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Supplementary Materials for

Engineering a highly elastic human protein–based sealant for surgical applications

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Other Supplementary Material for this manuscript includes the following:
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SUPPLEMENTARY METHODS

Scanning electron microscopy (SEM) study

Photocrosslinked MeTro hydrogel sample was lyophilized and mounted onto an aluminum holder. A 30 nm thick gold layer was spin-coated on the sample prior to imaging. Secondary electron imaging (SEI) was performed at 15 kV using a FEI/Philips XL30 FEG SEM. The effects of MeTro concentrations and methacrylation degrees on the pore size of MeTro gels were studied. Triplicates of 5, 10 and 20% (w/v) MeTro with medium methacrylation, as well as 10% (w/v) MeTro with high and low methacrylated samples were imaged. Pores were manually selected per image for pore sizing (n=50). ImageJ software was then used to calculate the apparent pore size of the MeTro gels.

Swelling ratio study

The swelling ratios of 5, 10 and 20% (w/v) MeTro with medium methacrylation, as well as 10% (w/v) MeTro with high and low methacrylated gels were evaluated in PBS at 37°C. 100 µl of MeTro solution was injected into a PDMS mold with a 7 mm diameter and 5 mm depth. The prepolymer solution was exposed to UV light for 3 minutes. MeTro gel was detached from the mold and immersed into PBS solution for 10 mins. The sample was lyophilized, and the dry weight was recorded. Samples were then placed in PBS at 37°C for different time points (4, 12, 24, 48 h). At each time point, samples were removed from PBS and weighed. The swelling ratio was calculated with the following equation: $(W_{wet}-W_{dry})/W_{dry} \times 100\%$, where W_{dry} is the weight after lyophilizing and W_{wet} is the weight after removal from PBS. Tests were performed in triplicate for each condition to ensure statistical significance.

Mechanical characterization

Tensile and compressive cyclic testing of MeTro gel were performed using a mechanical tester (Instron model 5542) with a 10 N load cell. Hydrogel was photocrosslinked in a PDMS model (dimensions 15 mm x 5 mm x 1 mm) for tensile test. Gels were then incubated in PBS at 37°C for 4 h prior to mechanical testing. Mechanical testing was performed on the samples prior to reaching maximum swelling ratio, to mimic the swelling state of MeTro gel when applying as a tissue sealant. The dimensions of the samples were measured with a digital caliper. To minimize dehydration during testing, a humidifier was used surrounding the testing device. The strain rate was 10 mm/min and performed until sample failure. Ultimate tensile stress was obtained at the failure point, maximum strain was obtained at the failure point, and elastic modulus was determined from the slope of the stress-strain curve. Compressive samples were prepared in the same molds used for swelling tests (7 mm in diameter and 5 mm in depth). Prior to the test, hydrogels were incubated in PBS for 4 h. The compressive strain rate was 10 mm/min and strain level was up to ~ 60% of the original height. The compression and load was recorded for 20 cycles. There is no significant change through all the cycles. The compressive modulus was obtained from the slope during loading on the 20^{th} cycle. The slope of the stress-strain curve was taken from the 0.1-0.3 mm/mm of the strain. The energy loss was calculated for the 20^{th} cycle based on the area between the loading and unloading curves, following the equation $\text{Energy Loss} = \frac{\text{Area below loading} - \text{Area below unloading}}{\text{Area below loading}} \times 100\%$. Samples were tested in triplicate for each condition.

Cell culture study

Mesenchymal stem cells (MSC, from Lonza) and epithelial progenitor cells (EPC) (EPC, Lonza) were used for in vitro studies. MSCs were cultured using mesenchymal stem cell growth medium (MSC-GM, Lonza) with 10% fetal bovine serum (FBS, Invitrogen), glutamine-penicillin-streptomycin (GPS, Invitrogen). EPCs were cultured in endothelial basal medium (EBM-2, Lonza) enriched with endothelial growth factors (BulletKit, Lonza), 10% FBS and 100 units/ml penicillin-streptomycin (Gibco). MSCs and EPCs were cultured in 5% CO_2 humidified incubators at 37 °C. Cells were passaged every 3 days and media changed every other day.

