Supplementary information for:

A defined heat pretreatment of gelatin enables control of hydrolytic stability, stiffness, and microstructural architecture of fibrin-gelatin hydrogel blends

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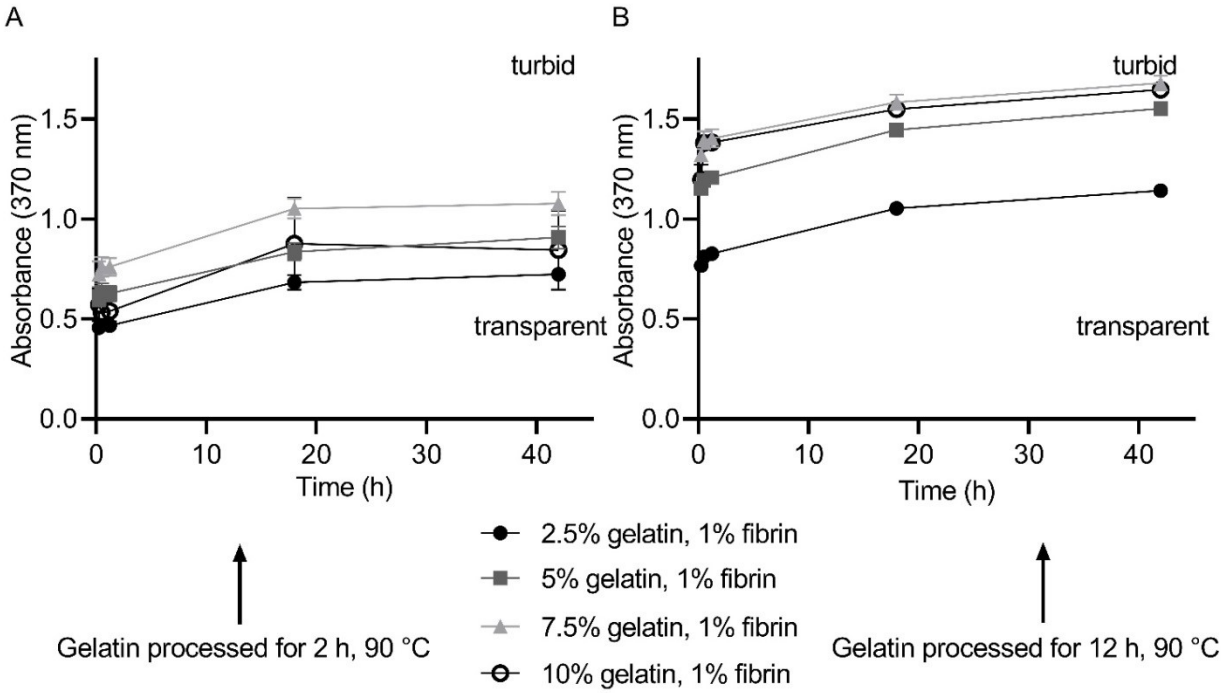
Table S1: Mixing protocols for 1 ml of the hydrogel blends

2.5, 5, 7.5, 10%Gelatin 1%Fibrin – all reagents and mixing at 37 °C						
	Substance	Concentration	[ml]			
1	PBS		0.52175	0.35515	0.18845	0.02175
2	CaCl2	250 mM	0.01			
3	Transglutaminase	60 mg/ml	0.0667			
4	Gelatin	150mg/ml	0.1667	0.3333	0.5	0.6667
5	Fibrinogen	50 mg/ml	0.2			
6	Tranexamic acid	100 mg/ml	0.0015			
Adjusting pH to 7.4 using 0.7 M NaOH and pre-incubation for 1h						
7	Thrombin	30 U/ml	0.03335			

Figure S1: Absorbance at 370 nm of fibrin-gelatin and fibrin-collagen blends. Measurements of absorbance at 450, 550 or 620 nm revealed identical patterns. A: Fibrin-gelatin blends with gelatin

processed for 2 h at 90 °C. B: Fibrin-gelatin blends with gelatin processed for 12 h at 90 °C.



