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Human textiles: A cell-synthesized yarn as a truly "bio" material for tissue engineering applications

Laure Magnan, Gaëlle Labrunie, Mathilde Fénelon, Nathalie Dusserre, Marie-Pierre Foulc, Mickaël Lafourcade, Isabelle Svahn, Etienne Gontier, Jaime H Vélez, Todd N Mcallister, et al.

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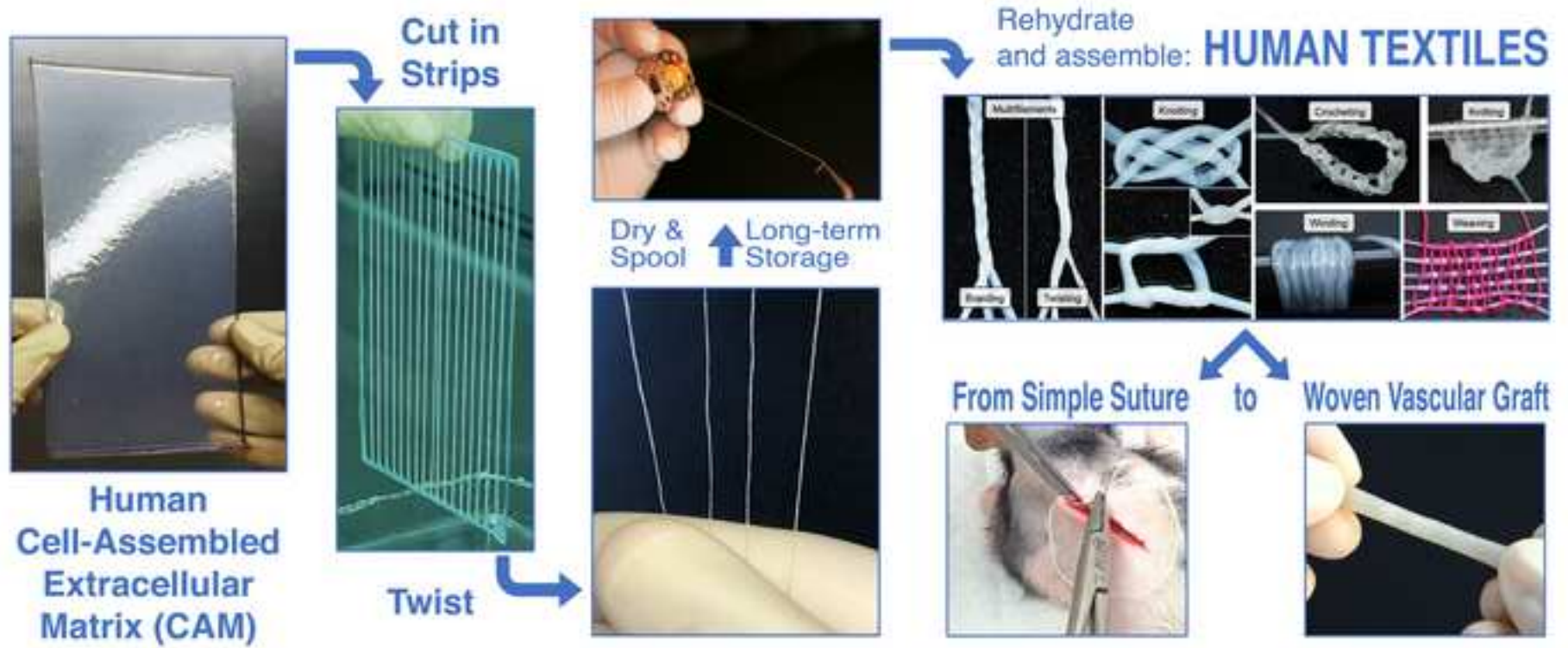
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Abstract: In the field of tissue engineering, many groups have come to rely on the extracellular matrix produced by cells as the scaffold that provides structure and strength to the engineered tissue. We have previously shown that sheets of cell-assembled extracellular matrix (CAM), which are entirely biological yet robust, can be mass-produced for clinical applications using normal, adult, human fibroblasts. In this article, we demonstrate that CAM yarns can be generated with a range of physical and mechanical properties. We show that this material can be used as a simple suture to close a wound or can be assembled into fully biological, human, tissue-engineered vascular grafts (TEVG) that have high mechanical strength and are implantable. By combining this truly "bio" material with a textile-based assembly, this original tissue engineering approach is highly versatile and can produce a variety of strong human textiles that can be readily integrated in the body.

Statement of Significance

Yarn of synthetic biomaterials have been turned into textiles for decades because braiding, knitting and weaving machines can mass-produce medical devices with a wide range of shapes and mechanical properties. Here, we show that robust, completely biological, and human yarn can be produced by normal cells *in vitro*. This yarn can be used as a simple suture material or to produce the first human textiles. For example, we produced a woven tissue-engineered vascular grafts with burst pressure, suture retention strength and transmural permeability that surpassed clinical requirements. This novel strategy holds the promise of a next generation of medical textiles that will be mechanically strong without any foreign scaffolding, and will have the ability truly integrate into the host's body.



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TITLE: Human Textiles: a cell-synthesized yarn as a truly “bio” material for tissue engineering applications.

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KEYWORDS:

Biomaterials, tissue engineering, extracellular matrix, human cells, vascular graft

ABSTRACT:

1
2 In the field of tissue engineering, many groups have come to rely on the extracellular matrix
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4 produced by cells as the scaffold that provides structure and strength to the engineered tissue.
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6 We have previously shown that sheets of cell-assembled extracellular matrix (CAM), which
7
8 are entirely biological yet robust, can be mass-produced for clinical applications using
9
10 normal, adult, human fibroblasts. In this article, we demonstrate that CAM yarns can be
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12 generated with a range of physical and mechanical properties. We show that this material can
13
14 be used as a simple suture to close a wound or can be assembled into fully biological, human,
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16 tissue-engineered vascular grafts (TEVG) that have high mechanical strength and are
17
18 implantable. By combining this truly “bio” material with a textile-based assembly, this
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20 original tissue engineering approach is highly versatile and can produce a variety of strong
21
22 human textiles that can be readily integrated in the body.
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1. Introduction

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3 Medical textiles, because of their remarkable versatility, have been an integral part of modern
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5 medicine. When used as implants, these can be as simple as sutures or as complex as covered
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7 stents for abdominal aorta aneurism repair. Depending on the application, they can be made
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9 of permanent polymers, such as polytetrafluoroethylene (GORE-TEX[®]) or polypropylene, or
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11 of biodegradable materials, such as polyglycolic acid or processed collagen. However, most
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13 permanent synthetic biomaterials are recognized as foreign by the innate immune system,
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15 which leads to the well-described “foreign body reaction” upon implantation. [1] While this
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17 response can be a positive contribution to the tissue repair strategy, such as for repairs of
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19 abdominal hernias with polypropylene meshes, it is often very detrimental for many
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21 applications. [2, 3] As for biodegradables, they are well-suited for repairs of tissues that heal
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23 quickly. However, their use is challenging for applications that require significant or complex
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25 tissue reconstruction, especially when mechanical properties are critical, because the body
26
27 needs to heal before the mechanical failure of the scaffold. [4-6] But what if we could provide
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29 a textile that is not recognized as foreign, that is long lasting, and that the body can slowly
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31 remodel to fit its needs?

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34 Traditionally, the field of tissue engineering has been dependent on synthetic biomaterials to
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36 provide the desired shape and mechanical properties of engineered tissues. But because the
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38 foreign body reaction culminates in a fibrous encapsulation that impedes the biological and/or
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40 mechanical function of the implant, researchers have sought alternatives to exogenous
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42 scaffolds for over 30 years. [7] In the last 20 years, tissue engineers have succeeded in
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44 replacing synthetic scaffolds with extracellular matrix (ECM) synthesized by cells cultured *in*
45
46 *vitro* to produce constructs with appropriate mechanical properties. [8-10] More recently,
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48 these efforts resulted in the production of clinically relevant tissue-engineered implants with
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50 promising outcomes. [11-14] Our group has produced entirely biological sheets of Cell-
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1 Assembled extracellular Matrix (CAM), synthesized by non-transformed human cells, which
2 have a complex, native-like ECM that provides substantial mechanical properties. [15] CAM
3 sheets have been rolled to form tubes that have been successfully used as tissue-engineered
4 vascular grafts in patients. [11, 16, 17] However, like most tissue-engineering approaches, this
5 method is complex, requires specifically designed equipment, and is limited to the production
6 of a precise type of construct. In this report, we show how a textile-based approach can allow
7 the production of an array of completely biological human products from this truly “bio”-
8 material that is the CAM. This new approach is faster, automatable, much more versatile than
9 the previously described sheet-based approach, and is an enabling technology for the mass
10 production of tissue-engineered human textiles.
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26 2. Materials and methods