In vitro 2D cell study

10% (w/v) MeTro solutions with medium methacrylation were photocrosslinked on 3-(trimethoxysilyl) propyl methacrylate *TMSPMA* coated glass slide with 20 μm spacers for 30s. Either single cell types (MSCs or EPCs) or co-culture of both cells (MSCs and EPCs) were seeded on the top of the MeTro gels placed in a 24 well plate. For the single type cell culture, the cell seeding density was 5×10^4 cell/well. For the co-culture, each of the cell types were seeded at 2.5×10^4 cell/well and premixed prior to administration on the MeTro gel. Cell viability and proliferation was studied on days 1, 4, and 7 of culture (co-culture). Media was changed every other day.

Cell viability study

Cell viability was performed with the LIVE/DEAD kit (Invitrogen, USA) following instructions from the manufacturer. Briefly, ethidium homodimer-1 (2 μl/ml) and calcein AM (0.5 μl/ml) were mixed in PBS. The well plate was then incubated for 15 minutes at 37 °C and imaged with an inverted fluorescence microscope (Nikon TE 2000-U, Nikon instruments Inc.). ImageJ was used to count the live and dead cells by using at least 4 images from different areas of 3 hydrogels for each MeTro condition. Cell viability was calculated by division of the number of live cells by the total number of stained cells.

Cell spreading study

Phalloidin (Invitrogen), endothelial cell markers CD31 (Abcam, Cambridge, MA, USA) and 4', 6-diamidino-2-phenylindole (DAPI; Sigma) staining was used to investigate cellular attachment and spreading on the surfaces of MeTro hydrogels. CD31 is expressed on the cell surface of EPCs when these cells differentiate into endothelial cells. To stain for F-actin or CD31, the cells were permeabilized in a 0.1% (w/v) Triton solution in PBS for 30 minutes and blocked in 1% (w/v) bovine serum albumin (BSA) for 1 h. The samples were then incubated in a solution of 1:40 ratio of phalloidin with 0.1% BSA for 45 minutes at room temperature to stain the actin cytoskeleton or CD31 endothelial surface staining. For nuclear staining, the samples were incubated in a 0.1% (v/v) DAPI solution in PBS for 10 minutes at 37°C. The stained samples were then washed three times with PBS before visualizing with an inverted fluorescence microscope.

Cell activity Study

Cell activity was measured with PrestoBlue cell viability reagents (Life Technologies) following the manufacturer's protocol. Each construct was incubated with 400 µl of a solution containing 10% PrestoBlue reagent and 90% respective cell media (MSC-GM media for MSCs and EBM-2 for EPCs) for 2 h at 37°C. The resulting fluorescence was measured at a wavelength of 560 nm (excitation) and 590 nm (emission) with a fluorescence reader (Synergy HT-Reader, BioTek). By subtracting the fluorescence values from the control wells without cells, the relative fluorescence values were calculated and plotted for each day. Higher fluorescence values correlate to greater total metabolic activity. Samples were tested in triplicate for each condition.

Engineering MeTro-MMP hydrogels and In vitro degradation test

20% MeTro-MMP hydrogel prepolymers containing MMP at various concentrations (0.1, 1 and 10 µg/ml) were prepared in water at 4 ºC. The solutions were then placed inside cylindrical polydimethylsiloxane (PDMS) molds (2 mm height ×8 mm diameter) and photopolymerized to form hydrogels for the in vitro degradation tests. Samples were then weighed and placed in DPBS at 37 °C for 20 days. 20% MeTro hydrogels without MMP hydrogels were used as control. The percentage degradation (D%) of the engineered hydrogels was calculated using the following equation:

$$D\% = \frac{W_0 - W_f}{W_f} \times 100\% , \quad (1)$$

where W_0 is the initial wet weight of the sample and Wf is the final weight of the sample after degradation (n=5). For the in vivo lung experiments, 20% MeTro was mixed with MMP prior to injection on the defect site and photopolymerized as explained previously.

