biomedicines

MDPI

# Supplementary Materials

## Porcine decellularized diaphragm hydrogel: a new option for muscle malformations

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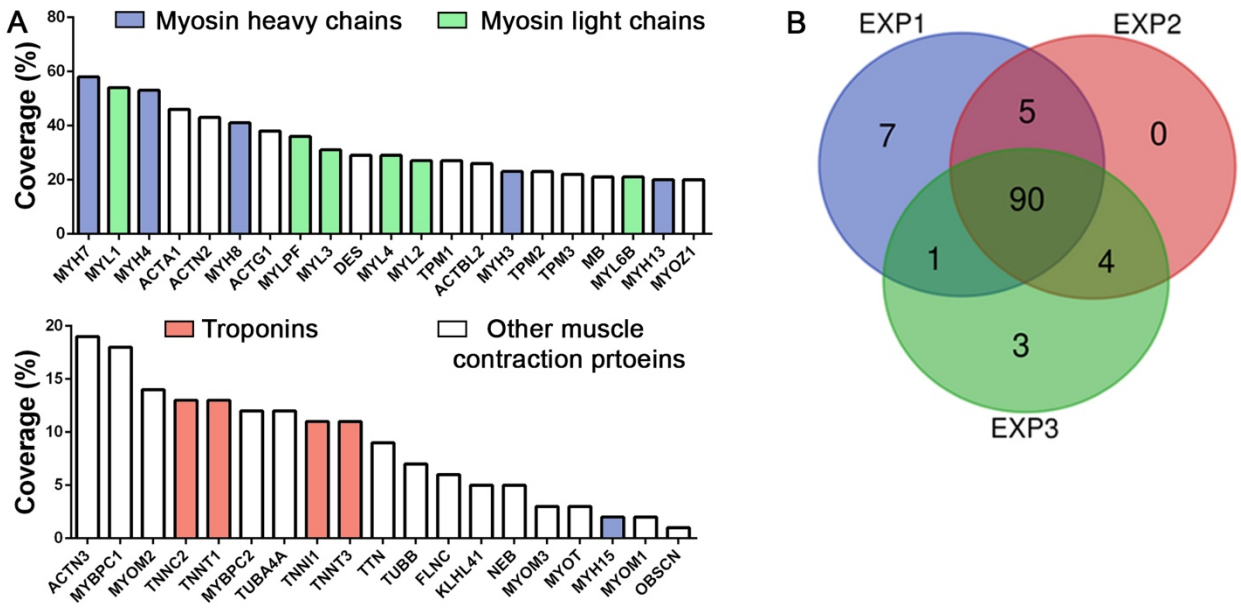
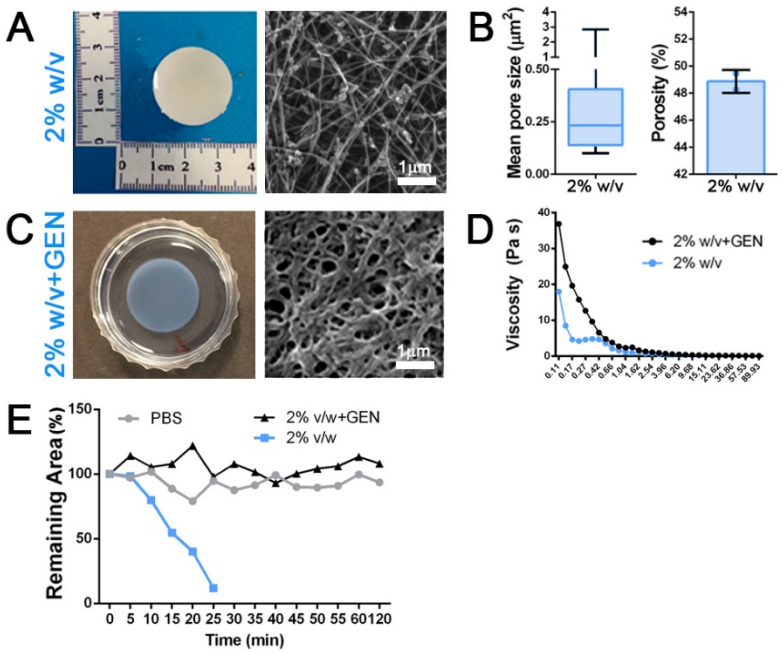
**Figure S1. Muscle-specific proteins in decellularized samples.**