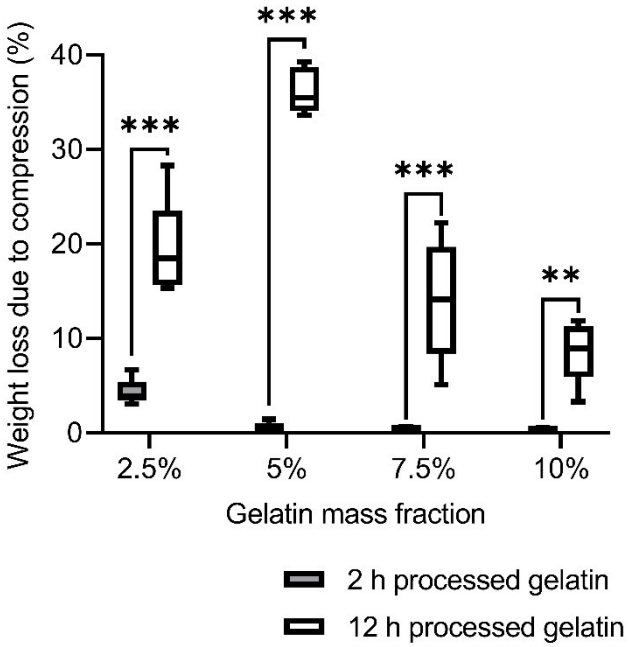


Figure S2: Weight/fluid loss due to compression of fibrin-gelatin blends. When gelatin in fibrin-gelatin blends was processed for 12 h instead of 2 h at 90 °C, weight loss significantly increased.

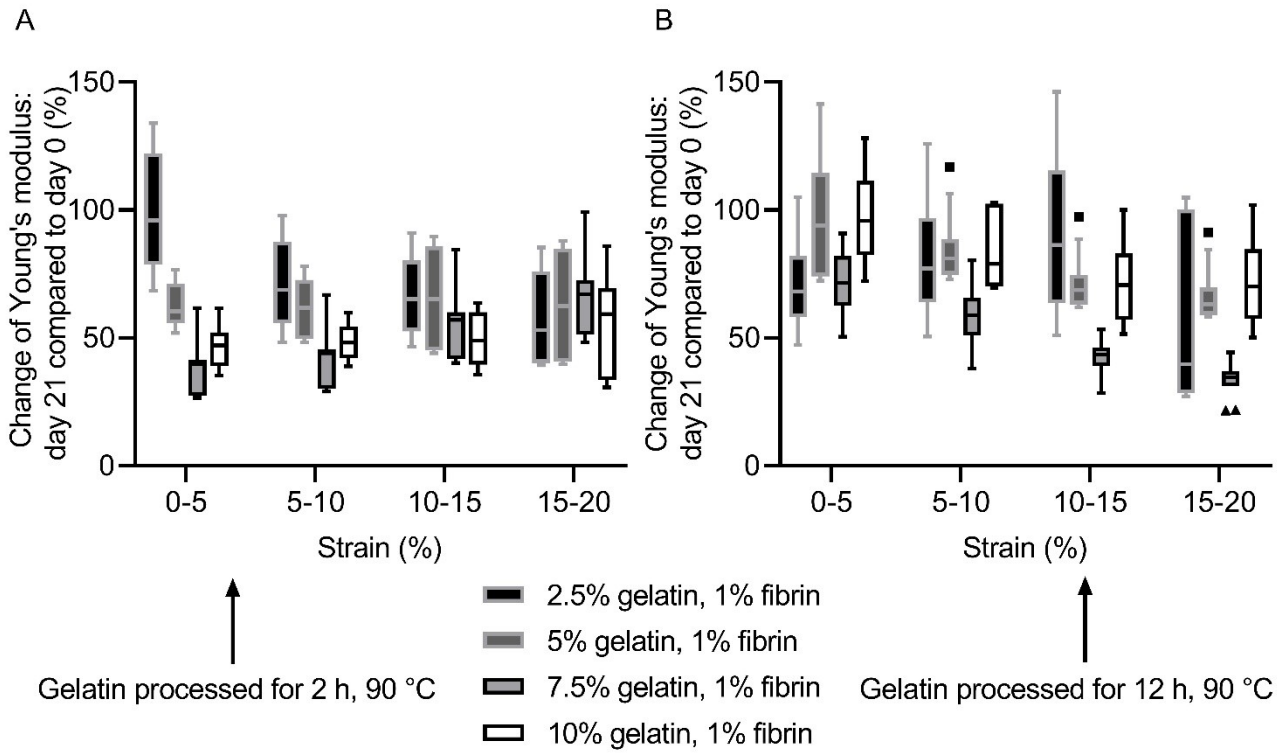


Figure S3: Change of Young's modulus of fibrin-gelatin blends after 21 days of in vitro degradation compared to initial Young's moduli. The blends retained most of their initial stiffness. A: Fibrin-gelatin blends with gelatin processed for 2 h at 90 °C. B: Fibrin-gelatin blends with gelatin processed for 12 h at 90 °C.

