile strength of QHM and QH polymers.

Group	Group	*P* value
0s UV QHM polymer	90s UV QHM polymer	0.000
0s UV QHM polymer	180s UV QHM polymer	0.000
0s UV QHM polymer	300s UV QHM polymer	0.000
90s UV QHM polymer	180s UV QHM polymer	0.000
90s UV QHM polymer	300s UV QHM polymer	0.000
180s UV QHM polymer	300s UV QHM polymer	0.133
0s UV QH polymer	90s UV QH polymer	1.000
0s UV QH polymer	180s UV QH polymer	1.000
0s UV QH polymer	300s UV QH polymer	1.000
90s UV QH polymer	180s UV QH polymer	1.000
90s UV QH polymer	300s UV QH polymer	1.000
180s UV QH polymer	300s UV QH polymer	1.000

Table S5. *P* values comparing tensile modulus of QHM and QH polymers.

Group	Group	*P* value
0s UV QHM polymer	90s UV QHM polymer	0.000
0s UV QHM polymer	180s UV QHM polymer	0.000
0s UV QHM polymer	300s UV QHM polymer	0.000
90s UV QHM polymer	180s UV QHM polymer	0.000
90s UV QHM polymer	300s UV QHM polymer	0.000
180s UV QHM polymer	300s UV QHM polymer	0.872
0s UV QH polymer	90s UV QH polymer	0.999
0s UV QH polymer	180s UV QH polymer	0.995
0s UV QH polymer	300s UV QH polymer	1.000
90s UV QH polymer	180s UV QH polymer	1.000
90s UV QH polymer	300s UV QH polymer	0.993
180s UV QH polymer	300s UV QH polymer	0.978

Table S6. *P* values comparing tensile strain at yield (0s UV QHM polymer) or failure (All other polymers) of QHM and QH polymers.

Group	Group	*P* value
0s UV QHM polymer	90s UV QHM polymer	0.001
0s UV QHM polymer	180s UV QHM polymer	0.000
0s UV QHM polymer	300s UV QHM polymer	0.001
90s UV QHM polymer	180s UV QHM polymer	1.000
90s UV QHM polymer	300s UV QHM polymer	1.000
180s UV QHM polymer	300s UV QHM polymer	1.000
0s UV QH polymer	90s UV QH polymer	0.706
0s UV QH polymer	180s UV QH polymer	0.482
0s UV QH polymer	300s UV QH polymer	0.824
90s UV QH polymer	180s UV QH polymer	1.000
90s UV QH polymer	300s UV QH polymer	1.000
180s UV QH polymer	300s UV QH polymer	0.999

Table S7. Compression properties (mean ± SEM) of QHM and QH polymers.

Polymer	Compressive strength (MPa)	Compressive modulus (GPa)	Strain at maximum compressive stress (%)
0s UV QH polymer	105 ± 0.8	2.7 ± 0.02	5.5 ± 0.26
90s UV QH polymer	118 ± 0.7	3.0 ± 0.02	6.5 ± 0.38
180s UV QH polymer	106 ± 0.7	2.7 ± 0.04	6.2 ± 0.11
300s UV QH polymer	108 ± 1.4	2.7 ± 0.03	6.7 ± 0.15
0s UV QHM polymer	58 ± 4.0	1.5 ± 0.10	5.7 ± 0.23
90s UV QHM polymer	82 ± 4.8	2.1 ± 0.11	5.8 ± 0.18
180s UV QHM polymer	109 ± 2.4	2.8 ± 0.05	6.8 ± 0.25
300s UV QHM polymer	121 ± 1.3	3.1 ± 0.04	6.0 ± 0.30

Table S8. *P* values comparing compressive strength of QHM and QH polymers.