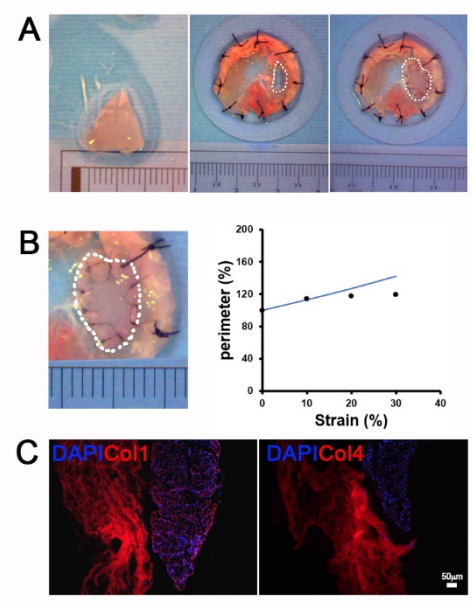

**A.** Percentage of coverage of specific muscle contraction proteins detected in decellularized samples. Highlighted with colors different isoforms of the same protein. **B.** Venn diagram showing the overlapping of detected proteins among triplicates of three different analyzed samples (EXP = experiment).

**Figure S2. 2% w/v dECM-derived hydrogel characterization.**



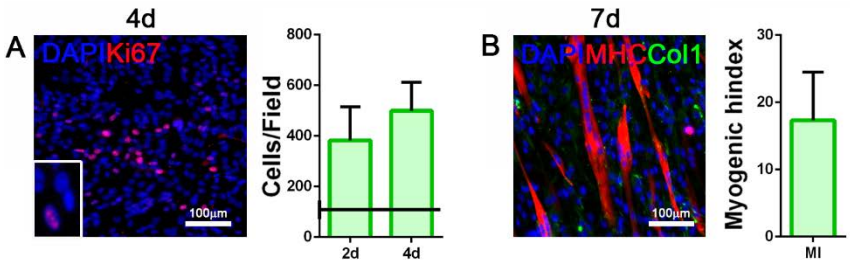

**A.** Gross appearance and ultrastructure of 2% w/v hydrogels. **B.** Mean pore size and porosity of 2% w/v hydrogels. **C.** Gross appearance and ultrastructure of 2% w/v hydrogels after crosslinking with genipin (+GEN). **D.** Viscoelastic properties under shear stress of 2% w/v hydrogels with and without genipin cross-linking. **E.** Degradation assay of 2% w/v hydrogels with and without genipin crosslinking using collagenase II. PBS: 2% w/v hydrogels without genipin and incubated with PBS (no collagenase II).

**Figure S3. Preparation and analysis of crosslinked 3% w/v dECM-derived hydrogel application in ex vivo CDH model.**




**A.** Gross appearance of crosslinked 3% w/v hydrogel patch (left panel), diaphragm defect (middle panel) and sutured patch on the defect (right panel). **B.** Calculated perimeter increasing during continuous stimulation up to 30% strain. Dots represent experimental results and solid curve is obtained from FE analysis. **C.** Immunofluorescence staining for the detection of Col1 and Col4 in crosslinked patches and damaged diaphragms after continuous mechanical stimulation.

**Figure S4. In vitro hydrogel biocompatibility.**




**A.** 2D culture of mixed hSKMC and hFb population after well coating with dECM-derived hydrogel. Quantification of cell growth (black line = number of seeded cells) after 2 and 4 days of culture. Proliferation marker (Ki67) in red. **B.** Differentiation of seeded cells and quantification of myogenic potential with calculation of myogenic index. Myosin heavy chain (MHC) in red; collagen 1 (Col1) in green. Nuclei are counterstained with DAPI (blue).