27 2.1. CAM Production

28 Human skin fibroblast (HSFs) were isolated from adult normal human skin as previously
29 described. [16] Fibroblasts were grown in DMEM/F-12 media (Gibco, #31331-028)
30 supplemented with 20% FBS (Biowest #S1810.500, Hyclone #SH30109.03) and 1X
31 Pen/Strep (Gibco #15140-122) and used at passages 3 or 4. To produce the CAM, HSFs were
32 cultured for 6 to 12 weeks with a supplementation of 500 μ M of sodium L-ascorbate (Sigma,
33 #A4034-500G) in 225 cm² flasks. To devitalize the tissue, CAM were quickly rinsed in
34 distilled water and dried at room temperature in a biosafety cabinet. Dried CAM was stored at
35 -80° C. Prior to use, CAM was rehydrated in water for at least 1 hr. The use of human tissues
36 was done in accordance with article L. 1243-3 of the code of public health and under the
37 agreement DC-2008-412 with the University Hospital Center of Bordeaux, France (update
38 10/10/2014).
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57 2.2. Yarns Production

1 Rehydrated CAMs were placed under a cutting device, blades were pressed on the sheet and
2 the device was pushed to rotate the blades and cut the CAM to obtain 3 to 10-mm-wide by 17-
3 cm-long ribbons (Fig.1). To create threads (twisted ribbons), one extremity of a rehydrated
4 ribbon was attached on a rotating device, while the manipulator held the other extremity. The
5 calibrated motor was turned on, for a set duration according to ribbon length, and the
6 manipulator gently massaged the thread during the twisting process. Ribbons were twisted at
7 2.5, 5, and 7.5 $\text{rev}\cdot\text{cm}^{-1}$. Ribbons were then dried under a laminar flow hood. For the
8 production a longer thread, CAM sheets were dried on a thin sacrificial plastic sheet with a
9 printed spiral pattern on the opposite side (to avoid ink transfer). Both sheets were cut
10 sterily with a scissor following the spiral. Using this approach, a continuous 3-meter-long
11 ribbon can be produced from a single 225 cm^2 sheet. For the production of the circumferential
12 thread used to weave the TEVG, this long thread was cut in half and the two segments were
13 twisted together at 5 $\text{rev}\cdot\text{cm}^{-1}$. The internal part of the spiral was twisted with the external part
14 of the other spiral to distribute any weakness points that can arise from the curvature points.
15 This two-thread approach is designed to protect the mechanical integrity of the graft in the
16 event of a tear occurring on the circumferential tread. With this design, the tear would not
17 progress throughout the entire thread.

41 2.3. Histology

42 Yarn were fixed in 4% paraformaldehyde (Antigenfix, Microm Microtech, France),
43 dehydrated through graduated baths of increasing ethanol and paraffin embedded. Tissues
44 were then sectioned with a microtome (7 μm), processed for Masson's trichrome staining
45 (*vert lumière*) to visualize collagen. Images were acquired using an Eclipse 80i microscope
46 (Nikon, Japan).

56 2.4. Transmission electron microscopy:

1 Yarn were fixed with 0.1 M sodium cacodylate buffer containing 2% glutaraldehyde, pH 7.5,
2 at 4°C overnight and post-fixed *en bloc* with 1% osmium tetroxide. Tissues were stained with
3
4 0.5% uranyl acetate for 1 h at 4°C. After dehydration, the samples were embedded in Epon
5
6 812. Ultrathin sections were mounted on formvar-coated grids and stained with uranyl acetate
7
8 and lead citrate. Samples were examined with a transmission electron microscope (H7650,
9
10 Hitachi, Tokyo, Japan) at 80kV.
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14 2.5. Cryo-electron microscopy

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16 Yarns were fixed with 2.5% glutaraldehyde/0.1M phosphate buffer. Samples were mounted
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18 with “tissue freezing medium”/colloidal graphite, and rapidly transferred into liquid nitrogen
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20 and then, into the preparation chamber (-140°C). Samples were sublimated (-95°C) and
21
22 coated with platinum (30 s/ 10 mA). Samples were then transferred to the microscope
23
24 cryostage (CRYO-SEM PP3010T, Quorum Technologies, England). Observations were done
25
26 at 3kV with a GeminiSEM 300_FESEM (ZEISS, Germany).
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31 2.6. Yarn characterization

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33 Diameter of fully rehydrated yarn was determined with a laser micrometer (Xactum,
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35 AEROEL). Then, yarn was clamped in the jaws of a tensile testing machine (Shimatzu, AGS-
36
37 X, force sensor 100N), pre-loaded at 20 mm/min until they reached 0.1N, and then pulled
38
39 until rupture at 60 mm/min, at room temperature. Samples remained hydrated throughout the
40
41 test. Strain was recorded using the TRViewX digital video extensometer and analysis
42
43 performed with the TrapeziumX software.
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48 2.7. Suturing of nude rat skin

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50 Stitches in overlock were made in the back of an adult female nude rat (n=1) to close a 3-cm-
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52 long wound with a curved surgical needle under gas anesthesia (isoflurane). Buprenorphine
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54 (30 µg/g) was administrated intraperitoneally 30 min before and the day after the procedure.
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58 All procedures and the animal treatment complied with the Principles of Laboratory Animal
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1 Care formulated by the National Society for Medical Research which are in-line with the EU
2 Directive 2010/63/EU. The studies were carried out in accredited animal facilities
3
4 (accreditation #B33-063-916) at the University of Bordeaux and were approved by the
5
6 Animal Research Committee of Bordeaux University (protocol authorization #
7
8 APAFIS#4375-2016030408537165 v8).
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10 11 12 2.8. TEVGs production and mechanical properties 13

14 TEVGs were produced using a basic weaving technique (plain 1/1) with a custom-made
15
16 circular loom. One circumferential yarn (forming the “weft”) was inserted between a movable
17
18 and a fixed set of tensioned yarns (the “warp”) to create the woven tube. The two sets of the
19
20 warp were then moved to cross over the weft, and the circumferential yarn was run again
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22 between the two set, and so on. TEVGs were produced with 49 longitudinal ribbons and one
23
24 double-filament thread (as described in section 2.2) as circumferential thread (see Fig. 6C).
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29 TEVGs were cannulated and pressurized with water or gas. Permeability was measured and
30
31 calculated as recommended by the Cardiovascular implants— Tubular vascular prostheses
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33 ANSI/AAMI/ISO 7198:1998/2001 standards. Suture retention and burst pressure were
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35 performed as previously described with some modifications. [18] Suture pull out was
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37 performed with using 7-0 Prolene® and only one site per vessel could be tested because of the
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39 failure mode of the TEVG. For burst pressure, a thin latex balloon was used in the TEVG to
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41 avoid leakage at high pressure. The pressure needed to dilate the balloon to a diameter larger
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43 than that of the TEVG (100 mmHg) was negligible compared to the burst pressure but was
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45 still subtracted from the latter.
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50 51 2.9. TEVG implantation 52

53 A 4.2 mm internal diameter human TEVG was implanted as an interpositional graft with end-
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55 to-end anastomosis (Prolene® 6-0) into a sheep’s carotid (n=1). Briefly, the carotid was
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57 exposed. Heparin was administered (~200 units/kg) prior to sectioning the carotid to maintain
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1 activated clotting time of > 300 seconds. Papaverin was administered if vasospasm were
2 observed. The TEVG was sutured on the distal segment of the carotid with running sutures.
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4 The graft was flushed by releasing the distal hemostat and the proximal anastomosis was
5 performed. Light bleeding was observed at the anastomosis but hemostasis was achieved with
6 simple dabbing with a gaze. The animal was sacrificed to following day under anesthesia
7 (Buthanasia, I.V.). The implantation was performed at BioSurg, Inc. (Winters, California,
8 USA) with Institutional Animal Care and Use Committee (IACUC) approval.
9

10 2.10. Statistical analyses

11 All statistical analyses were performed with GraphPad Prism, Version 6 (GraphPad Software
12 Inc. USA). Data is presented as mean \pm standard deviation. Differences between the groups
13 were determined on basis of analysis of variance (ANOVA) with Tukey's multiple
14 comparison tests. Differences were considered significant at $p < 0.05$.
15