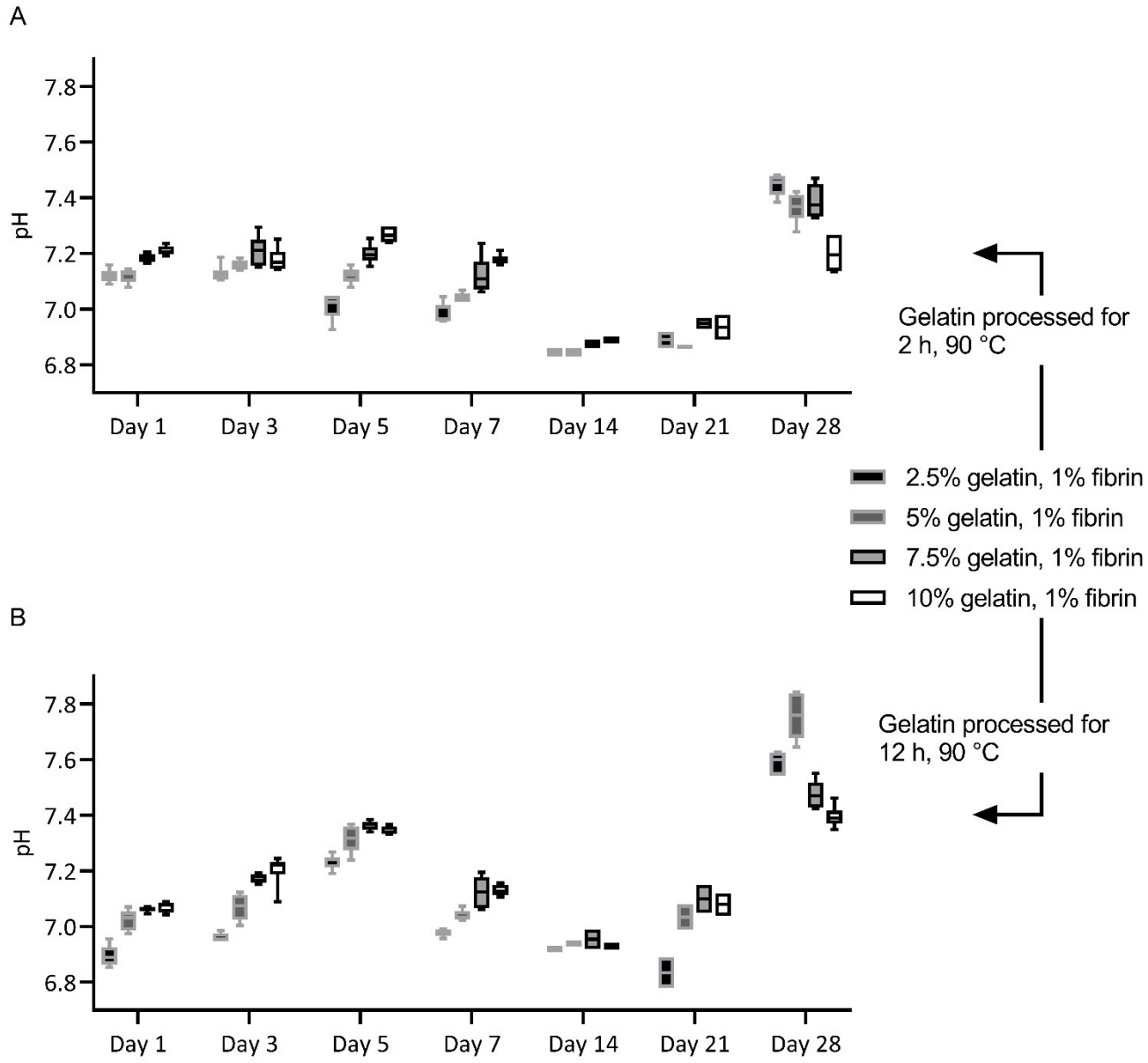


Figure S4: pH value of PBS buffer used for in vitro degradation experiments for fibrin-gelatin blends. The shift to higher pH of day 28 indicated progressed degradation. A: Fibrin-gelatin blends with gelatin processed for 2 h at 90 °C. B: Fibrin-gelatin blends with gelatin processed for 12 h at 90 °C.