**Table S1.** List of proteins found in dECM with mass spectrometry.

| Accession | Description | GENE | Coverage [%] | Peptides [#] |
|---|---|---|---|---|
| A0A5G2R3E4 | Acyl-CoA dehydrogenase very long chain | ACADVL | 5 | 2 |
| P16276 | Aconitate hydratase | ACO2 | 10 | 5 |
| A0A5S6G831 | Actin, alpha skeletal muscle | ACTA1 | 46 | 14 |
| A0A287A4R1 | Actin beta like 2 | ACTBL2 | 26 | 6 |
| A0A287AA77 | Actin Gamma 1 | ACTG1 | 38 | 11 |
| F1RHL9 | Alpha-actinin-2 isoform 1 | ACTN2 | 43 | 31 |
| A0A5G2RET9 | Actinin Alpha 3 | ACTN3 | 19 | 15 |
| A0A286ZQ79 | Adenylate kinase isoenzyme 1 | AK1 | 10 | 2 |
| A0A287BFY0 | Fructe-bisphphate aldolase | ALDOA | 29 | 9 |
| A0A287B8Z2 | Fructe-bisphphate aldolase | ALDOC | 12 | 3 |
| A0A286ZJV6 | Annexin | ANXA2 | 8 | 2 |
| A0A287B5C0 | CMP/dCMP-type deami-nase domain-containing protein | APOBEC2 | 20 | 2 |
| A0A5G2R940 | Calcium-transporting ATPase | ATP2A1 | 27 | 21 |
| P11607 | Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 | ATP2A2 | 19 | 16 |
| A0A287BBS4 | ATP synthase subunit alpha | ATP5F1A | 32 | 13 |
| K7GLT8 | ATP synthase subunit beta | ATP5F1B | 38 | 14 |
| A0A287AHM1 | ATP synthase subunit gamma | ATP5F1C | 15 | 4 |

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|---|---|---|---|---|
| Q9GKQ6 | biglycan | BGN | 5 | 2 |
| Q5S1S4 | Carbonic anhydrase 3 | CA3 | 27 | 6 |
| F1RJW7 | Calsequestrin | CASQ1 | 13 | 3 |
| Q5XLD3 | Creatine kinase M-type | CKM | 11 | 3 |
| A0A286ZQI3 | Creatine kinase | CKMT2 | 15 | 5 |
| A0A5G2QQE9 | Collagen alpha-1(I) chain | COL1A1 | 39 | 34 |
| F1SFA7 | Collagen alpha-2(I) chain | COL1A2 | 38 | 23 |
| A0A286ZWS8 | Collagen alpha-1(II) chain | COL2A1 | 12 | 9 |
| A0A286ZQ85 | Collagen alpha-1(III) chain | COL3A1 | 17 | 15 |
| A0A5G2QW87 | Uncharacterized protein | COL6A1 | 10 | 6 |
| I3LQ84 | Collagen aplpha-2(VI) chain | COL6A2 | 4 | 2 |
| I3LUR7 | Collagen aplpha-3(VI) chain | COL6A3 | 4 | 7 |
| F1S902 | Cartilage oligomeric matrix protein | COMP | 7 | 3 |
| A0A287ATJ4 | Alpha(B)-crystallin | CRYAB | 32 | 7 |
| P02540 | Desmin | DES | 29 | 10 |
| A0A286ZNV2 | Elongation factor 1-alpha 2 | EEF1A2 | 19 | 7 |
| I3LII3 | Tr-type G domain-containing protein | EEF2 | 3 | 2 |
| Q1KYT0 | Beta-enolase | ENO3 | 34 | 8 |
| A0A286ZRJ3 | Electron transfer flavoprotein subunit alpha | ETFA | 8 | 2 |
| A0A287AAL6 | four and a half LIM domains 1 | FHL1 | 24 | 9 |
| F1SMN5 | Filamin-C isoform X4 | FLNC | 6 | 10 |
| F1S6B5 | Fibromodulin | FMOD | 9 | 3 |