16 3. Results

17 To produce the CAM, we preferentially used fibroblasts from normal adult human skin,
18 although we have shown that other mesenchymal cells can be used. [8] Fibroblasts, in a 225
19 cm^2 flask, formed a strong and cohesive 18 x 10 cm living sheet that was used for yarn
20 production after 8 weeks of culture (Fig. 1A). Strips of the CAM were produced by cutting
21 the sheet longitudinally with a custom device equipped with a series of circular blades that
22 were evenly spaced to achieve the desired width (Fig. 1B). While these "ribbons" readily
23 collapsed into a thin structure when taken out of the medium, they could also be twisted at
24 various rates of revolutions per unit length to form more compact, uniform, threads (Fig. 1C).
25 The sheet could also be cut in two connected spiral patterns to obtain a 3-meter-long
26 continuous ribbon (Fig. 1D). Either yarn (ribbons or threads) were devitalized by drying and
27 were then spooled and stored at -80°C for extended periods of time with no apparent
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1 degradation. This process produced a robust new biological yarn without the need for any
2 exogenous scaffolding or any chemical crosslinking that would denature this human ECM and
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4 make it a target for rapid degradation once implanted (Fig. 1E).
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7 Cryo-scanning electron microscopy (cryo-SEM) revealed the smooth and homogenous
8
9 surface of the CAM ribbons after a drying-freezing-rehydration cycle (Fig. 2A). Despite this
10
11 devitalization and storage process, no cracks, nor holes, nor cell or ECM debris detaching
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13 from the CAM were observed. At this magnification, the decrease in diameter of the yarn as
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15 the result of twisting was obvious. These observations suggest that tissue compaction was
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17 increased until $5 \text{ rev}\cdot\text{cm}^{-1}$ but seemed to plateau thereafter. Cross sections of paraffin-
18
19 embedded yarn illustrated how the CAM layers are compacted into a thread (Fig. 2B and
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21 **Suppl. Fig. 1**). The *vert lumière* Masson's trichrome staining specifically stained the collagen
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23 in blue-green highlighting the very dense network of fibers and the compaction of the matrix.
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29 Transmission electron microscopy (TEM) imaging revealed the typical striation of collagen
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31 fibrils cut longitudinally as well as the very dense network of collagen fibrils organized in
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33 parallel bundles oriented in multiple directions, which is typical of physiological, strong,
34
35 mature, human connective tissue (Fig. 2C). [19, 20] Furthermore, microfibrils, an important
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37 sub-component of most ECMs [21], were observed throughout the matrix demonstrating that
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39 the CAM is much more than a collagen network. TEM observation also clearly revealed that
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41 twisting led to an increase in fibril density and an orientation of the fibrils and microfibrils in
42
43 the longitudinal axis of the thread.
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48 In order to confirm the decrease in diameter seen in cryo-SEM, rehydrated yarn diameters
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50 were measured with a biaxial laser micrometer (Fig. 3A). **Twisting reduced ribbons diameter**
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52 **significantly but the average diameter difference between the twist levels were not statistically**
53
54 **significant.** **We performed a mechanical characterization of the yarn using uniaxial testing**
55
56 **(typical stress-strain curves are shown in Suppl. Fig. 2).** Twisting slightly increased the force
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1 at failure and the effect also seemed to peak or plateau at $5 \text{ rev}\cdot\text{cm}^{-1}$ (Fig. 3B). The ultimate
2 tensile stress (UTS) increased much more dramatically than the force at failure, which also
3 seemed to peak at $5 \text{ rev}\cdot\text{cm}^{-1}$, highlighting the considerable tissue compaction (Fig. 3B). As
4 would be expected, strain at failure was increased with twisting, with the effect becoming
5 significant and maximal at $7.5 \text{ rev}\cdot\text{cm}^{-1}$ (Fig. 3C). Higher twist levels were not tested because
6 they started creating secondary coiling that made handling more difficult but these could still
7 be produced if needed to achieve higher stretchability for specific applications. A simple way
8 to increase yarn force at failure was to increase the width of the initial strip of CAM (Fig.
9 3D). Using CAM from cell lines from 4 donors, a linear relationship between ribbon width
10 and force at failure was demonstrated in all cases, as one would expect with any material.
11 Finally, another way to control yarn force at failure is to control CAM sheet age (time of
12 culture) since the tissues will get thicker and stronger practically linearly with time in culture
13 (Fig. 3E). These experiments exemplify how a wide range of physical characteristics and
14 mechanical properties can be achieved when creating human yarn.

15 Human yarns are compatible with many assembly techniques classically used for creating
16 textiles (Fig. 4). For example, multifilament yarn can be created by braiding or twisting
17 individual ribbons or threads in order to achieve a set of specific mechanical properties as is
18 commonly done in the suture industry (Fig. 4A). The yarn can be tied off using different types
19 of knots as would be done with standard sutures (Fig. 4B). Knot-based assembly techniques
20 such as crocheting and knitting can be performed to create truly three-dimensional geometries
21 as is currently done in the medical implant industry (Fig. 4C). The yarn could also be tightly
22 wound around a synthetic surface to create a hybrid implant with increased
23 integration/biocompatibility potential (Fig. 4D). Weaving is an age-old assembly method
24 where a set of tensed yarn filaments (called “warp”) is intertwined with a single moving
25 filament (called “weft) to produce a dense structure without using knots (Fig. 4E). As seen

1 here, the warp and the weft can be made of different yarns (the warp can even contain
2 multiple types of yarn), which allows for directional control of mechanical properties.
3

4 **In order to confirm the biocompatibility of the CAM, rehydrated ribbons were seeded with**
5 **human endothelial cells from the umbilical vein (Fig. 4F and 4G). Three sets of ribbons were**
6 **seeded with three endothelial cells lines that were labelled (lentivirus-based stable**
7 **transduction) with either green fluorescent protein or red fluorescent protein,** or left unlabeled.
8
9

10 After 3 days of culture, multifilaments were produced by braiding or twisting three ribbons
11 together (one from each set). Using a fluorescence binocular microscope, each cell line could
12 be imaged on their respective ribbons (the nuclei of unlabeled cells were stained blue using
13 DAPI).
14

15 To demonstrate the basic usability of human yarn, a 5-mm-width ribbon was used to close a
16 wound on the back of a nude rat (Fig. 5). The ribbon was used as a typical suture material and
17 tied off using a classic surgeon's knot. The "suture" dried and fell off naturally, not unlike the
18 remnant of the umbilical cord (Fig. 5B, C). By two weeks, practically all the external parts of
19 the sutures were gone and the wound remained closed and healed normally. **Histology at 1**
20 **month (Fig. 5D, E) showed that the CAM suture was still clearly visible in the dermal layer**
21 **with no signs of inflammation or degradation.**
22
23

24 As a proof of concept, a tissue-engineered vascular graft was developed to show that this
25 technology can be used to create complex tissues and organs. One of the key advantages of a
26 textile approach is the possibility to automate tissue fabrication. In Fig. 6A and B, a tube was
27 automatically braided with dried human threads using a 48-carrier braiding machine. The
28 result was a tube that was too porous to be used as a vascular graft but this assembly method
29 could be useful for other applications **such as an external sheath to provide mechanical**
30 **support to vascular other types of grafts. [22, 23]** By using a basic weaving method (Fig. 6C)
31 and a circular loom (Fig. 6D), a TEVG was produced with an internal diameter of 4.2 mm, a
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1 very dense wall, and ends suitable for suturing (Fig. 6E, F). The longitudinal thread count of
2 the graft was $16.3 \pm 0.9 \text{ cm}^{-1}$ (n=5 individual vessels), i.e. a 5.5% coefficient of variation
3
4 indicating already a very good reproducibility for these hand-made prototypes. The primary
5 challenge for a completely biological TEVG is to display sufficient mechanical strength to
6
7 support safe clinical use. Mechanical characterization data is summarized in Table I. The
8
9 burst pressure of these TEVGs was $5968 \pm 732 \text{ mmHg}$ (n=5) which compares favorably to
10
11 that of the human internal mammary artery ($3196 \pm 1264 \text{ mmHg}$), a vessel widely used for
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13 heart bypass surgery. [18] Another important mechanical property of a vascular graft is its
14
15 ability to resist pull out of the sutures used for anastomosis. The suture pull-out strength (Fig.
16
17 6G) of these grafts was actually superior to that needed to break the synthetic sutures in used
18
19 for the test in 2 of the 5 vessels (over 600 gf or 6 N). The three vessels that provided data had
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21 an average suture retention force of $566 \pm 17 \text{ gf}$. These grafts had a water permeability of $10 \pm$
22
23 $8 \text{ ml} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ (n=4), which is far below the threshold where a pre-clothing step is required
24
25 ($50\text{-}350 \text{ ml} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$). [24, 25]
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33 To demonstrate the actual implantability of these TEVG, a 6-7 cm-long segment was
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35 implanted as an interpositional graft in the carotids of sheep. The procedure was performed
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37 using standard protocols and tools. Suturability, low transmural permeability, and initial
38
39 resistance to arterial pressure were confirmed visually and palpation (Fig. 6H).
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44 4. Discussion

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47 The goal of this study was to demonstrate the potential of a textile-inspired approach for the
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49 production of completely biological products using CAM. We have previously shown that the
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51 CAM can be used as a sheet to produce TEVGs by rolling sheets around a mandril followed
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53 by a two-month long maturation phase, in a bioreactor, to allow the fusion of the rolled sheet
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55 layers through cellular deposition of ECM. [8, 16] These TEVG were the first completely
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57 biological TEVG implanted in humans and showed promising clinical results as arteriovenous
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1 shunts. [11, 17] A textile-based approach has significant advantages over the sheet-based
2 approach. One of the most important is that the biological textile does not require one or more
3 maturation phases for the yarn to fuse together to form a cohesive construct. As a result, this
4 assembly strategy avoids the use of costly and bulky bioreactors, dramatically accelerates
5 productions time, and does not rely on cellular activity, which can vary from donor to donor.
6 In addition, this assembly strategy does not produce a laminated structure, which are always
7 susceptible to delamination when exposed mechanical loads. In fact, the mechanical
8 properties of textiles can be easily tuned locally and directionally, which is very difficult to
9 achieve with a sheet-rolling strategy. Moreover, porosity of the textile can also be controlled
10 by adjusting assembly parameters. Finally, the textile industry has a long expertise in
11 automating assembly, which would significantly reduce production costs of tissue-engineered
12 product.