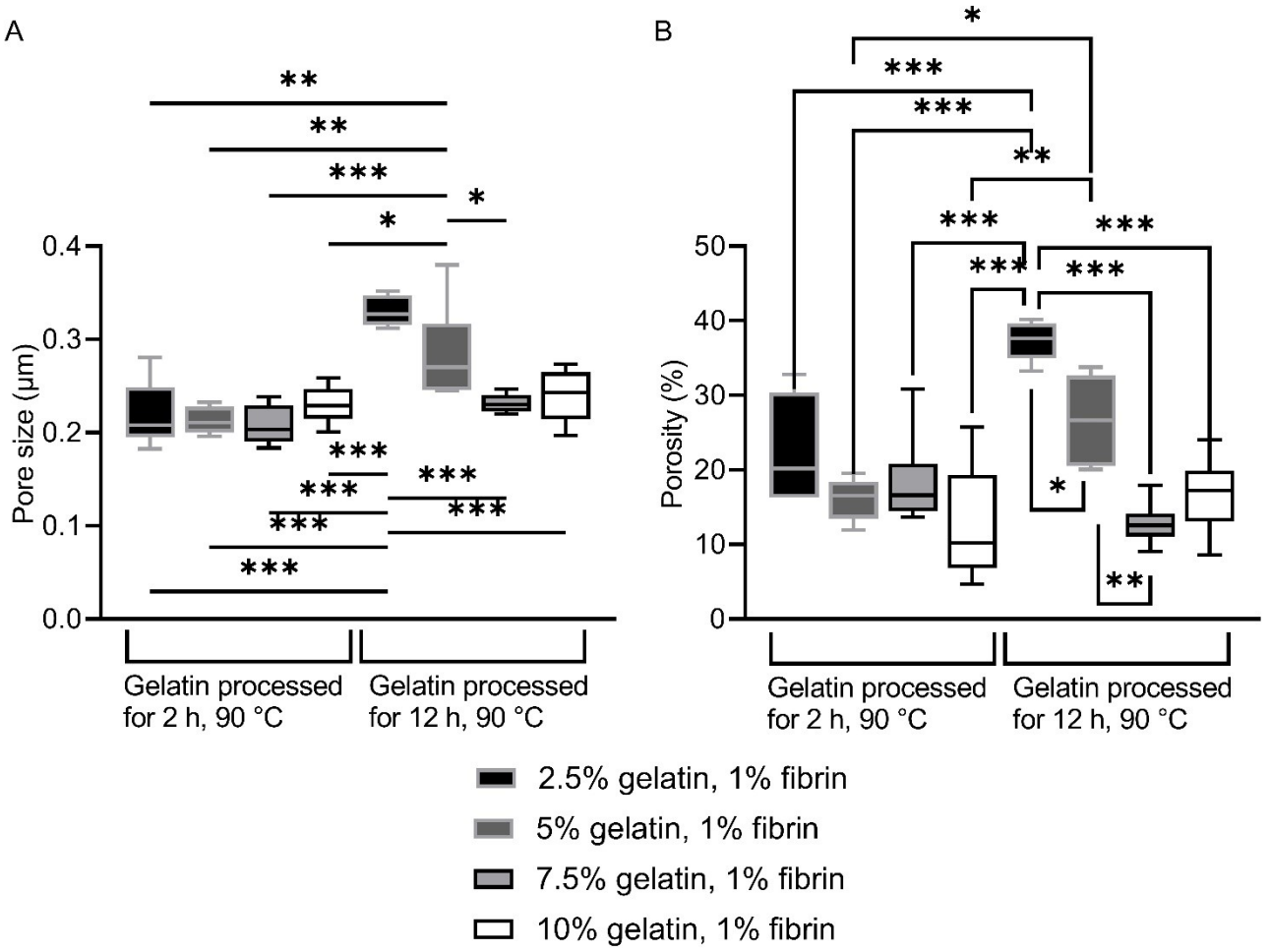


Figure S5: A: Analysis of pore size of fibrin-gelatin blends. Gelatin fibrin blends with gelatin processed for 12 h instead of 2 h show significantly larger pore sizes at 2.5% and 5% (w/v) gelatin. B: Analysis of porosity of fibrin-gelatin blends. Gelatin fibrin blends with gelatin processed for 12 h instead of 2 h show significantly higher porosity at 2.5% and 5% (w/v) gelatin.