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|---|---|---|---|---|
| F1SS24 | Fibronectin | FN1 | 8 | 12 |
| A0A287BG23 | Glyceraldehyde-3-phphate dehydrogenase | GAPDH | 40 | 8 |
| F1RM74 | Glyceraldehyde-3-phphate dehydrogenase | GAPDHS | 11 | 3 |
| A0A287BH33 | Aspartate aminotransferase | GOT2 | 18 | 5 |
| A0A5G2QML3 | Histone H2B | H1-3 | 8 | 2 |
| P00348 | Hydroxyacyl-coenzyme A dehydrogenase | HADH | 13 | 2 |
| Q29554 | Trifunctional enzyme subunit alpha | HADHA | 5 | 2 |
| F1SDN2 | Acetyl-CoA Acyltransferase | HADHB | 6 | 3 |
| A0A5G2QRW3 | Hemoglobin subunit beta | HBB | 33 | 7 |
| F1RII6 | Hemoglobin subunit epsilon | HBE1 | 18 | 2 |
| A0A5S6G3Y8 | Heat shock 27 kDa protein | HSPB1 | 12 | 4 |
| A0A287AQR8 | SHSP domain-containing protein | HSPB6 | 36 | 4 |
| A0A286ZYE6 | Isocitrate dehydrogenase | IDH2 | 25 | 9 |
| A0A287AIE4 | Kelch Like Family Member 41 | KLHL41 | 5 | 2 |
| F1S911 | 60S ribomal protein L40 | KXD1 | 8 | 2 |
| A0A286ZXT7 | L-lactate dehydrogenase | LDHA | 25 | 8 |
| F1SR05 | L-lactate dehydrogenase | LDHB | 7 | 2 |
| F1RGX4 | GLOBIN domain-containing protein | LOC100737768 | 54 | 4 |
| F1SCU3 | Matrilin 3 | MATN3 | 5 | 2 |
| P02189 | Myoglobin | MB | 21 | 3 |
| A0A5G2RGL7 | Malate dehydrogenase | MDH2 | 34 | 9 |

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|---|---|---|---|---|
| A0A287B5J2 | Myosin Binding Protein C1 | MYBPC1 | 18 | 17 |
| A0A5G2QJC1 | Myosin binding protein C2 | MYBPC2 | 12 | 12 |
| F1SS66 | Myosin heavy chain 13 | MYH13 | 20 | 41 |
| I3L675 | Myosin Heavy Chain 15 | MYH15 | 2 | 5 |
| A0A5G2QTZ6 | Muscle Embryonic Myosin Heavy Chain | MYH3 | 23 | 53 |
| Q9TV62 | Myosin-4 | MYH4 | 53 | 109 |
| P79293 | Myosin-7 | MYH7 | 58 | 115 |
| A0A5G2R196 | Myosin-2 | MYH8 | 41 | 83 |
| A0A286ZVM3 | Myosin light chain 1 | MYL1 | 54 | 9 |
| Q8MHY0 | myin regulatory light chain 2 | MYL2 | 27 | 3 |
| F1SNW4 | Myosin light chain 3 | MYL3 | 31 | 5 |
| F1RRT2 | Myosin light chain 4 | MYL4 | 29 | 3 |
| A0A5G2QUW6 | Myosin Light Chain 6B | MYL6B | 21 | 5 |
| A0A5G2R327 | Myosin light chain, phos-phorylatable, fast skeletal muscle | MYLPF | 36 | 6 |
| A0A480KXA1 | Myomesin-1 isoform a | MYOM1 | 2 | 3 |
| A0A286ZK06 | Myomesin 2 | MYOM2 | 14 | 8 |
| A0A287ANE3 | Myomesin 3 | MYOM3 | 3 | 3 |
| A0A287AJY7 | Myotilin | MYOT | 3 | 2 |
| Q4PS85 | Myozenin-1 | MYOZ1 | 20 | 3 |
| A0A287BN18 | Nebulin | NEB | 5 | 20 |
| A0A287AQG1 | Proton-translocating NAD(P)(+) transhydrogen-ase | NNT | 4 | 3 |