SUPPLEMENTARY FIGURES

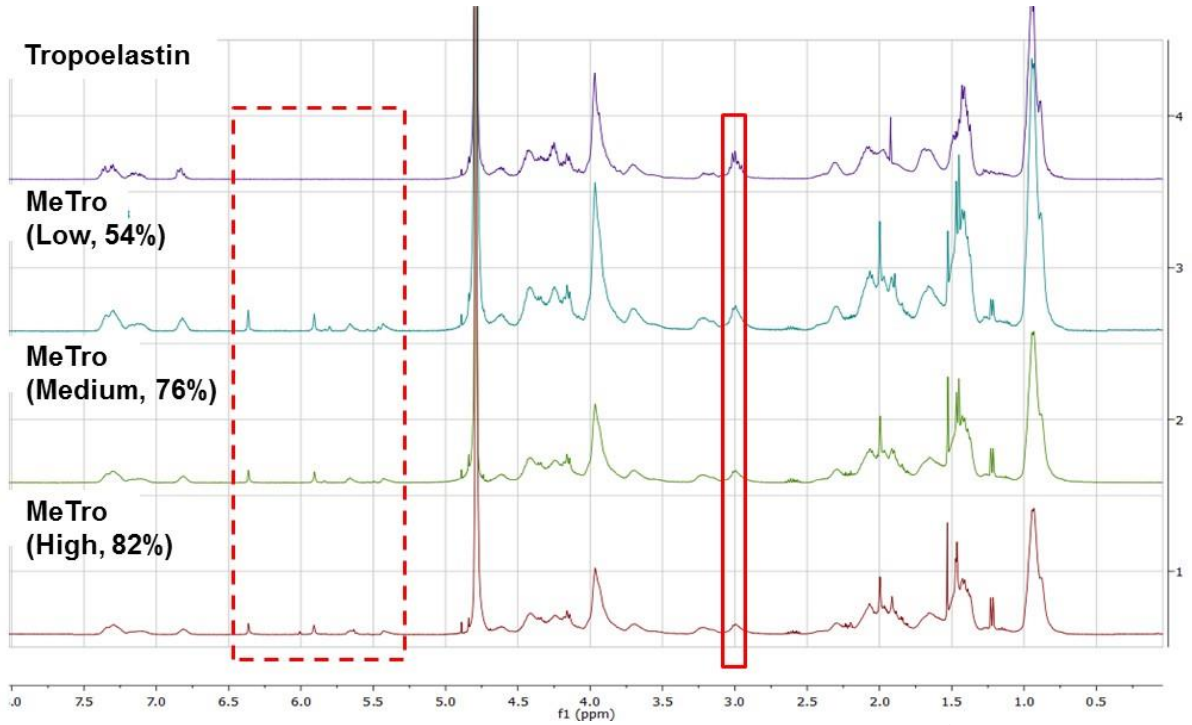

Fig. S1. Effect of methacrylation on tropoelastin. Representative ^{1}H NMR spectrum of tropoelastin and MeTro solutions measured at 4°C. The methacrylation degree of MeTro is provided in parentheses. Peaks that correspond to methacrylate groups (between 5 and 6.5 ppm) grew in intensity at the expense of the lysine-specific peak in tropoelastin at 3 ppm.

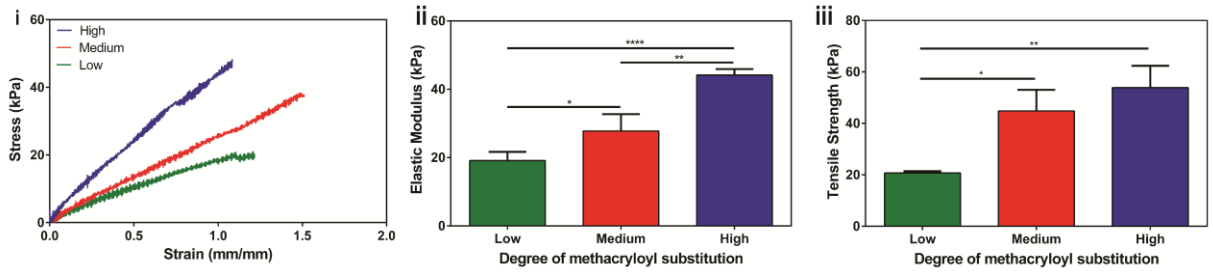

Fig. S2. Tensile test on MeTro hydrogel with different degrees of methacryloyl substitution. (i) Representative graph of tensile test, (ii) elastic modulus, and (iii) maximum tensile strength.