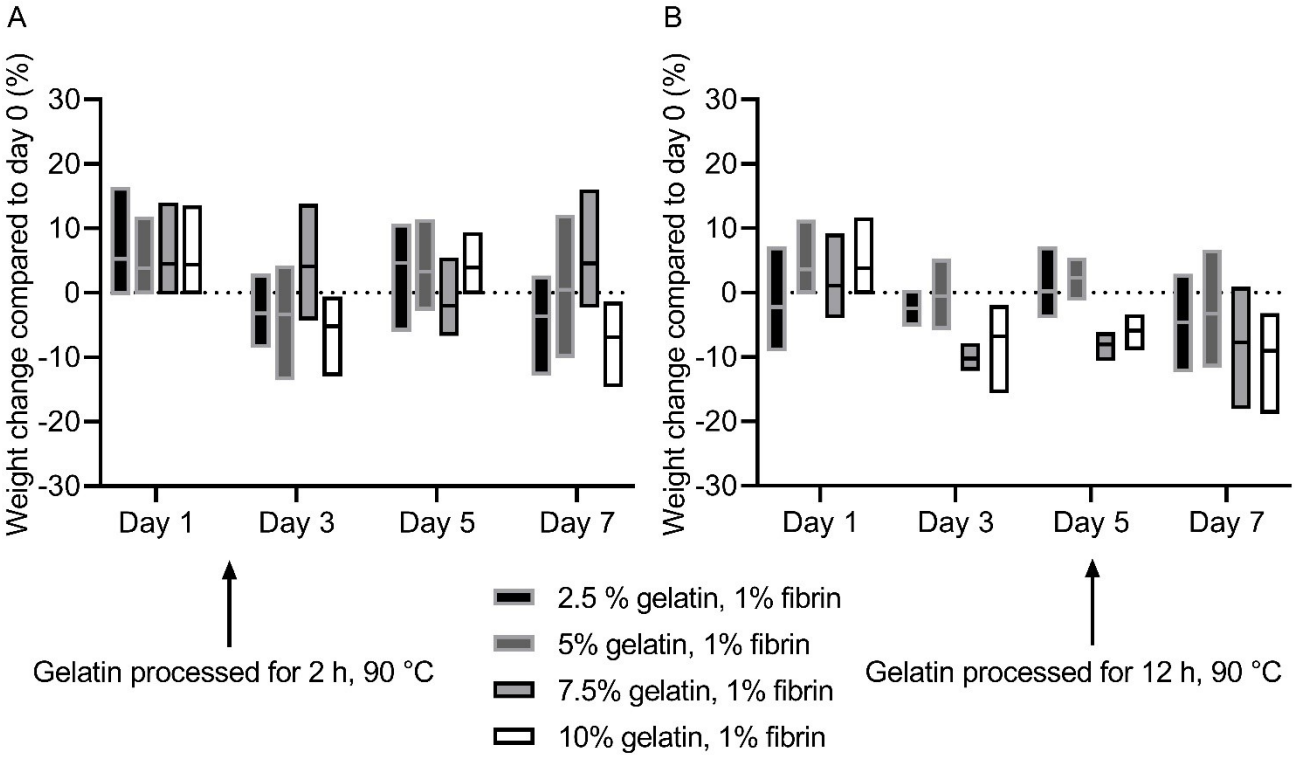


Figure S6: In vitro degradation of HUVEC-laden fibrin-gelatin blends over a proliferation duration of 7 days. Deviations are within the range of statistical variance as no significant weight change of lyophilized blends was found, indicating hydrogel stability during HUVEC proliferation. A: Fibrin-gelatin blends containing gelatin processed for 2 h at 90 °C. B: Fibrin-gelatin blends containing gelatin processed for 12 h at 90 °C.