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30 In this study, we have shown that we can control the mechanical properties of the basic
31 building block of the biological textile: the yarn. We have shown that the strength of the yarn
32 can be controlled by the width of the ribbons and by the age of the CAM sheet. Twisting
33 ribbons into threads was shown to significantly compact the material and change the yarns
34 stretchability (strain at failure) and UTS. Finally, we have shown that multifilaments can be
35 produced by braiding or twisting, which offer additional ways to control yarn mechanical
36 properties (although these were not explored in this study). However, it is clear that this
37 biological yarn will never have a UTS similar to that of synthetic polymers such a
38 polypropylene, used in popular commercial sutures Prolene®, which are in the 400 MPa
39 range while our yarn's UTS is about 40 times lower. This means that this biological yarn
40 cannot replace directly these polymers in any applications, however, it is unclear that such
41 supraphysiological strength is necessary in all applications. In fact, this yarn has a UTS that is
42 in the range of native soft tissues, which suggest that it could be a useful reconstruction tool.

1 [26] The real advantage of this biological yarn would be found in the absence of inflammation
2 upon implantation, which could allow long-term persistence without chronic inflammation.
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4 These may be very valuable tools for specific applications.
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11 There have recently been exciting efforts to create living fibers and to assemble those using
12 textile approaches. [27-29] However, these approaches still rely significantly on the use of
13 exogenous biomaterials which will create an inflammatory response that will disrupt tissue
14 architecture once implanted. While demonstrating a very fine resolution, these fibers, and
15 resulting tissues, have not been shown to have the mechanical strength needed to produce
16 tissues that play a significant and sustained mechanical role, which can be key for a successful
17 implantation. The uniqueness of the approach described here is that, based on our previous
18 animal and clinical work with CAM [11, 12, 16, 17, 30], the tissue that will be implanted will
19 largely remain intact and will only be very slowly remodeled.
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37 The impressive mechanical strength of this CAM-based biomaterial is due to its fibrillar
38 collagen backbone. Its native-like ultrastructure is in contrast with the well-known collagen
39 gels produced from solubilized collagen where only collagen is present and where only an
40 extremely low density of collagen fibrils can be seen in TEM. [31, 32] Tissues from
41 processed collagen, even at high concentration of collagen, are rapidly degraded in vivo
42 because the innate immune system recognizes this material as “damaged”. [33, 34] There
43 have been previous attempts to produce collagen yarn from solubilized collagen but these all
44 had to resort to chemically cross-linking the collagen to achieve significant mechanical
45 properties. [35-38] In addition, when TEM was presented, the structure of the collagen was
46 far from native-like. [35, 36] Both these denaturation processes (solubilization and cross-
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1 linking) are very likely to bring about an aggressive degradation of the implanted material.
2 While, as mentioned before, this can be acceptable for some applications, our goal is to
3 produce a biomaterial that will integrate and persist in the host to avoid having to heavily
4 depend on rapid remodeling responses from the patient since these can be variable and
5 dependent on age, sex, health status, and other unknown factors. [39]
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15 However, the CAM is more than a collagen scaffold. For example, we have shown that it also
16 includes a microfibrillar network, an important sub-component of most ECMs. [21] In fact,
17 using mass spectrometry, over 50 components of the ECM in the CAM were identified
18 demonstrating how this very complex matrix has a physiological-like organization and
19 composition, which suggests that it could support a variety of biological functions. [15]
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32 Our strategy is to work with devitalized yarn for ease of storage and manufacturing
33 considerations. [15] Our clinical work with vascular grafts using devitalized CAM [12, 30],
34 as well as current animal work (data not shown), strongly suggests that the fibroblast
35 remnants, even allogeneic, do not create a significant immune response. Other groups have
36 shown that allogeneic fibroblasts can be accepted/tolerated in tissue-engineered skin implants.
37 [40, 41] We have expressly avoided using a decellularization process to actively remove
38 cellular components because these extraction processes will modify the ECM by, among other
39 things, removing some of its components. [15, 42] While this approach offers the
40 commercially attractive “off the shelf” option, in some cases, living cells maybe be needed to
41 achieve a therapeutic goal. Here, CAM yarns were shown to be easily seeded with cells as a
42 proof-of-concept to demonstrate that any complex human textile could be seeded with the
43 relevant cells. However, simple CAM sutures could also be used as a delivery system for
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adherent cells that could outperform injection of cells in suspension since the latter is associated with a very low survival rate. [43]

In this study, we have shown that we can use a weaving approach to produce TEVG with the required mechanical properties for safe *in vivo* implantation. With an average burst pressure nearing 6000 mmHg, these vessels are stronger than human internal mammary artery (around 3000 mmHg) or other biological TEVG. [13, 14, 44, 45] While this supra-physiological strength may seem overkill, this safety margin could compensate for any partial weakening due to some level of remodeling *in vivo*. These vessels also displayed an impressive suture retention strength was 3 to 5-fold higher that of human arteries or other biological TEVG. [13, 14, 18] Finally, TEBVs had a water permeability far below the threshold where a pre-clothing step is required (50-350 ml·min⁻¹·cm⁻²). [24, 25] Transmural permeability is an obvious concern for a woven or knitted vascular grafts and this is why modern commercial grafts are typically sealed using a protein coating. [46]. In order to demonstrate the overall implantability of the graft, a short-term xenogeneic implantation was performed with no complications. Taken together, these results show that this textile-inspired assembly strategy is faster, automatable, highly tunable, and can produce completely biological, human, TEVG with the requisite properties for *in vivo* use.

Future avenues of research include the study of long-term *in vivo* remodeling of CAM-based TEVGs and of other textile forms. Indeed, while clinical data proved that CAM is long-lived *in vivo* [11, 12, 17, 30], the clinical setting was not conducive to a systematic and mechanistic study of the remodeling process. In this study, we have used a xenogeneic implant model (human in ovine) to show the implantability of the graft (sutureability, wall

1 impermeability, and initial resistance to arterial pressure). However, this model is not suitable
2 for remodeling or outcome studies since we expect this xenogeneic tissue to cause an acute
3 rejection based on our previous experience. [16] We are currently developing a TEVG using
4 ovine CAM to allow long-term studies in an allogenic model, which would be more relevant
5 to the anticipate clinical application. Of particular interest would be to determine if CAM-
6 based textiles can grow, which is critical for pediatric applications where permanent synthetic
7 materials often have to be replaced at great risk to the patient. Another avenue to explore
8 would be to determine if the CAM can be sterilized without significantly damaging its native-
9 like structure. Indeed, while yarn can be produced and assembled sterilely, the manufacturing
10 process of complex tissues would be considerably simplified if terminal sterilization was an
11 option.
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30 5. Conclusion

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32 In this study, we demonstrated how a tissue-engineering strategy can produce yarn of
33 unmodified, human, native-like ECM that have substantial and highly tunable mechanical
34 properties. This yarn was shown to be compatible with many textile assembly strategies and
35 easily seeded with cells. To illustrate the potential this truly “bio”-material, we used it as a
36 simple suture material *in vivo* and to produce a robust, yet completely biological, implantable
37 TEVG. This rapid, automatable, and highly tunable platform technology can create constructs
38 with a wide range of shapes, mechanical properties and porosities. These human textiles offer
39 a unique level of biocompatibility and represent a new generation of completely biological
40 tissue-engineered products.
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10 **CONFLICT INTERESTS:**

11 The authors have no competing interests to declare.
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18 **DATA AVAILABILITY:**

19 The raw/processed data required to reproduce these findings cannot be shared at this time due
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21 to technical or time limitations. However, the corresponding author will be happy to share any
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23 data used to prepared this manuscript with any researcher upon simple request.
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30 **LEGENDS:**

31 Fig. 1. Human yarn production. (A) A fresh sheet detached from the flask after 8 weeks of
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33 culture stretched over a frame. (B) Seventeen 5-mm-wide ribbons obtained by longitudinally
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35 cutting a CAM sheet. (C) Ribbons could be twisted to obtain threads. From left to right:
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37 ribbon (no twist), 2.5, 5, and 7.5 rev·cm⁻¹. (D) One continuous 3-meter-long ribbon was
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39 obtained by cutting a sheet in two connected spirals (inset). The biological yarn could be
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41 dried, spooled, and stored until needed. (E) A thread (7.5 rev·cm⁻¹) tied to, and lifting, a
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43 metal weight of 200 g.
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52 Fig. 2. Microscopic view of human yarn. (A) Cryo-SEM shows the smooth surface of the yarn
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54 and the changes induced by twisting. Scale bar = 200 μm. (B) Cross sections of yarn stained
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56 with Masson's trichrome revealed the dense collagen network (blue-green). Three layers of
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58 the ribbon are visible (2 are stuck together). Twisting at 5 rev·cm⁻¹ produced a very dense
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1 tissue. Scale bar = 100 μm . (C) TEM of yarn cross sections show the typical striated
2 pattern of the collagen fibrils arranged in dense parallel arrays to form fibers. Microfibrils are
3 also visible throughout the tissue but concentrated in some areas (arrow). Twisting clearly
4 compacted the fibrils but also seemed to align them in the longitudinal direction. Scale bar =
5 400 nm.
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14 Fig. 3. Factors influencing the mechanical properties of yarn. (A) Biaxial laser micrometry
15 confirmed that twisting produced significantly more compact and uniform yarn (**: $p < 0.01$,
16 compared to 0, $n=4$, 5-mm ribbons). (B) The force at failure was significantly higher for 5
17 $\text{rev}\cdot\text{cm}^{-1}$ threads than for ribbons (by $\approx 17\%$). The UTS also appeared to peak at 5 $\text{rev}\cdot\text{cm}^{-1}$
18 (*: $p < 0.05$, **: $p < 0.01$, ****: $p < 0.0001$, compared to 0 unless otherwise indicated, $n=4$, 5-mm
19 ribbons). (C) Twisting also increased the strain at failure which became significant at 7.5
20 $\text{rev}\cdot\text{cm}^{-1}$ ($p < 0.01$, compared to 0, $n=4$, 5-mm ribbons). (D) The force at failure of 5-mm-wide
21 ribbons could also be tuned by changing ribbons width as demonstrated here with 4 different
22 cell lines ($n=5-18$). Correlation R^2 ranged from 0.91 to 0.98. (E) The force at failure of the
23 yarn could also be controlled by the culture time of the sheet (sheet age) as seen here with 5-
24 mm-wide ribbons using finite cell lines from 4 donors ($n=8-16$). Correlation R^2 ranged from
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46 Fig. 4. Human textiles. (A) More complex yarn could be produced by braiding and twisting
47 ribbons. (B) Complex or surgical knots could be tied and firmly tightened (inset) without
48 breaking the yarn. (C) Crocheting and knitting could be used to produce complex structures.
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51 (D) One ribbon was wound around a mandrel to cover its surface. (E) A loose weave is shown
52 with colored yarn to show the assembly principle. Six ribbons (light pink) were woven with a
53 two-filament thread (dark pink). (F,G) Three ribbons, each seeded with a separate culture of
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1 human endothelial cells (labeled in red, green or blue), were assembled into a multi-cell-line
2 construct by twisting (F) or braiding (G).