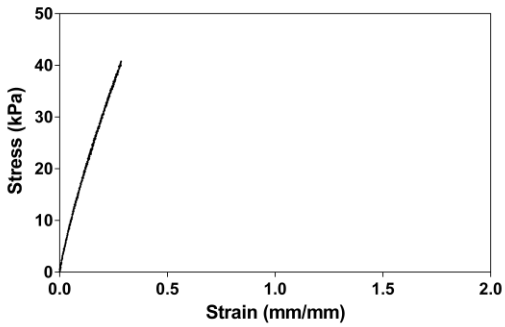

Fig. S3. Tensile test for Progel. Representative graph of tensile test on Progel.

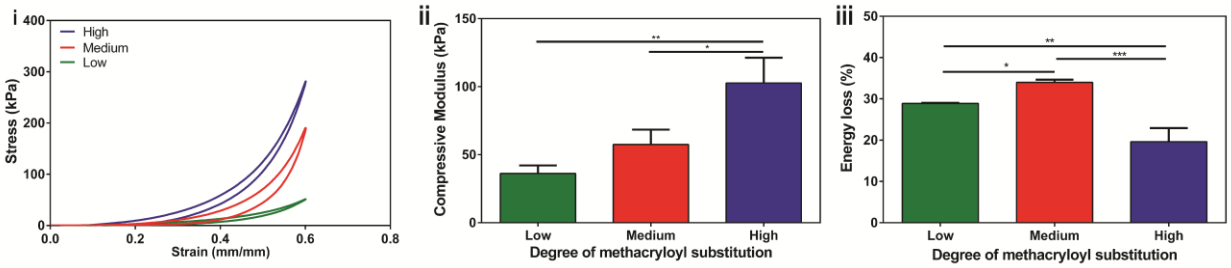

Fig. S4. Compression test on MeTro hydrogel with different degrees of methacryloyl substitution. (i) Representative graph of compression test, (ii) compressive modulus, and (iii) energy Loss.