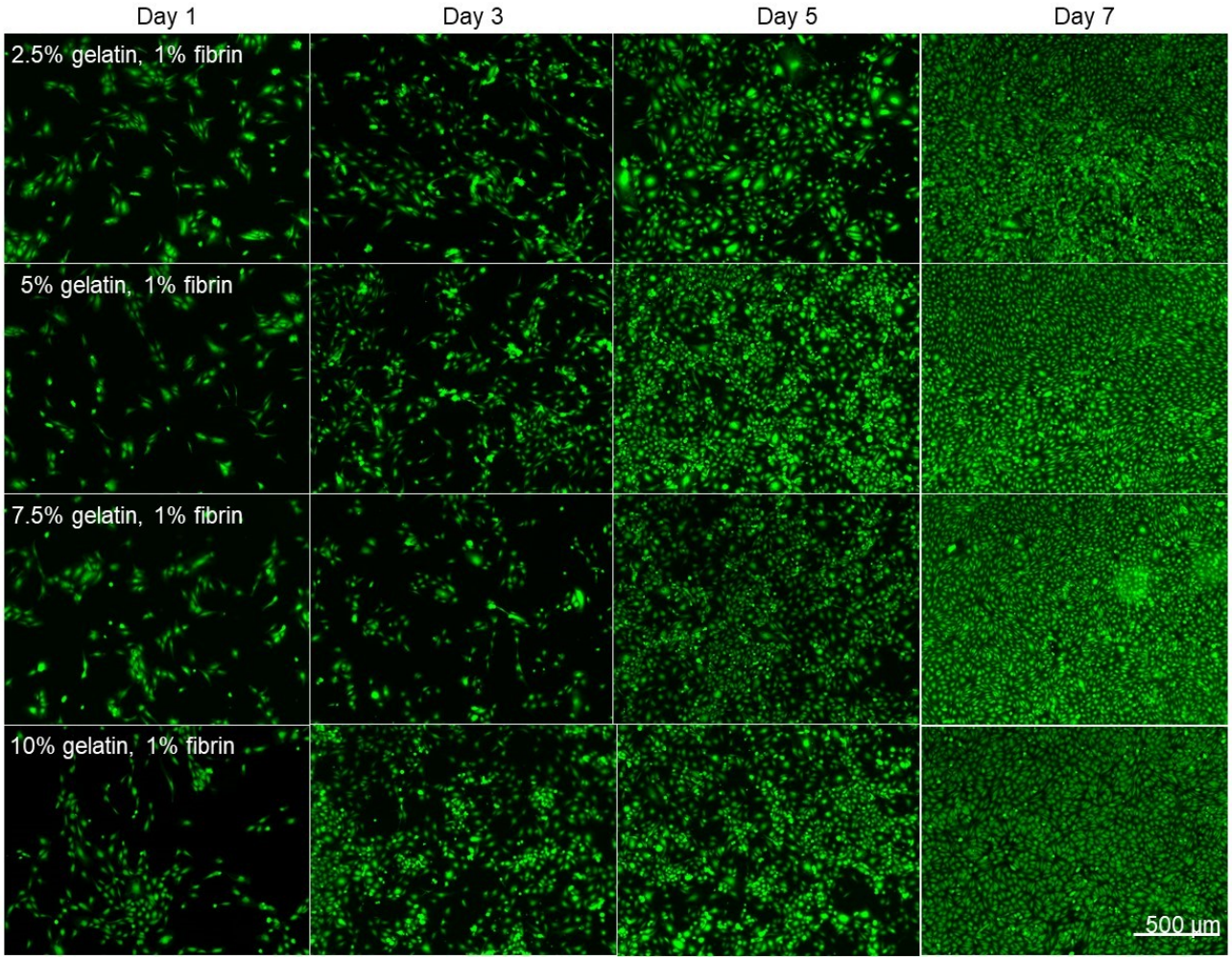


Figure S7: Fluorescence images of calcein-AM stained HUVECs on fibrin-gelatin blends composed of gelatin processed for 2 h at 90 °C. Dead cells detach from the gels and are hence not visible in the pictures. After 7 days, a monolayer was formed on all gels. Scale bar identical for every picture.