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7 Fig. 5. Human yarn could be used as a suture material. (A) A 5-mm-wide ribbon was threaded
8 on a curved surgical needle to suture a wound on the back of a nude rat. The ribbon could be
9 firmly pulled to close the wound. (B) 3 days after the suture, knots were still visible and the
10 wound was solidly closed, no macroscopic inflammation was visible. (C) At 14 days, the
11 external part of the suture was all but gone and the wound was closed. (D) Histology of a
12 human ribbon used as suture material in a nude rat at 1 month after implantation (Masson's
13 Trichrome stain). The CAM ribbon used as suture material is clearly visible in the dermal
14 layer of the skin (arrow). Scale bar = 500 μm . (E) At higher magnification, the different
15 collagen-rich layers of the ribbon are clearly visible. Layer separation could be a histology
16 processing artefact. There are no signs of inflammation or degradative response and moderate
17 cell penetration of the CAM. Scale bar = 100 μm

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39 Fig. 6. Completely biological, human, textile-based, tissue-engineered vascular grafts. (A, B)
40 Automated manufacturing of a braided tube with human threads (48 spools). While this
41 demonstrated the feasibility of automation, the resulting tube was clearly too porous for use as
42 a vascular graft. (C) Schematic representation of the basic weaving technique used with a
43 manual circular loom. One circumferential thread (weft) was inserted between a movable and
44 a fixed set of tensioned ribbons (warp) to create the woven tube. (D) Production of the TEVG
45 with, partly rehydrated, devitalized yarn: 49 longitudinal ribbons and one double-filament
46 thread (2 ribbons twisted together at 5 rev $\cdot\text{cm}^{-1}$) as circumferential thread. (E) Vascular grafts
47 were fully rehydrated before removal from the mandrel yielding a robust and flexible tube. (F)

The tightly packed yarn produced a dense, homogenous, and watertight wall (scale bar = 1 mm). (G) The ends of the graft were “finished” to produce a clear and even ring for suturing (internal diameter of 4.2 mm). (H) A human woven TEVG (internal diameter 4.2 mm) was implanted in the carotid of a sheep as interpositional using 6-0 Prolene® using standard surgical techniques (inset). The graft was leak-proof and allowed normal blood flow.

Table I. Mechanical characterization of the human woven grafts and Human Internal Mammary Artery .

	Burst Pressure (mmHg)	Suture Retention Strength (gf)	Transmural Permeability (ml·min ⁻¹ ·cm ⁻²)
TEVG	5968 ± 732 n = 5 Max: 6545 Min: 4755	566 ± 17 n = 3 (5*) Max: 576 Min: 546	10 ± 8 n = 4 Max: 18.5 Min: 0.1
Human Internal Mammary Artery [18]	3196 ± 1264 n = 16 Max: 5688 Min: 534	138 ± 50 n = 6 Max: 205 Min: 78	N/A

*Suture broke before pull-out in two cases that were not included in the calculation (around 600 gf).

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Fig 1

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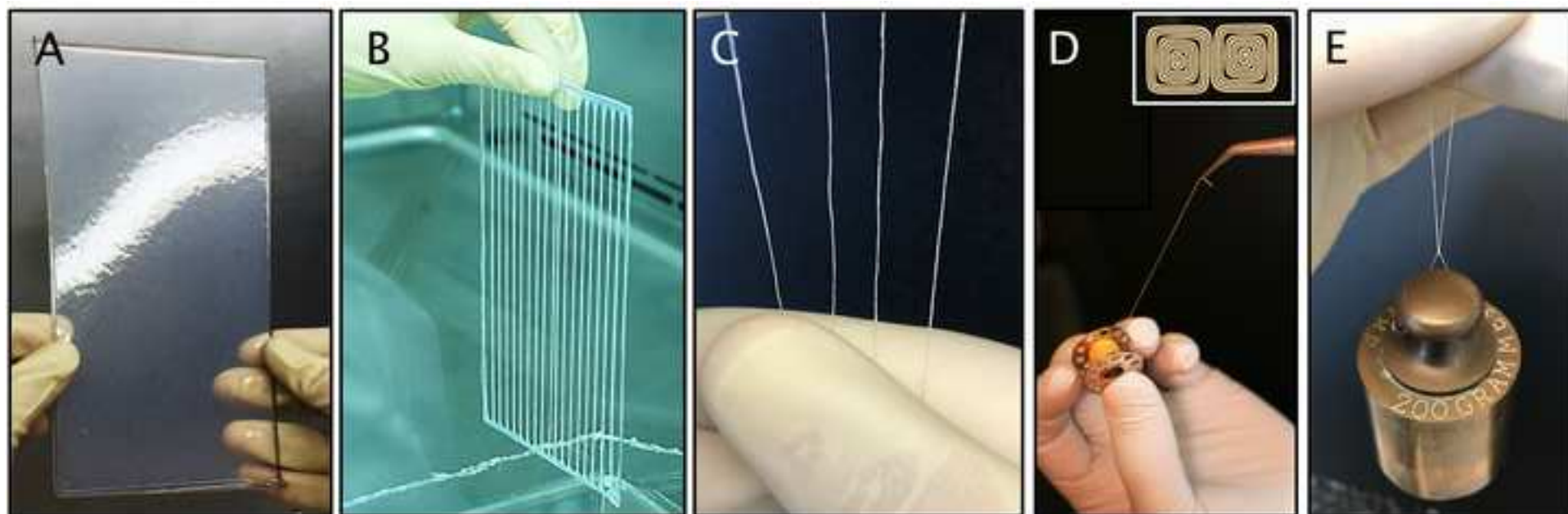


Fig 2

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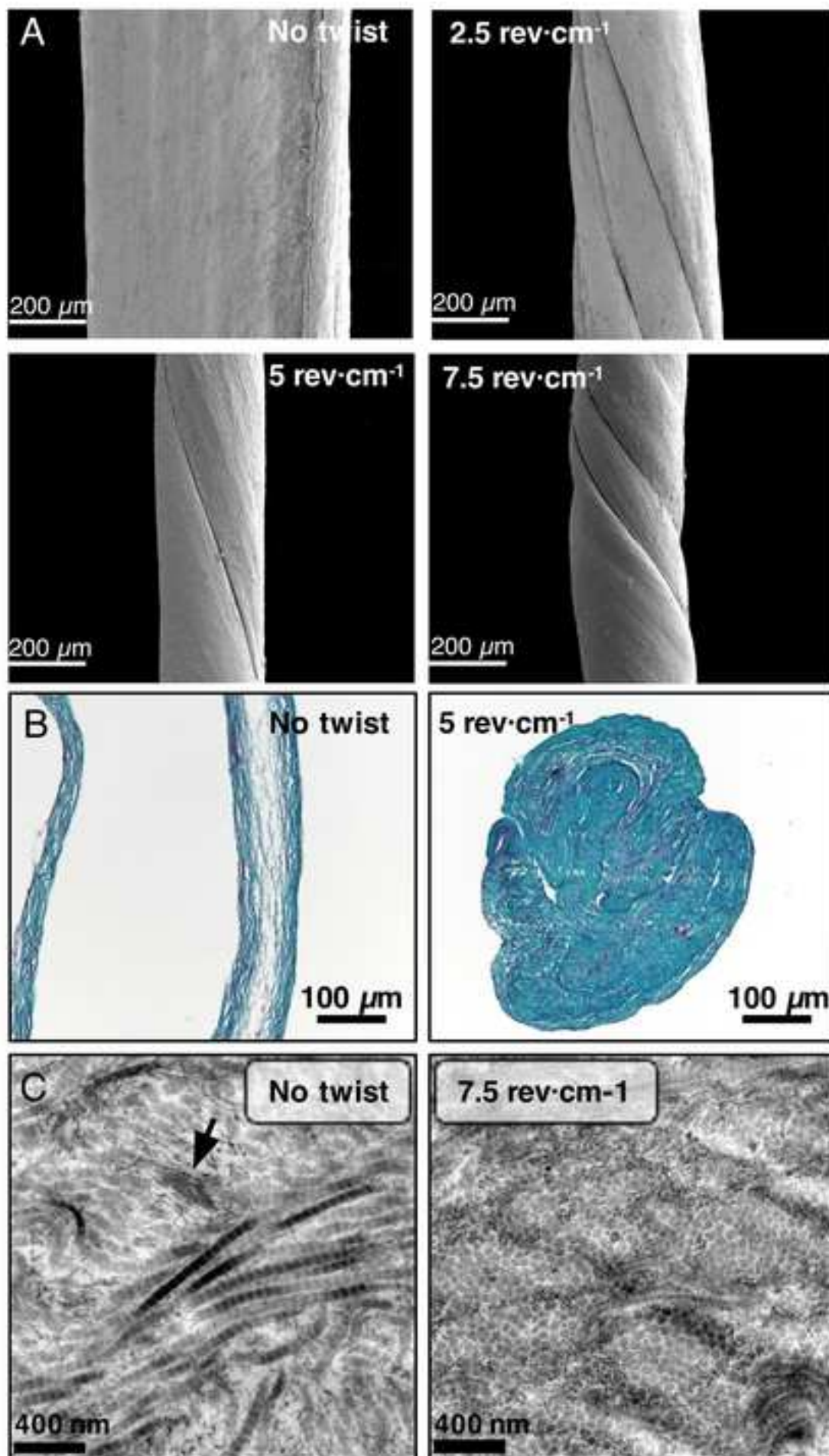