Group	Group	*P* value
0s UV QHM polymer	90s UV QHM polymer	0.053
0s UV QHM polymer	180s UV QHM polymer	0.000
0s UV QHM polymer	300s UV QHM polymer	0.000
90s UV QHM polymer	180s UV QHM polymer	0.017
90s UV QHM polymer	300s UV QHM polymer	0.003
180s UV QHM polymer	300s UV QHM polymer	0.021
0s UV QH polymer	90s UV QH polymer	0.000
0s UV QH polymer	180s UV QH polymer	0.950
0s UV QH polymer	300s UV QH polymer	0.670
90s UV QH polymer	180s UV QH polymer	0.000
90s UV QH polymer	300s UV QH polymer	0.003
180s UV QH polymer	300s UV QH polymer	0.944

Table S9. *P* values comparing compressive modulus of QHM and QH polymers.

Group	Group	*P* value
0s UV QHM polymer	90s UV QHM polymer	0.000
0s UV QHM polymer	180s UV QHM polymer	0.000
0s UV QHM polymer	300s UV QHM polymer	0.000
90s UV QHM polymer	180s UV QHM polymer	0.000
90s UV QHM polymer	300s UV QHM polymer	0.000
180s UV QHM polymer	300s UV QHM polymer	0.029
0s UV QH polymer	90s UV QH polymer	0.047
0s UV QH polymer	180s UV QH polymer	0.994
0s UV QH polymer	300s UV QH polymer	1.000
90s UV QH polymer	180s UV QH polymer	0.240
90s UV QH polymer	300s UV QH polymer	0.074
180s UV QH polymer	300s UV QH polymer	0.999

Table S10. *P* values comparing compressive strain at maximum stress for QHM and QH polymers.

Group	Group	*P* value
0s UV QHM polymer	90s UV QHM polymer	1.000
0s UV QHM polymer	180s UV QHM polymer	0.064
0s UV QHM polymer	300s UV QHM polymer	0.987
90s UV QHM polymer	180s UV QHM polymer	0.111
90s UV QHM polymer	300s UV QHM polymer	0.998
180s UV QHM polymer	300s UV QHM polymer	0.360
0s UV QH polymer	90s UV QH polymer	0.123
0s UV QH polymer	180s UV QH polymer	0.388
0s UV QH polymer	300s UV QH polymer	0.017
90s UV QH polymer	180s UV QH polymer	0.998
90s UV QH polymer	300s UV QH polymer	0.991
180s UV QH polymer	300s UV QH polymer	0.828

Table S11. *P* values comparing C2C12 cell numbers cultured in the presence of DMEM media containing degradation products at 5 days.

Group	Group	*P* value
Day 5 HBSS	Day 5 0s UV QHM polymer	0.259
Day 5 HBSS	Day 5 90s UV QHM polymer	0.902
Day 5 HBSS	Day 5 180s UV QHM polymer	0.756
Day 5 HBSS	Day 5 300s UV QHM polymer	0. 957

Table S12. FibreWire 4-0 suture migration (mean ± SEM) during cyclic loading.

Group	100 cycles (mm)	200 cycles (mm)	300 cycles (mm)	400 cycles (mm)	500 cycles (mm)
ADM	0.99 ± 0.12	1.24 ± 0.15	1.30 ± 0.16	1.41 ± 0.13	1.42 ± 0.12
0s UV QHM polymer	0.34 ± 0.12	0.41 ± 0.14	0.45 ± 0.15	0.49 ± 0.15	0.51 ± 0.15

Table S13. FibreWire 4-0 suture migration (mean ± SEM) during load to failure (at 25 N).

Group	Migration (mm)
ADM	1.92 ± 0.26
0s UV QHM polymer	0.54 ± 0.11

Table S14. Tensile properties (mean ± SEM) of ADM- or QHM-repaired supraspinatus muscle-tendon-bone unit.

Group	Ultimate Load (N)	Normalized Tensile Stiffness (N/(mm/mm))
ADM	31.6 ± 2.0	33.5 ± 6.5
0s UV QHM polymer	31.1 ± 3.3	54.3 ± 6.7