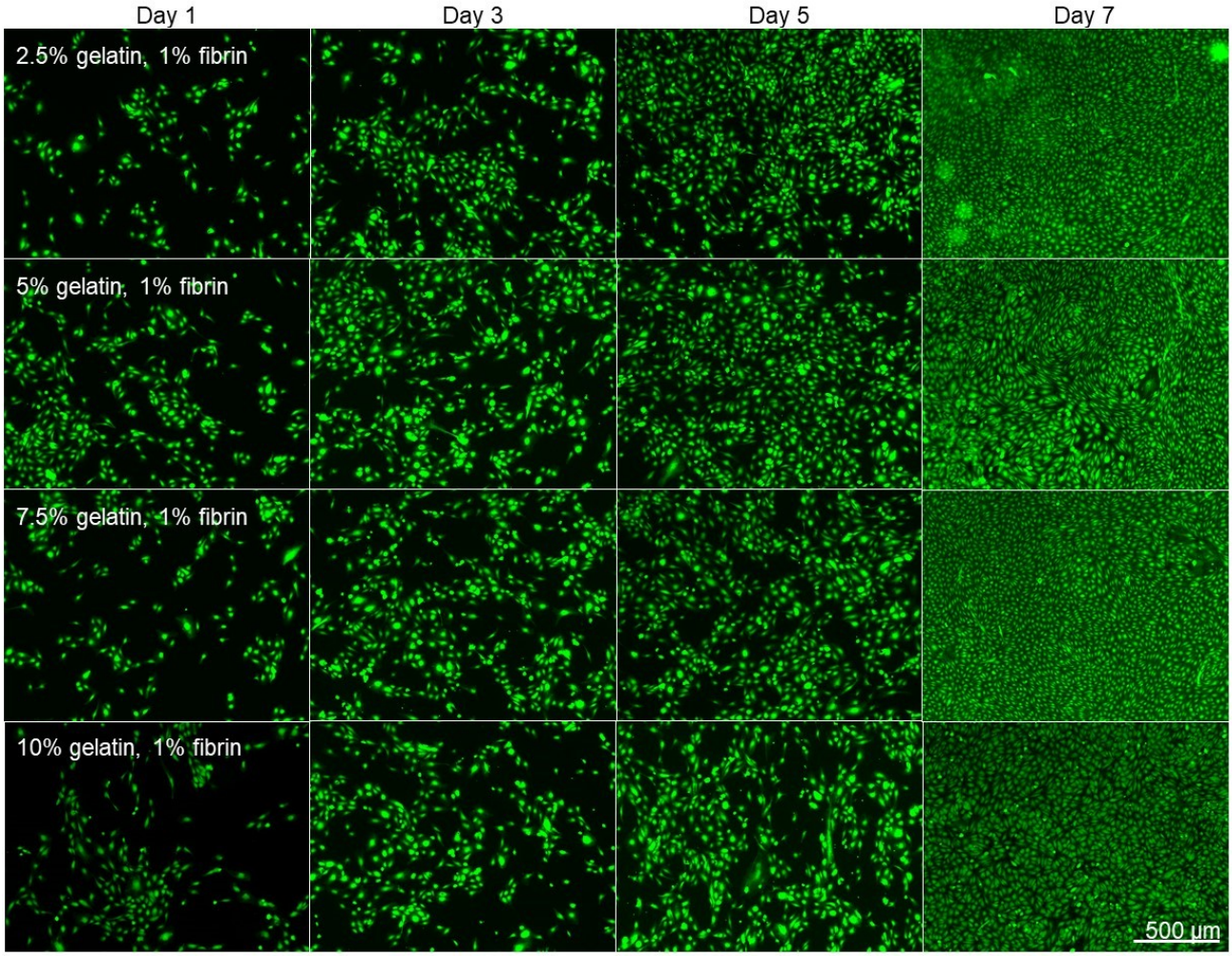


Figure S8: Fluorescence images of calcein-AM stained HUVECs on fibrin-gelatin blends composed of gelatin processed for 12 h at 90 °C. Dead cells detach from the gels and are hence not visible in the pictures After 7 days, a monolayer was formed on all gels. Scale bar identical for every picture.