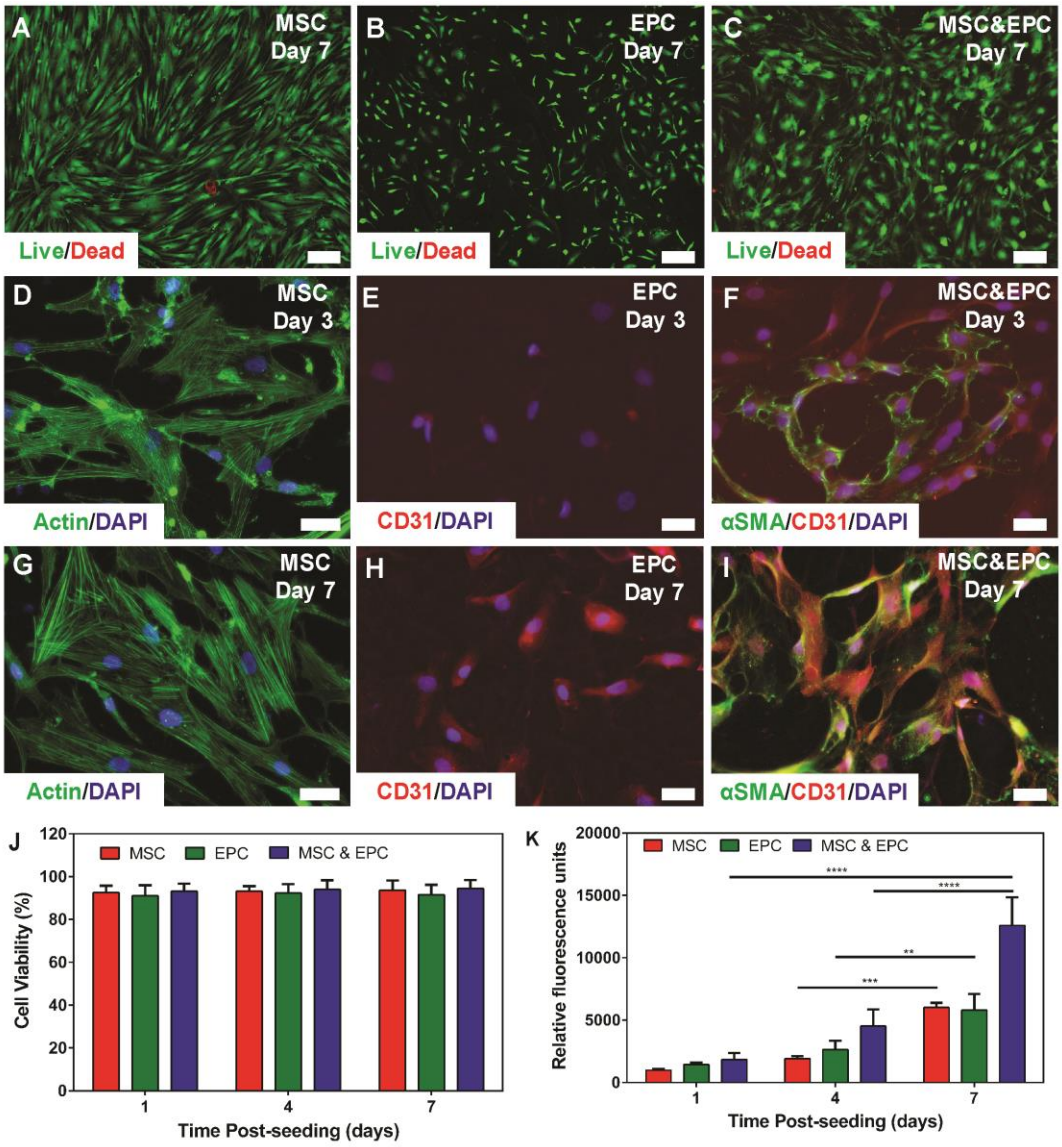

Fig. S5. In vitro two-dimensional studies on MeTro hydrogels. 10% MeTro concentration using mesenchymal stem cells (MSCs), epithelial progenitor cells (EPCs), and a co-culture of both MSCs and EPCs. (a-c) Live/dead images from the surfaces of MeTro hydrogels seeded with monoculture (a) MSCs, (b) EPCs, and (c) co-culture MSCs and EPCs on day 7 (scale bar = 200 μm). Rhodamine-labeled phalloidin/DAPI staining for F-actin/cell nuclei of MeTro seeded with MSCs on day (d) 3 and (g)7 of culture; CD31/DAPI staining for MeTro seeded with EPCs on day (e) 3 and (h)7 of culture; α-SMA/CD31/DAPI staining for MeTro seeded with MSCs and EPCs coculture on day (f) 3 and (i) 7 of culture (scale bar = 50 μm). Clearly staining for α-SMA and CD31 was found in day 3 of MSCs and EPCs coculture indicating that by coculture these two types of stem cells on MeTro gels, MSC can differentiate to smooth muscle cells and EPCs can differentiate to endothelial cells after day 3. By day 7 of culture, a confluent layer of coculture was formed on MeTro gel indicating a strong interaction between differentiated MSCs and EPCs, which can lead form capillary-like structures. **(j)** Quantification of cell viabilities 1, 4 and 7 days after cell seeding. **(**k**)** Quantification of metabolic activity by PrestoBlue at day 1, 4 and 7 after cell seeding (***P* < 0.01, ****P* < 0.001, *****P* < 0.0001).