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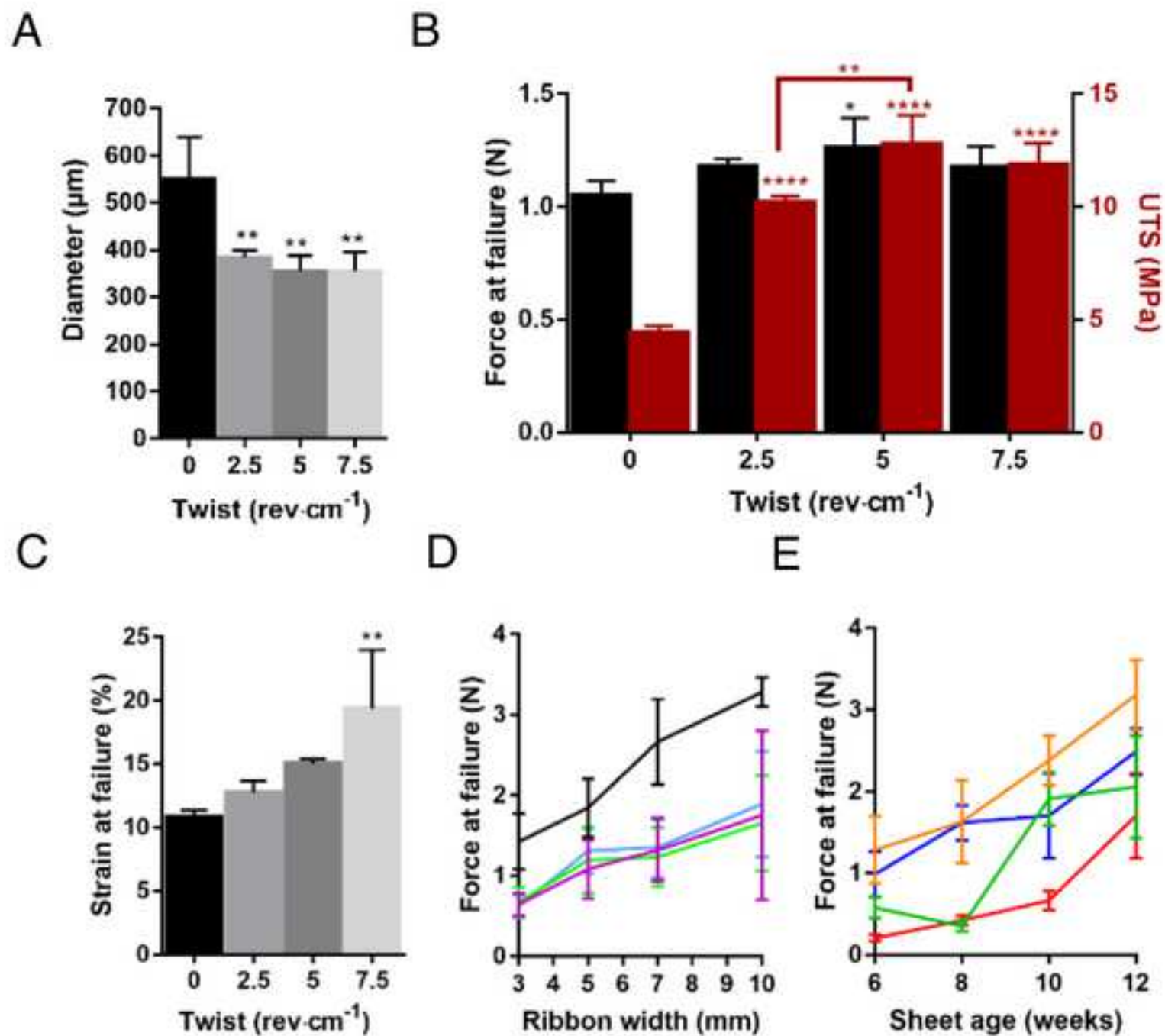


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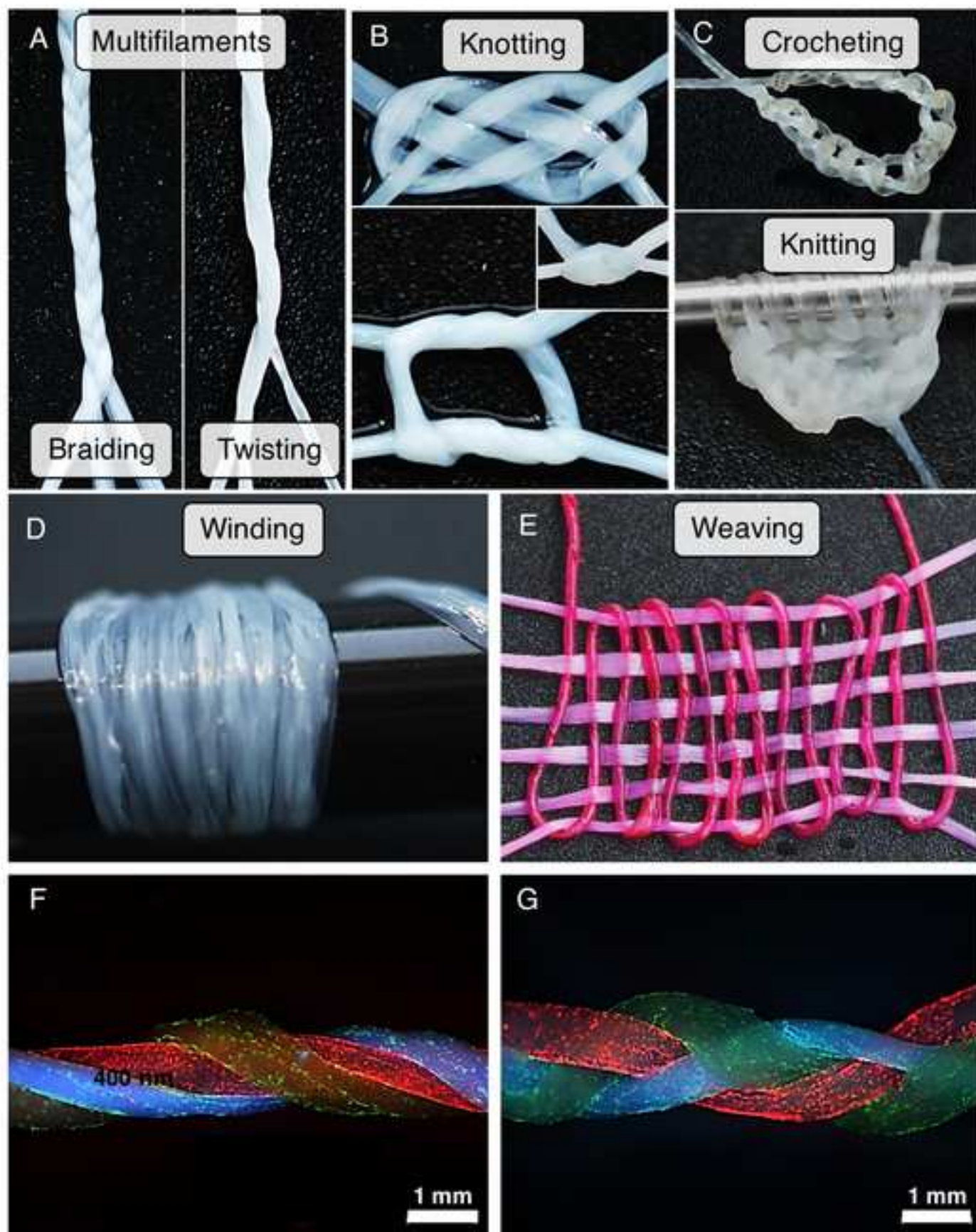