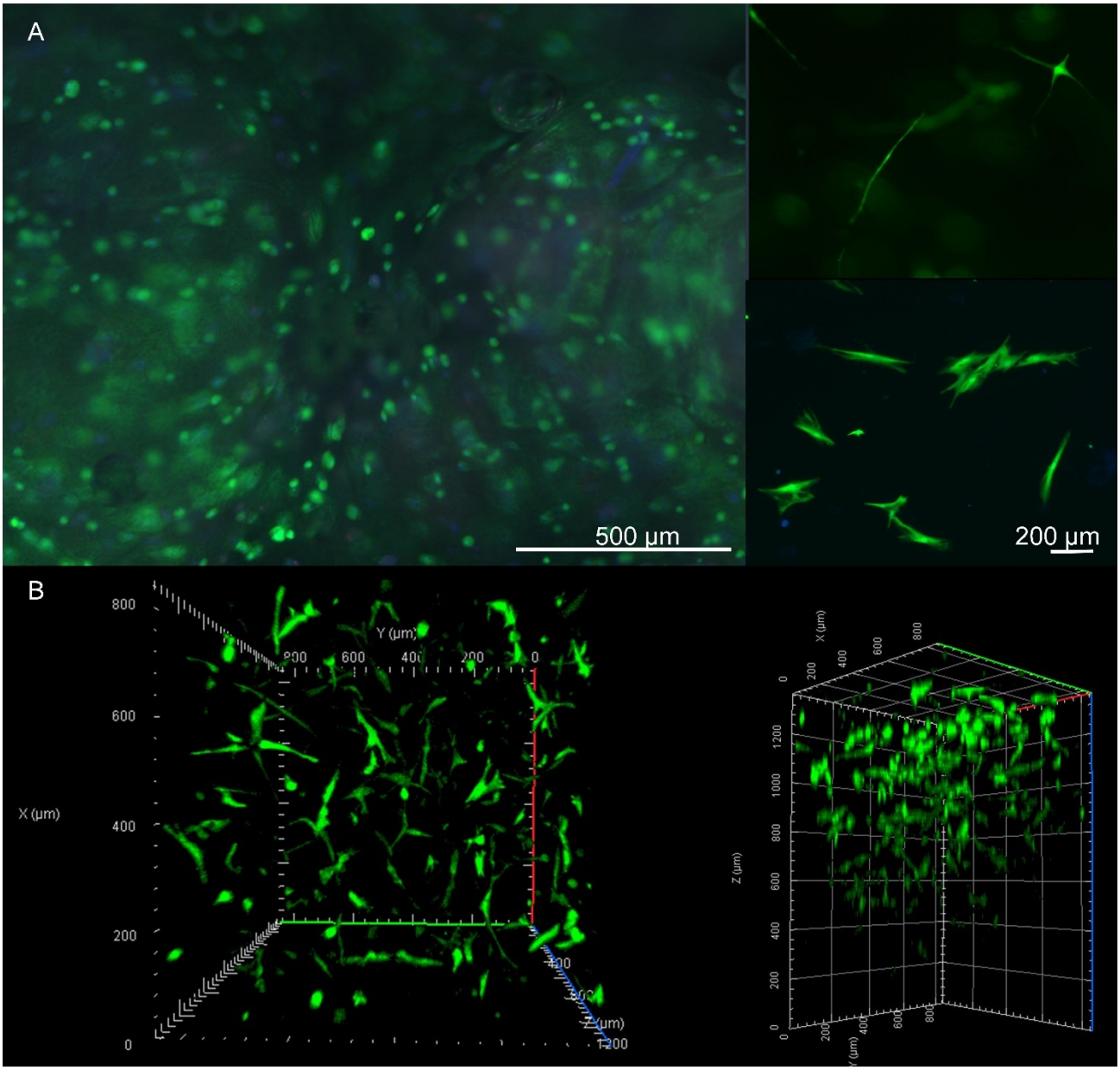


Figure S9: Smooth muscle cells encapsulated in dense fibrin-gels with high viability (left) and stretching (right) after 7 days of cultivation. A: Imaging by fluorescence microscopy. B: Imaging by confocal microscopy – transparent hydrogel blends enable penetration depths of >1 mm. Images were taken using a Zeiss LSM 710 (Carl Zeiss AG, Oberkochen, Germany) and the software Zen 2012 (Carl Zeiss AG, Oberkochen, Germany) with a 10x objective for a z-stack of 91 slices over 1.35 mm. Excitation and emission wavelengths were 488 nm and 538 nm, respectively, and detection wavelength 494-582 nm.

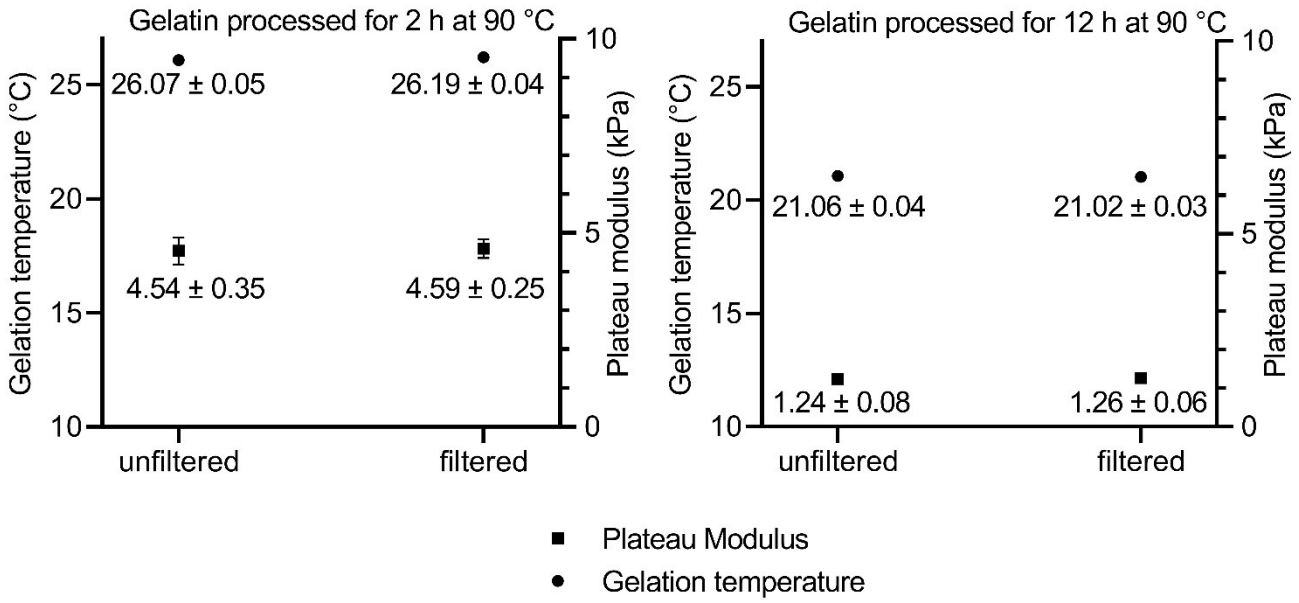


Figure S10: Rheometry of gelatin solutions (150 mg/ml) processed for either 2 h or 12 h at 90 °C. No statistical differences were found in gelation points and plateau moduli of unfiltered and filtered gelatin. Hence, the filtering procedure did not shift the initial gelatin mass fractions.