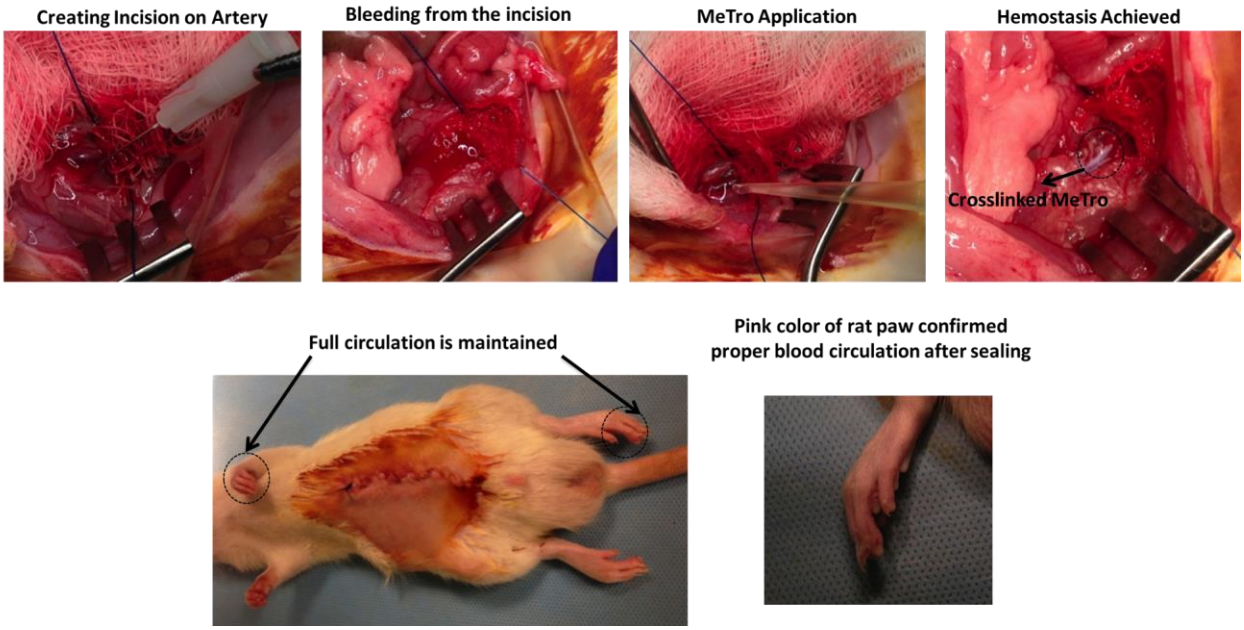

Fig. S6. Images of in vivo tests on rat artery sealed by MeTro.

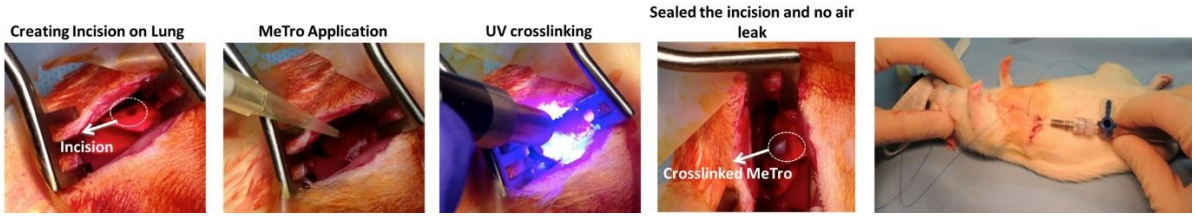

Fig. S7. Images of in vivo tests on rat lung sealed by MeTro.