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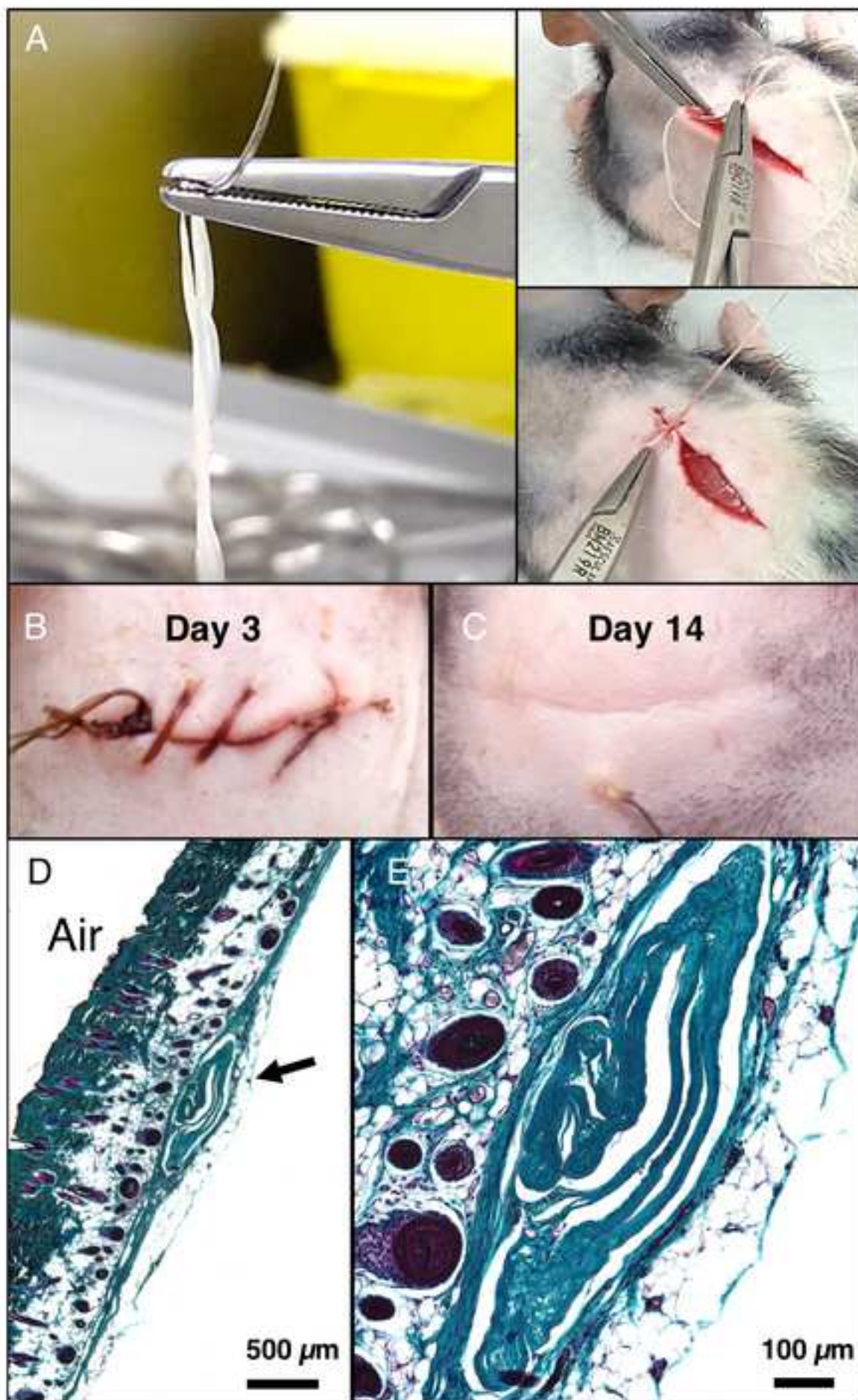


Fig 6

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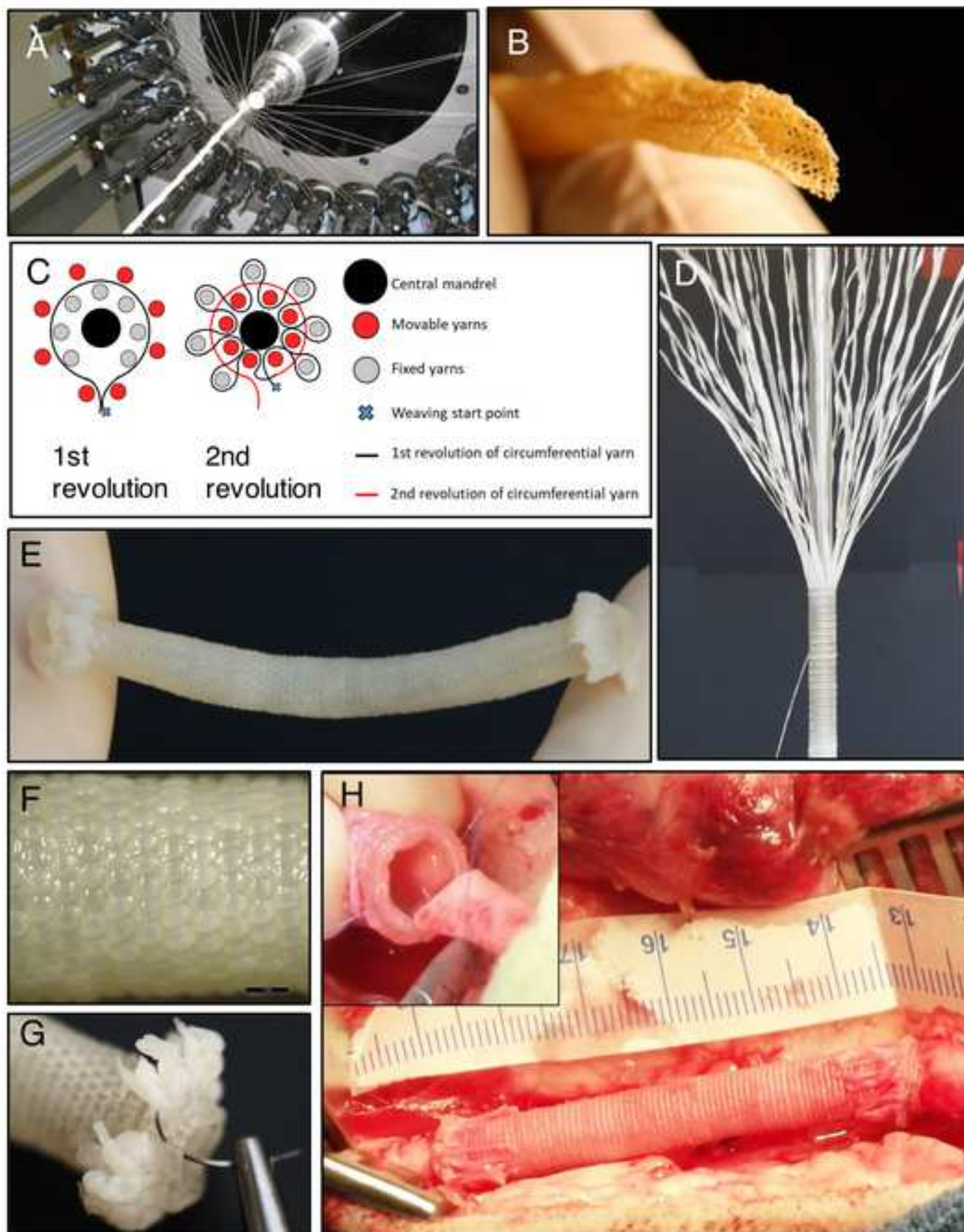