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|---|---|---|---|---|
| A0A5G2QZ79 | Obscurin, Myosin Light Chain Kinase | OBSCN | 1 | 2 |
| A0A286ZIJ9 | ATP-dependent 6-phphofructokinase | PFKM | 8 | 5 |
| B5KJG2 | Phphoglycerate mutase 1 | PGAM2 | 9 | 3 |
| F1RPH0 | Phphoglycerate kinase | PGK1 | 8 | 2 |
| A0A287A7R4 | Pyruvate Kinase M1/2 | PKM | 11 | 9 |
| A0A287B9H1 | Plectin | PLEC | 2 | 4 |
| A0A287AT94 | Alpha-1,4 glucan phphorylase | PYGB | 9 | 6 |
| A0A287B6I2 | Alpha-1,4 glucan phphorylase | PYGM | 23 | 28 |
| P16960 | Ryanodine receptor 1 | RYR1 | 1 | 2 |
| A0A287BGP7 | Solute carrier family 25 member 3 | SLC25A3 | 6 | 2 |
| A0A286ZIE8 | Solute carrier family 25 member 4 | SLC25A4 | 24 | 5 |
| P20112 | Sparc | SPARC | 8 | 3 |
| F1RK48 | Sarcalumenin isoform X1 | SRL | 7 | 4 |
| F1SNZ7 | Succinate--CoA ligase | SUCLG1 | 6 | 2 |
| P02587 | Troponin C, skeletal muscle | TNNC2 | 13 | 2 |
| A0A286ZI93 | Troponin I | TNNI1 | 11 | 2 |
| A0A5G2R687 | Troponin T, slow skeletal muscle | TNNT1 | 13 | 3 |
| A0A5S6I3K1 | Troponin T, fast skeletal muscle | TNNT3 | 11 | 3 |
| A0A287BHM1 | Tropomyosin alpha-1 chain | TPM1 | 27 | 10 |
| A0A287AN33 | Tropomyosin 2 | TPM2 | 23 | 10 |

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|---|---|---|---|---|
| A0A287BDL6 | Tropomyosin alpha-3 chain | TPM3 | 22 | 8 |
| A0A5G2QM05 | Titin | TTN | 9 | 4 |
| A0A5G2R655 | Tubulin alpha 4a | TUBA4A | 12 | 5 |
| F2Z5B2 | Tubulin beta chain | TUBB | 7 | 2 |
| F1RPD2 | Cytochrome b-c1 complex subunit 2, mitochondrial | UQCRC2 | 9 | 2 |
| A0A5S6HVC8 | Voltage-dependent anion-selective channel protein 1 | VDAC1 | 22 | 6 |
| Q9MZ15 | Voltage-dependent anion-selective channel protein 2 | VDAC2 | 10 | 2 |
| A0A5G2QH97 | Voltage-dependent anion-selective channel protein 3 | VDAC3 | 19 | 5 |
| A0A5S6H025 | Vimentin | VIM | 5 | 2 |

**Table S2.** List of primary and secondary antibodies used.

| Name | Source | Dilution |
|---|---|---|
| Laminin | Sigma-Aldrich | 1:200 |
| Phalloidin (F-actin) | Abcam | 1:1000 |
| Col1 | Abcam | 1:100 |
| Col4 | Abcam | 1:200 |
| Ki67 | Abcam | 1:100 |
| MHC | R&D Systems | 1:50 |
| Anti rabbit IgG (H+L)-Alexa 594 | Invitrogen | 1:200 |
| Anti mouse IgG (H+L)-Alexa 594 | Invitrogen | 1:200 |
| Anti mouse IgG (H+L)-Alexa 548 | Invitrogen | 1:200 |