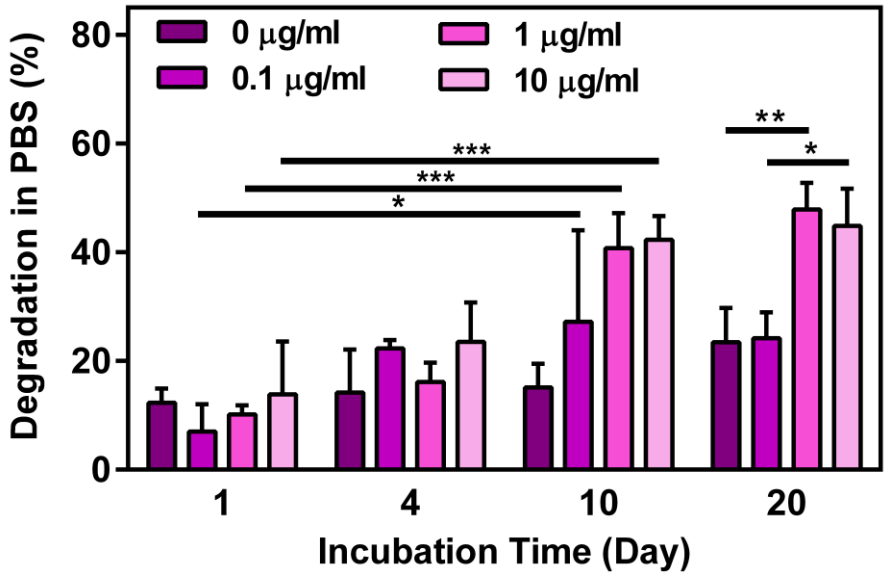

Fig. S8. In vitro degradation of MeTro and MeTro-MMP hydrogels. Tested in PBS at 37°C, depending on different MMP concentrations over 20 days (n = 4). 20 % MeTro sealants with high methacryloyl substitution were used. Data are means ± SD (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001).

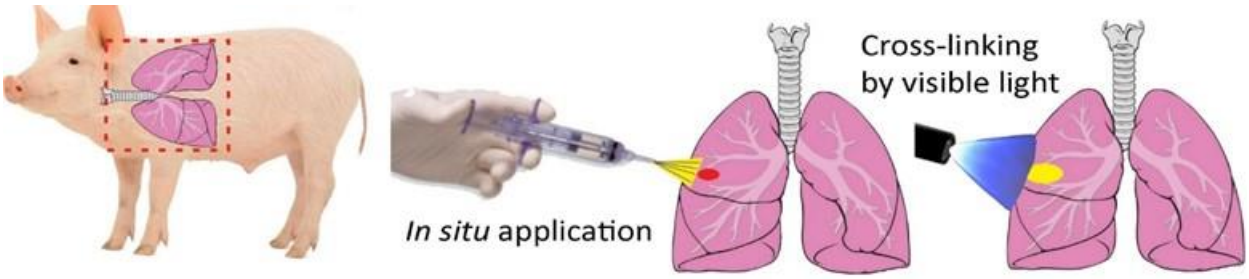

Fig. S9. Schematic of porcine lung leakage sealing using MeTro.

Table. S1. Comparison of surgical adhesives/sealants on the market.

Name	Major Components	Advantages	Disadvantages
Cyanoacrylate adhesives	Cyanoacrylate monomer	- Low cost - High adhesion - Fast polymerization	- Toxic monomer - Highly rigid - Non-degradable
Bioglue (CryoLife Inc.)	Bovine albumin and glutaraldehyde	- High adhesion - Hemostatic properties	- Toxicity of glutaraldehyde
Coseal (Cohesion Technologies, Inc.)	Glutaryl-succinimidyl ester and thiol-terminated PEG	- Biocompatibility - Low cost	- Low adhesion (<30kPa) - Low burst pressure (<2 kPa) - Low shear strength (<70 kPa)
Fibrin-based sealants (Evicel, Ethicon Inc.)	Fibrinogen and thrombin	- Biocompatibility - Hemostatic properties	- Possible disease transmission from blood products - Low burst pressure (<4 kPa) - Low adhesion (<30 kPa)
FocalSeal (Genzyme)	Eosin-based primer and PEG	- Biocompatibility - Bioabsorbable	-Additional of primer -complex delivery and crosslinking procedure
Progel (Davol Inc.)	Human albumin and a NHS-activated PEG	- Medium burst pressure (~4 kPa) - High adhesion (~75 kPa) - High shear strength (~200 kPa)	- Possible disease transmission from blood products - High cost - High chance of recurring leaks (~65%)