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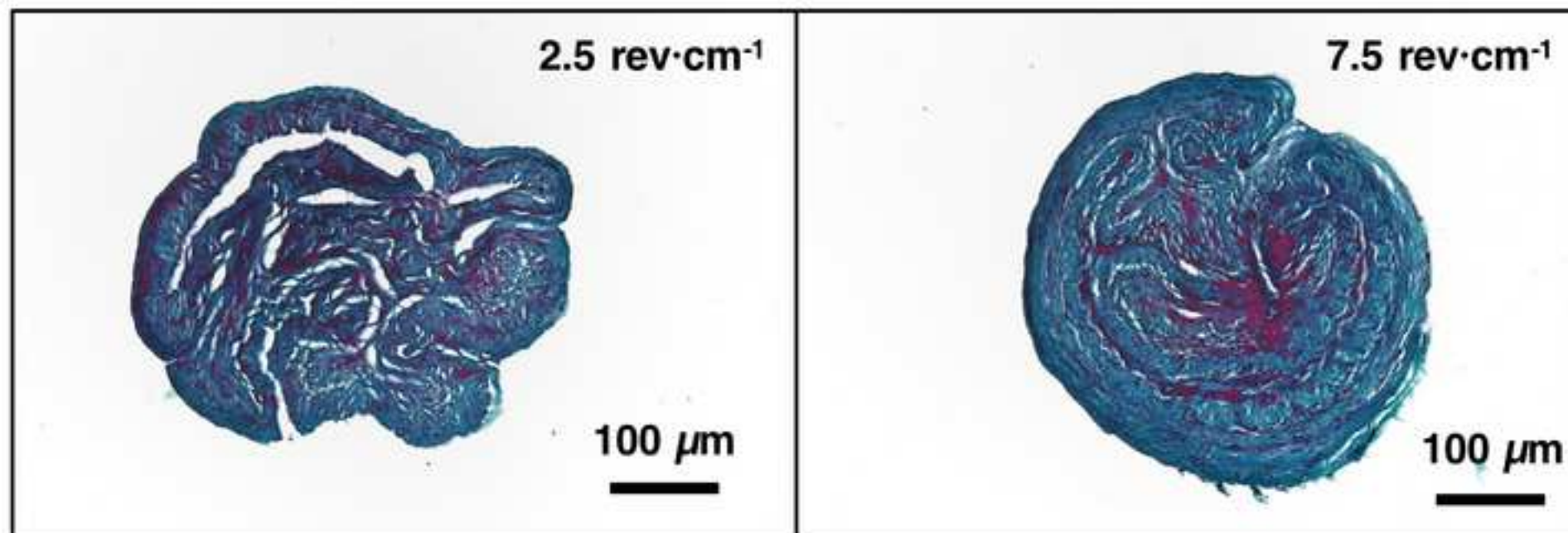


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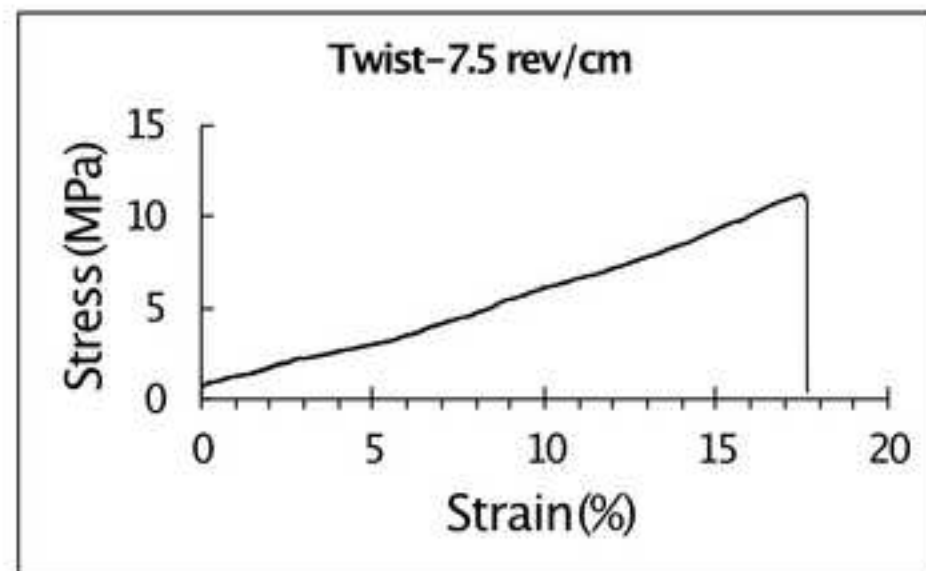
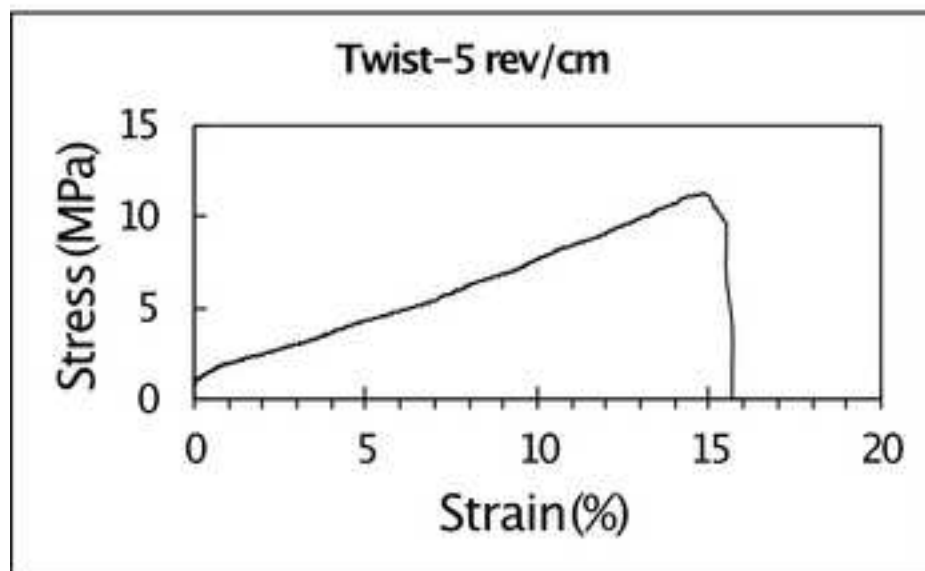
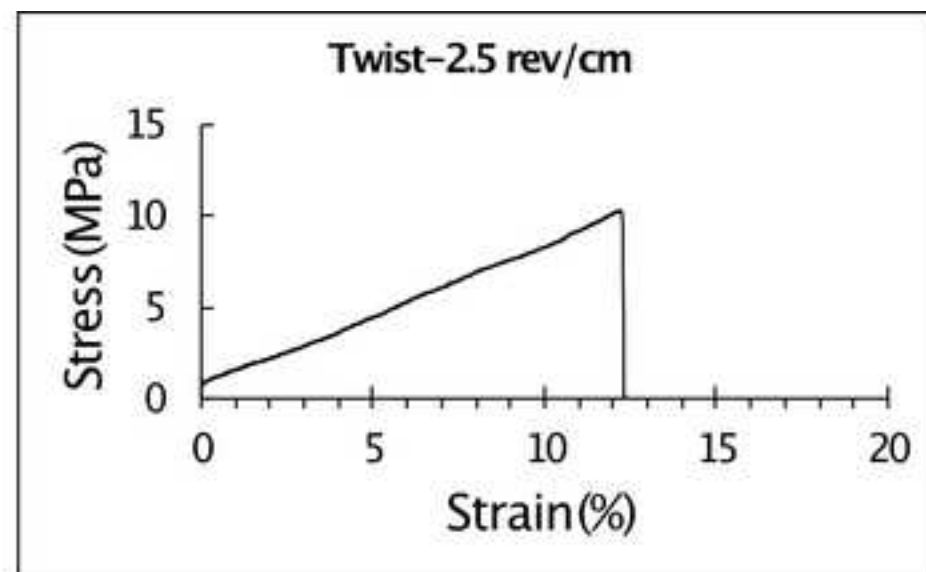
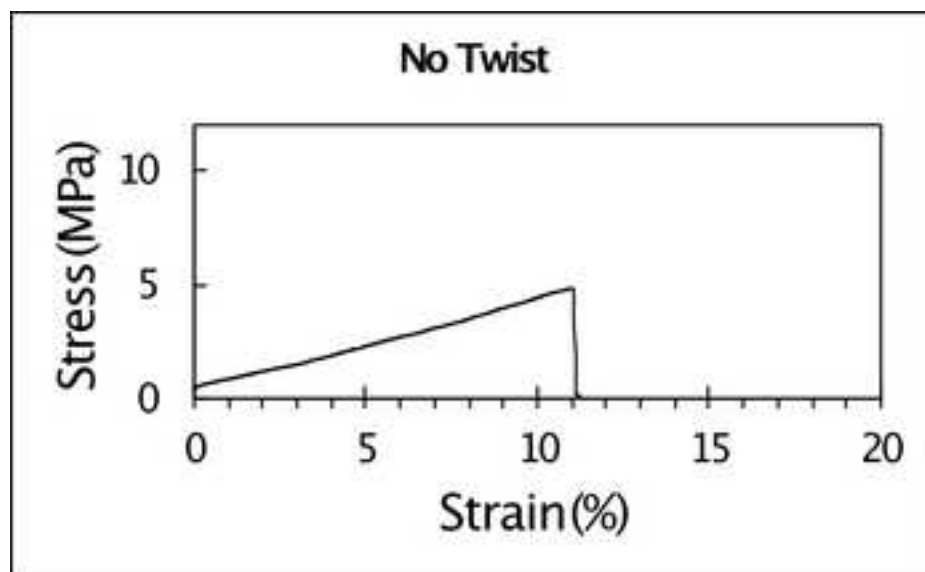


Table 1. Mechanical characterization of the human woven grafts and Human Internal Mammary Artery .

	Burst Pressure (mmHg)	Suture Retention Strength (gf)	Transmural Permeability (ml·min ⁻¹ ·cm ⁻²)
TEVG	5968 ± 732 n = 5 Max: 6545 Min: 4755	566 ± 17 n = 3 (5*) Max: 576 Min: 546	10 ± 8 n = 4 Max: 18.5 Min: 0.1
Human Internal Mammary Artery [18]	3196 ± 1264 n = 16 Max: 5688 Min: 534	138 ± 50 n = 6 Max: 205 Min: 78	N/A

*Suture broke before pull-out in two cases that were not included in the calculation (around 600 gf).

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: