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Multiaxial filament winding of biopolymer microfibers with a collagen resin binder for orthobiologic medical device biomanufacturing

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Abstract

Multiaxial filament winding is an additive manufacturing technique used extensively in large industrial and military manufacturing yet unexplored for biomedical uses. This study adapts filament winding to biomanufacture scalable, strong, three-dimensional microfiber (3DMF) medical device implants for potential orthopedic applications. Polylactide microfiber filaments were wound through a collagen 'resin' bath to create organized, stable orthobiologic implants, which are sized for common ligament (e.g. anterior crnciate ligament) and tendon (e.g. rotator cuff) injuries and can be manufactured at industrial scale using a small footprint, economical, high-output benchtop system. Ethylene oxide or electron beam sterilized 3DMF samples were analyzed by scanning electron microscopy (SEM), w1de1went ASTM1635-based degradation testing, tensile testing, ISO 10993-based cytocompatibility, and biocompatibility testing, quantified for hwnan platelet-rich plasma (PRP) absorption kinetics, and examined for adhesion of bioceramics and lyophilized collagen after coating. 3DMF implants had consistent fiber size and high alignment by SEM. Negligible mass and strength loss were noted over 4 months in culture. 3DMF implants initially exceeded 1000 N hydrated tensile strength and retained over 70% strength through 4 months in culture, significantly stronger than conventionally produced implants made by fused fiber deposition 3D printing. 3DMF implants absorbed over *3x* their weight in PRP within 5 **min,** were cytocompatible and biocompatible in vivo in rabbits, and could readily bind tricalcium phosphate and calciwn carbonate coatings discretely on implant ends for fmther orthobiologic material functionalization. The additive manufacturing process further enabled engineering implants with suture-shuttling passages for facile arthroscopic surgical delivery. This accessible, facile, econmnical, and rapid microfiber manufacturing platform presents a new method to engineer high-strength, flexible, low-cost, bio-based implants for orthopedic and extended medical device applications.

1. Introduction

Tendon retears following surgical rotator cuff repairs (RCR) occur in up to 45% of patients [1, 2], an unacceptable reoccmrence rate given that approximately 500,000 RCR procedures are performed annually in the U.S. alone (3]. Rotator cuff injuries do not heal well, resulting in limited limb mobility and persistent pa.in, significantly impacting a patient's ability to return to regulaT activity or work [4, 5]. Many researchers have sought to improve RCR outcomes via therapeutics or devices, ranging from biological

injections to a myriad ofbiomaterial implants tested with various degrees of success in basic research through clinical use [6]. Today, RCRs commonly use only sutures and anchors for repairs [7] and the re-tear rate remains alarmingly high.

Historically, allograft tissue has been used to augment rotator cuff tendon repair and reconstruction surgeries. Yet, the benefits of allograft tissue in this context are limited by variable donor quality, lack of biomimetic stTuctures, challenges associated with artluoscopic surgical delivery, and impaired healing [8]. Moreover, the use of allograft tissue for augmentation of RCRs has not been reliably associated with significant clinical benefits compared to suture-only repairs [9]. Recently, a reconstituted freeze-dried bovine type I atelocollageu sponge exhibited a 6--12 month *in situ* remodeling period to help promote RCR healing via collagen biointegration. The collagen remodeled intoregularly oriented connective tissue in a sheep rotator cuff model over 52 weeks, with thickened, dense connective tissue replacing the implant [10] at around 9 months. Yet a dried collagen sheet lacks early repair strength and is troubled with challenging smgical delivery and trouble-prone staple fixation.

More recently, microfibrous tissue-engineered medical products biomanufacturing,includingadditive manufacturing technologies, has been widely investigated in orthopedic research [11]. Particularly in sports medicine-related soft tissue research, collagen-containing microfibrous implants have been developed to mimic the aligned collagen-fiber features of native ligaments and tendons (12-16]. Fiber alignment is important for strength and guided remodeling, so collagen is chosen for biological integration and cell attadnnent [17]. Microfibrous scaffolds for promoting ligament and tendon healing are made by myriad methods, including electrospinning, wet extrusion, dry spinning, fused fiber fabrication (FFF) tluee-dimensional (3D) printing, pnemnatospinning, and conventional biotextile approaches (braiding, knitting, and weaving), among other modalaties [12, 15, 16, 18, 19]. However, the existing methods have Val-ious disadvantages; for example, electrospm1 material clinical translation has been limited by scaling alld manufacturing complications like high batch variability, poor *in vivo* cellulal· infiltration due to the limited porosity of produced scaffolds, and the use of hal·mful processing solvents used in most, but not all, applications. By additive malmfacturing, FFF printing has been explored for making ligament and tendon analogs. Yet, FFF is inherentlylimited to simple fused structures and does not resemble native tissue structures or composition. Likewise, biotextiles are complicated and expensive for academic labs or non-specialized compal1ies to produce, presenting a need for more accessible fiber-based additive manufactming technologies.

To counter current limitations in the fibrous implant manufacturing field for orthopedics R&D and elsewhere, we investigated adapting filan1ent winding to create biologically compatible, scalable, high-strength medical device implants. Multi-axial filament winding is an additive manufacturing technique used widely in lal·ge industrial and military applications ranging from carbon fiber bike frames to missile casing al1d helicopter propeller malmfacttuiug and more (20-22]. This work reports on the successful first adaptation of multi.axial filament winding to biomauufactmer scalable, strong, 3D microfiber (3DMF) medical device implants with extraordinary tensile strength in a process that can be manufactured at all industrial scale using an accessible al1d affordable beuchtop system. Adapting the multi.axial filament winding system (figme 1) used in industrial al1d military applications will prospectively allow for a wet winding process to create biologically compatible and strong implal1ts that are cytocompatible, mechanically mimic native ligalnent and tendon properties, and can absorb biological fluids, such as platelet rich plasma (PRP) to enhance healing. The 3DMF implal1t is all innovation that most closely mi1nics native tendons in its fiber al1d alignment with the added benefit of exponential strength and absorption properties to incorporate healing factors. Additionally, future renditions can have varying layers alld widths to customize to various joint spaces like Achilles or ACL.

2. Methods

2.1. 3D microfiber filalnentproduction

Clinically releval1t Lactoprene™ (Poly (L-lactide) and Tri.methylene Carbonate} microfiber yal·n from PolyMed Inc (Anderson, SC) with an average diameter of 14 µm was used for this study, along with polydioxanone, Dyneema (ultra-high molecular weight polyethylene), al1d 1nicrofibrillated cellulose (nal1ocellulose) fibers for prototyping. Biopolymer fibers were passed through a collagen 'resin' binder at 5 mg1111-1 (Advanced Biomatrix, Carlsbad, CA) and pouredinto a trough with rollers to guide the fiber wetting (siinplified diagraln in figme 2(A) actual device in 2(B) and (C)) ii1 multi.axial filan1ent winding. Collagen resin-bound microfibers passii1g through the trough, rollers, al1d with a robotic al'.111 were then placed on a mandrel al1d controllably iiicorporated on all X-Wii1der™ as guided by custom sofuvare (X-Wii1der LLC, New Mexico, USA). Filalnents were wotmd via a program to create tendon- al1d ligament repai.J·-sized iinplants (2 x 3 x 0.2 mm and 1 x 3 x 0.2 mm, respectively}. Discretely wotmd iinplants were then gelled via incubation at 37 °C to form a stable, cohesive implal1t (figtues 2(D) and (E)) or fmther postprocess by coating the ends with calcium cal·bonate or beta-tricalcimn phosphate (Regenity, Oaklal1d, NJ) or by submersion in 9 ml of 5 mg ml-1 collagen

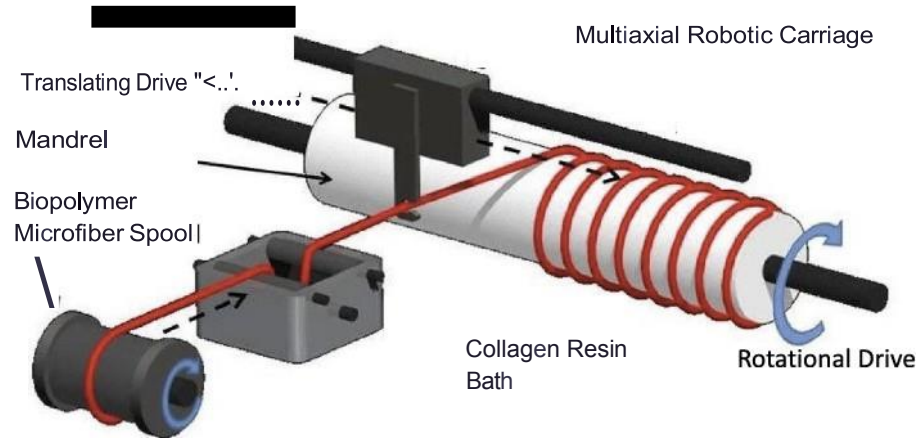

Figure 1. Diagram of a 4-a-.cis 3DMF multiaxial filament winding machine. Biopolymers from a bobbin ,ue passed through a collagen resin bath through a robotic head and collected on a translating and rotating mandrel.

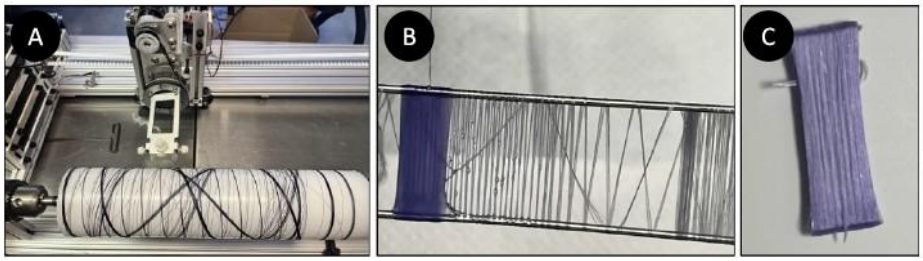

Figure 2. 3DMF Ha.rd.ware. 3DMF hardware is pictured (A) with varied winding geometries on a drum (A) and across posts (B) and the resulting 3DMF in1plant post-collagen gelation at 37 °C (C).

(Advanced Biomatrix) for lyophilization in a tray. Collagen fibrils were self-assembled at neutral pH into bundled fibers, typically 12-120 nm diameter, that crosslink to produce a matrix structure that ultimately forms a hydrogel in a water-based solvent and heat at 37 °C to gel and stabilize. The final anisotropic, organized collagen-fiber prints without additional modifications are pictured in figure 2(F). The rotating mandrel and translating drive moving together created a 1 cm wide single implant analog in 97 s. Samples were formed in four unique production runs and spilt into groups for tmsterilized, electron beam (e-beam) (EBeam, Cranbury, NJ) or ethylene oxide (EO) (Boulder Sterilization, Boulder, CO) terminal sterilization, with samples sealed in Tyvek pouches havingsterilization indicators, thenstored in ambient conditions for 1-3 months for subsequent testing.

For comparison to a clinically relevant reference material (polylactic acid filament), conventional FFF in1plants also sourced from PolyMed (Anderson, SC) were obtained for printing on a custom Prusa MK4 3D printer (Prague, Czech Republic). A CAD file was designed to print in1plants with fiber lines that approximated the size and shape of 3DMF prints. Printing nozzle, speed, and height were optimized to produce the finest possible 'fibers' at the tightest possible packing while preventing fusing to create a material reference of a 3DMF or native ligament/tendon tissue architecture. As with 3DMF, the FFF-produced implants weresubsequently coated in collagen (Advanced Biomatrix) by submerging them in collagen and placing them at 37 °C to promote collagen gel formation on the printed surface. FFF-produced implants were also terminally sterilized, as described above. Electrospun collagen-polylactide and pure lyophilized collagen sheets were also produced for tensile testing.

2.2. Scanning electron microscopy (SEM) imaging

Collagen-coated 3DMF microfibers and FFF printed clinical grade PLA prints were structurally analyzed by a JEOL JSM6490 SEM (University of South Florida, LMW Microscopy Core, Tampa, FL). The 3DMF samples were each placed onto the sample holder using the double-sided SEM tape and then gold sputter coated. SEM images were analyzed at 200x and lO00x at 10 kV beam strengili. The fiber alignment and mia-oscopic fiber topology were analyzed with relative measurement of fiber thickness in lmageJ FIJI (NIH Shareware). Unsterilized 3DMF samples and iliose treated with ethylene oxide and electron beam were imaged for ImageJ FIJI (**NIH** Shareware) analysis.

2.3. Cytocompatibility

3DMF implants and PLA feedstock fibers alone were wetted in growth media (GM) made as 89% Dulbecco's Modification of Eagle's Medium with 4.5 g 1-^{1} glucose (Gibco, Grand Island, NY), L-glutam.ine, & sodium pyruvate (DMEM) (Gibco, Grand Island, NY), 10% Fetal Bovine Serum (FBS) (Gibco, Grand Island, NY), 1% Antibiotic-Antimycotic l00X (A.BAM) (Gibco, Grand Island, NY). Separately, 0.5 x 10^{5} C2Cl2 cells (American Type Cultme Collection, Gaithersburg, MD) were added to quadruplicate wells and incubated at 37 °C and 5% CO2. Prest0Bh1ern Cell Viability Reagent (Invitrogen, Carlsbad, CA) was added to each test well, incubated for 4 h, observed for color changes, and transferred to the biological safety cabinet in low light. 100 µl of eachsample was pipetted *3x* ontoa 96-well plate to create triplicates for each test sample for plate reading at 560 nm EM, 590EX on a Tecan M200 Pro monoduomator plate reader to determine metabolic activity over 3 d in cultme. Microscopic images of the cells in the wells were captured on a Keyence ECHO (Itasca, IL) fluorescence microscope for cells stainedwith668.4 g$_{11101}$-^{1}propidium iodide to assess cell density, health, and smvival. Positive control for cytotoxicity was cells exposed to GM alone,compared to the negative (kill) control group of the C2Cl2 cells treated with 10% Triton X-100 (Gibco, Grand Island, NY), following the ISO 10993 guidelines.

2.4. Degradation testing

The 3DMF and FFF-printed PLA degradation testing is based on the ASTM Fl635-16 'Standard Test Method for *in vitro* Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms for Smgical Implants.' hi 6-well plates, 3DMF samples were placed for 2, 8, and 16 weeks to assess long-term biomechanical stability and degradation vi.a mass loss. Under sterile conditions in a biosafety cabinet, masses were measured for $T = 0$ and recorded. The 3DMF and FFF printed PLA samples were submerged in 5 ml of PBS (Dulbecco's phosphate-buffered saline) (Gibco, Grand Island, NY) and incubated at 37 °C. At approximately every 3 weeks, 3-5 ml of PBS were replenished to maintain full implant coverage. At each tin1e point, the respective samples were removed from the incubator, where the PBS's pH was measured upon removal, and the samples were rinsed 3 times with DI water, dried under vacuum for 8 **h,** and then weighed.

2.5. Tensiletesting

Biomechanical testingwas based on the 'standard test method for tensile properties of polymer matrixcomposite materials' or ASTM D3039M-0l 7. Tosimulate smgical fixation for tensile testing, 2 mm of high-performance line Dyneema fiber (SEAC, Miami, FL) was looped inside 3DMF implants for attachment to wire wrnpping grips on a 20 kN HST Universal Mechanical tester (Jinan Hensgrand Instrument Co., Jinan, China) using a calibrated 5 kN load cell. MaxTest (Physical Test Solutions, Culver City, CA) software was loaded with the ISO527 tensile properties standards. Three samples were analyzed per time point using 1 cm wide, 3 cm long, and 0.2 cm thick samples. The load control speed was set to 2 N s-^{1} with a tighten speed of 10 mm min-^{1}. Thecyclic preload was set at six ti.mes to 50 N load and released until open loop displacement testing was performed on $n = 4$---6 samples at 5 mm min-^{1}until failme, with image and video recording of the failures (iPhonel3, Apple, Cupertino, CA).

2.6. PRP wicking

Filament wound 3DMF samples were weighed, and each dry sample's initial mass was recorded. Then, 10 mL of PRP from precision for medicine (Fredrick, MD) was added to a 50 mL beaker with the fully submerged 3DMF sample. Across tin1es 1, 3, 5, 10, 15, and 30 min, the sample was cleared of drippage and removed from the beaker. Its mass was recorded before resubmerging to continue until PRP absorption was weighed by mass for the full 30 min. This experin1ent was repeated with $n = 6$ samples in total.

2.7. Bioceramic coating

Carbonate apatite and beta-a·icalciwn phosphate microgranules, a generous gift from Regenity (Oakland, NJ), were tested for adhesion to 3DMF printed consa·ucts by post-processing emersion of the implant ends into a tray of the bioceramics independently, followed by thermal gelling of the construct at 37 °C for 15-20 min, with the experiment performed in duplicate.

2.8. Lyophilizedcollagenshell

An encasing and integrating shell of collagen was formed arnw1d the 3DMF implant fibers ($n = 3$) by submersion on 10 mg $_{1111}$-^{1}of liquid atelocollagen in 0.05 M acetic acid, freezing at -80 °Cand lyophilizing to dryness on a USA Lab 6L scientific shelf freeze dryer (Livonia, MI) or prima1y and secondaiy diying to a residual moisture level under 6%.

2.9. Biocompatibilitytesting

Sterilized 3DMF implants ($n = 14$) were implanted subcutaneously in New Zealai1d white rabbits at NAMSA (No1wood, Ohio) following ISO 10993 per an approved IACUC protocol.Samples were collected at 2 weeks in life, and paraffin was embedded, sectioned, and stained with H&E for imaging and ISO 10993 biocompatibility assessment.

2.10. Statisticalanalyses

For degradation strength, mass loss cl1ai1ges, and PRP absorption, a paired t-test was used. *A priori,*

p values < 0.05 were defined as significant. All tests were performed using GraphPad Prism 9 (GraphPad by Dotmatics, Boston, MA), and all parameters are expressed as mean ± standard error of the mean (S.E.M.).

3. Results

3.1.3D microfiberfilament (3DMF) multi-axial filament windingproduction

Filament winding was originally advanced for large-scale winding of military and industrial applications such as carbon fiber, so robust and large amounts of material are usually used for winding without damage and with utmost precision. This makes it difficult when scaling down to microfibers for biomedical applications because they require proper tension and resistance to stayaligned and taught on the mandrel without ripping whilst also stacking layers linearly. The addition of a collagen bath for coating required complete filling and staggered roller setup to ensme collagen did not drop off of the fibers before being collected on the mandrel. Adjusting for tension, speed, and collagen bath fill, filament winding successfully produced RCR-sized (2 x 3cm) 3DMF implants in 97 s per implant using PLA microfiber yarn and collagen resin. This output perfonnance scales to 180 implants made in 6 h, 900 per week, and around 46 000 yi·-^{1} manufacturable on a benchtop (1 x 1 x 1.5 m) 3DMF microfiber printer shown in figure 1. Polydioxanone and nanocellulose microfibers were also 3DMF filament wound, showing the process is amenable to other material types. However, PLA-collagen was the focus of this investigation and was used in subsequent studies.

3.2. SEMimaging

SEM in1ag:ing revealed remarkably high fiber alignment in 3DMF constrncts, with collagen resin 'bridges' noted between fibers, showing the binder presence in the non-sterilized, elect1·on beam, and ethylene oxide treated samples (figure 3). 3DMF implants sterilized with ethylene oxide displayed an alteration to the highly aligned fibers in the unsterilized and electron beam samples, with a characteristic waviness to the fibers observed both microscopically and macroscopically. Compai·ed to FFF 3D printed PLA assessed by SEM, 3DMF prints had higher alignment. On average, FFF PLA printed 'fibers' were measmed over 300 µm in diameter relative to just over 14 µm fibers 3D printed in 3DMF filainent wound in1plants.

3.3. Cytocompatibility

Cytocompati.bility assessment (figure 4) presents the sterilized PLA microfiber feedstock and 3DMF (PLA microfibers plus collagen discretely filament wound) materials to exhibit consistent and high metabolic activity for implants that remained through 3 d of culture, along with healthy cellulai· morphology for the C2Cl2s cultures exposed to these materials. The cellular activity and morphology suggested the 3DMF process ai1d resulting implants as cytocompatible upon the biomaimfacturing processing and post-sterilization as an industry-standard *in vitro* test for material compatibility towai·ds biological applications.

3.4. Degradation testing

Degradation analyzes the long-term stability and integrity of the implant in a hydrated state to mimic *in vivo* conditions associated with a tendon over the healing period. ASTM Fl 635-16 standai·d for degradation of sainples was used to analyze the viability of the 3DMF ai1d FFF prints over 16 weeks, associated with the postoperative healing periodcommonly seen in soft tissue orthopedic injuries to require biomechanical suppo1t. Over 16 weeks, the average total mass losswas 3.68% for 3DMF samples aiid 2.90% for FFF-printed PLA relative to mass at time zero. High stability was exhibited physically at 16 weeks (figure 5), with no signs of material failure (a-acking, breaking, thinning) for either 3DMF or FFF print PLA; massretention further validated the absence of material failure. 3DMF implants demonstrated statistically significantly higher ($p < 0.05$) chai1ge in mass at week 2 of degradation in media but not at weeks 8 or 16 relative to time zero. The FFF 3D printed PLA consistently demonstrated a statistically significai1t higher change in mass over 16 weeks (table **1**).

3.5. Mechanical testing

The controllable filament winding of fibers in a stacked linear fashion is meant to represent the linear alignment of native tendon fibers ai1d maximize tensile strength. During mechai1ical testing, the fibers ai·e stretched cyclically and also to failure, mimicking the strain and movement experienced with a tendon in its native environment. Upon 50 N cyclic preloading of the 3DMF implant during tensile testing, a hysteresis curve, as present in native tendon tissue (bovine Achilles tendon tested and reported here), was noted (figure 6). While FFF PLA 3D printed exhibited an average maximum suture retention strength of 11.4 ± 6.8 N initially ($n = 6$), this dropped to 9.3 ± 4.1 N dming the 16 weeks of incubation in culture media (figure 7(A)). This strength contrasts dramatically with 3DMF implants that initially averaged a maximum peak load at break of 1332 ± 50.3 N of suture retention strength and retained 1,068 ± 18.82 N of this strength (over 80%) through 16 weeks hydrnted at 37 °C in culture (figure 7(B)). 3DMF implants exhibited high elasticity, with failure at over 70% strain, and failed through a 'horsetail' implant failure mechai1ism, defined as explosive, gage, middle, or XGM, per ASTM D3039/D3039M-17 'Standard test

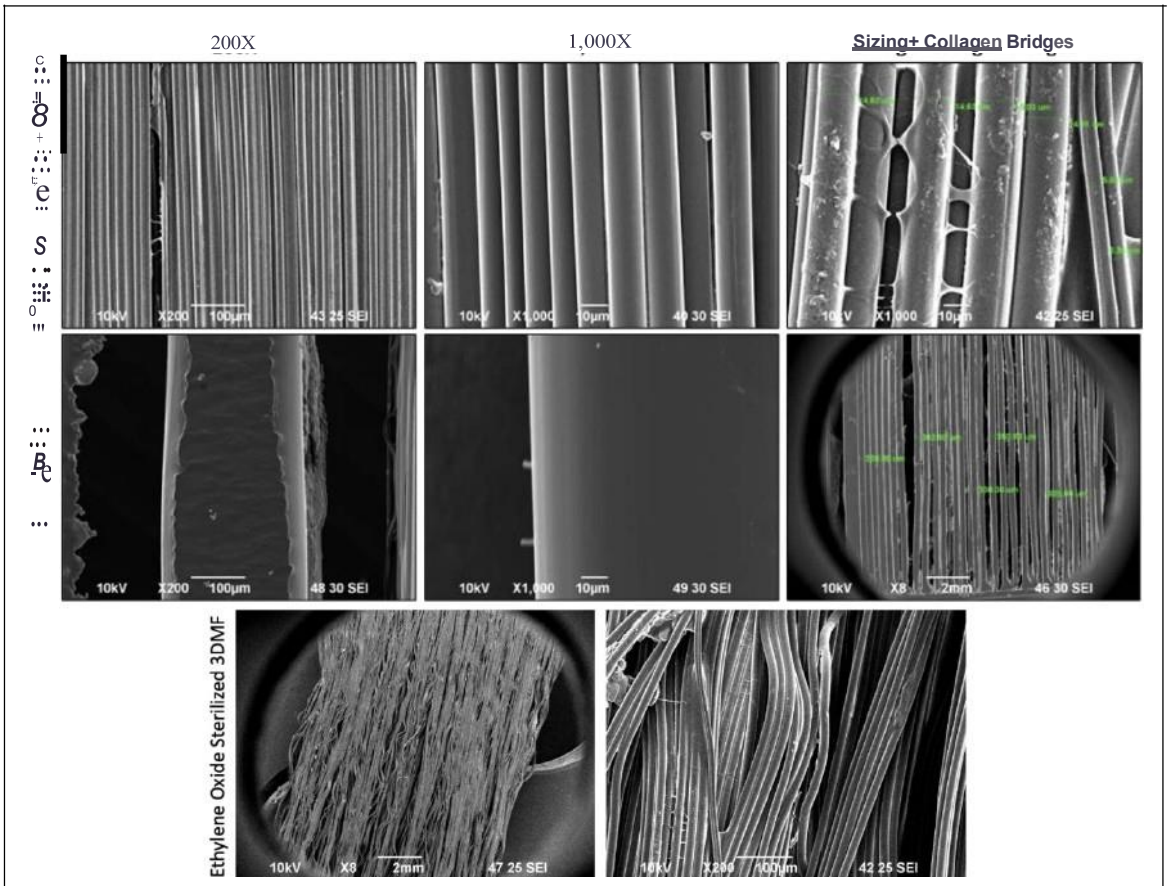

Figure 3. Scanning electron microscopy (SEM). SEM of the 3DMF and the FFFclinical grade PI.A 3D prints at 8, 20, 0, and l000x. Consistent and finer fibers are present in the 3DMF in1plants (14.21 ± 1.24 µm vs. 300.1 ± 41.83 µm) relative to the FFF PI.A prints. Alsoseen ai·e the collagen 'resin' bridges holding and binding the m.icrofilamentsin the 3DMF saiuples. (Magnifications differ to best reveal the features of each implant manufacturing method at different sizescales.

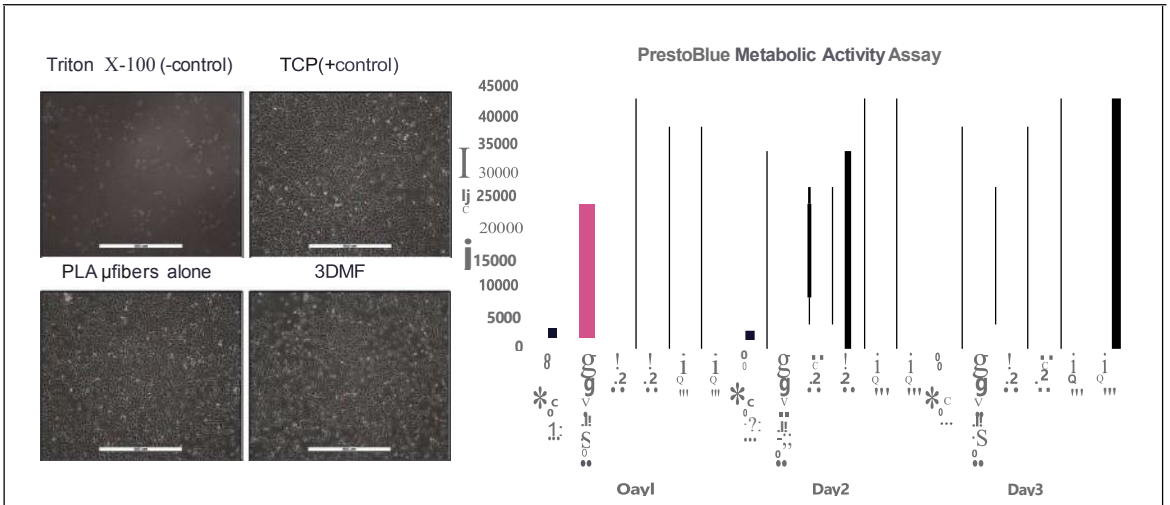

Figure 4. Cytocompatibility. Cytocompatibilitytesting per ISO 10993shows healthy cells from both thebase PI.A microfibers used in 3DMF, and the collagen-resin bound 3DMF constructs through 3 din culture. Four unique wells (n = 4) were assessed based on cell morphology and metabolic activity with PrestoBluen.•, with no statistical difference between the positive control (cultme media alone) ai1d test articles (media withscaffolds).

method for tensile properties of polymer matrixcomposite materials; which is highly analogous to native ligament and tendon ruphues.

Relative to lyophilized collagen and electrospun collagen/polymer sheets of matched dimensions, in diI·ect, head-to-head tensile testing on a calibrated machine followiI1g standard testiI1g methods, 3DMF fmther exhibited significantly higher strength iinplant strengtli and sutme retention properties iI1 tensile testiI1g. With matched scaffold dimensions to

Figure 5. 3DMF Culture Stability. A long-term stabilityassessment was done based on the ASTM1635 standard for 16 weeks to determine that the base filament wound fibers of 3DMF implants had maintained hydrated stability via the collagen 'resin' alone. FFF (top) and 3DMF implants retained their shape and structure, shown here, air-dried after 4 months of hydration, with samples used for assessing degradation by mass loss (table 1 below) and subsequent for long-term tensile testing to determine strength retention.

Table1. ASTM 1635-based mass loss of 3DMF and FFF PLA from 2-16 weeks relative to time rero.

Mass loss timepoint	3DMF average	Standard deviation	FFFPIA3D print average	Standru·d deviation
2 weeks	4.694%	0.9%	2.762%	0.666%
8weeks	5.356%	6.048%	2.529%	1.509%
16weeks	3.681%	1.439%	2.898%	0.621%

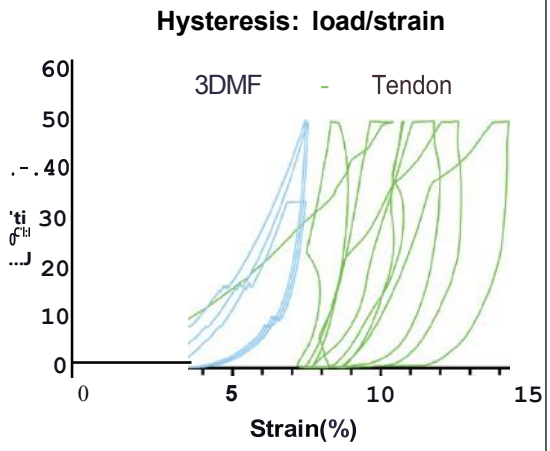

Figure 6. 3DMF Hysteresis. (A) Tensile cyclic testing at 50 N preload of 3DMF implants relative to bovine tendons indicating the hysteresis patterns of native and biosynthetic tissue analog.

cover the com.mon supraspinatus tendon repair footprint (2 x 3 cm), 3DMF implants exhibited over 2,000 times higher suture retention strength and over 150 times higher overall implant strength relative to electrospun and lyophilized collagen. 3DMF implants further exceeded the suture retention property of the humansupraspinatus tendon from the literature [23-27]. 3DMF hydrated strength overall approximated the native rotator cuff tendon at 753 N compared to 779 N on average for the load at failure for the native tissue (table 2), and dramatically higher implant strength relative to electrospun implants or reconstituted collagen sheets which had negligable inheret strength in the implants as gripped on the edges and pulled to pailure.

3.6. PRP wicking

PRP wicking on 3DMF implants (Left) exhibited rapid biological fluid absorption between time 0 and time 5 min, with an average relative absorption change of 338% (approximately 1 ml) of PRP absorbed by 5 min and held at least at this level of retention throughout the time course (figure 8). Conversely, the FFF PLA prints (Right) exhibited inconsistent biological fluid uptake between time 0 and 30 with very little relative absorption change of 86% (approximately 0.1 ml) of PRP absorbed by 5 min but did fluctuate in its retention level through the time course.

3.7. **BiologicCoating, andBiocompatability** Adding carbonate apatite and beta-tricalcium phos- phate coating directly onto the ends of the 3DMF implants and FFF PLA prints during post-processing yielded localized surface coating (figure 9) that remained integrated upon hydration. Lyophilization of 3DMF implants in a tray with collagen produced a collagen sponge layer around the filament wound implant, with passages on the endsmolded in, capable of passingsuture or suture tapes to facilitate shuttling the in1plants into a surgical space prospectively.

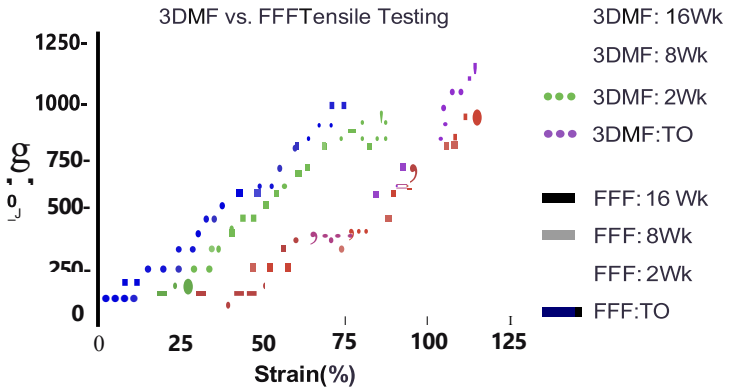

Figure 7. 3DMF tensile testing. Load/strain average values of the FFF print PLA and 3DMF implants degraded over 2, 8, and 16 weeks, relative to time zero (TO), with 3DMF implants showing over1500 times higherload holding capacity through 4 months in culture.

Table2. Average human tissuestrength and suture retention strength compared to 3DMF.

Material type	Maxsuture retention load (N)	Load at failure (N)	Sti·ess at failure (MPa)	Youngs modulus (MPa)
3DMF	1332 ± 50.3	753 ± 86.2	14.1 ± 1.3	43.8 ± 11.2
Electrosptm collagen-polylactide	0.652 ± 0.043	4.556 ± 1.426	0.023 ± 0.003	1.0±0.342
Lyophilized collagen	1.163 ± 0.152	4.958 ± 2.244	0.098 ± 0.057	0.7 ± 0.122
Hwnan rotator cuff supraspinatus tendon[23-25, 28, 29]	104-262	779.2 ± 218.9	21.1 ± 5.4	181 (M)' 210 (F)'

M-Male.
F-Female.
• lnte1preted from graph dataset.

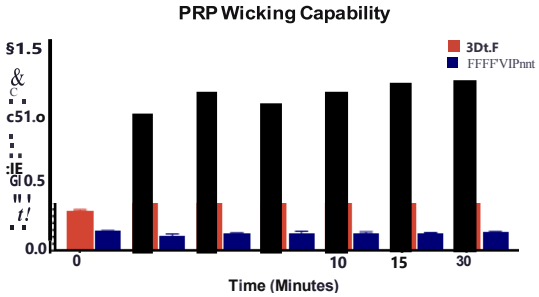

Figure8. 3DMF Biological Flu.id Wicking. 3DMF and FFF PIA in1plants (r, = 6) were weighed diy, thendipped in hwnan PRP to assess biological flu.idwicking capacity over 1, 3, 5, 10, 15 and 30 min. 3DMF san1ples consistently absorbed 3x their weight in PRP in 1 min, and peaked abso1ption at arotmd *Sx* their weight in PRPfrom 5-10 min whilst **PIA** prints remained arow1d its original weight with peak abso1ption at close to lx the.ir weight in PRP at 30min

3.8. Biocompatibilitytesting

Finally, subcutaneous 3DMF implants present the implants as highly cellularized and vascularized at only 2 weeks in life, with no severe adverse iinmtmological response noted ($n = 12$)., 1SO10993 biocompatability testing histology (figure 10) show the collagen coated 3DMF iinplants to be highly compatible ii1 rabbits, with market cellular ii1filtration, vascularizatio, and new collagen deposited arnw1d the microfibrous implant.

4. Discussion

This fu-st report on adaptii1g multi-axial filament windmg technology for medical device additive manufacturing describes the scalable orthobiologic iinplants made from strong, bioabsorbable microfibers, controllable manufactmed and bound with collagen 'resin' to create medical device iinplants. For iinplant manufactming speed, 3DMF iinplants made by adaptii1g multi-axial filament windii1g polylactide fibers with collagen resin additively manufactured organized microfibrous iinplants were generated m about 1.5 mii1 per implant. This output tnmslates at scale to over 40 000 implants manufactured ii1 a year with a small-footprint tabletop machme. This output could be further economically scaled out (with multiple machmes) or scaled up (such as with multiple filaments ,vindii1g simultaneously) to achieve tremendous output ii1 a small form factor with miiumal associated costs and no risk of hazardous volatile chenucal exposme. Advanced robotic filament wiI1diI1g can nuther make inegular 3Dshapes (such as spheres or angled 'elbows') or patterns (such as figme-of-eight), as made mdustrially for carbon fiber and other non-medical materials. Robotic control to make varied patterns and nonchemical fusion (e.g. ultrasonic weldmg) can also be employed here with 3DMF for biological iinplants made at high output, low cost, high spatial control,

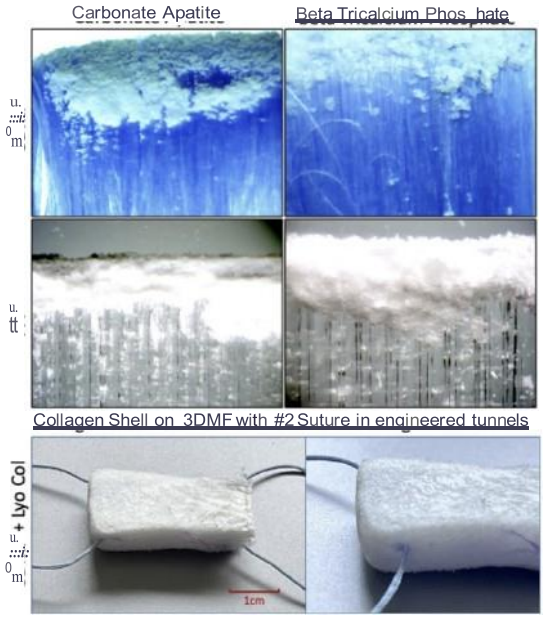

Figure 9. 3DMF Biological Coating. 3DMF implants (Top) and FFF PLA 3D prints (Middle) were discretely coated on one end with carbonate apatite, and beta tricalciwn phosphate to provide a template for osseointegration at the enthesis (*4x* magnification under stereom.icroscopy). The implants are further shown (Bottom) as encased in lyophilized collagen (3DMF + Lyo Col) with suture passage through the engineered tunnels to facilitate arthroscopic deliveryas a further extension of th.is manufacturing technology.

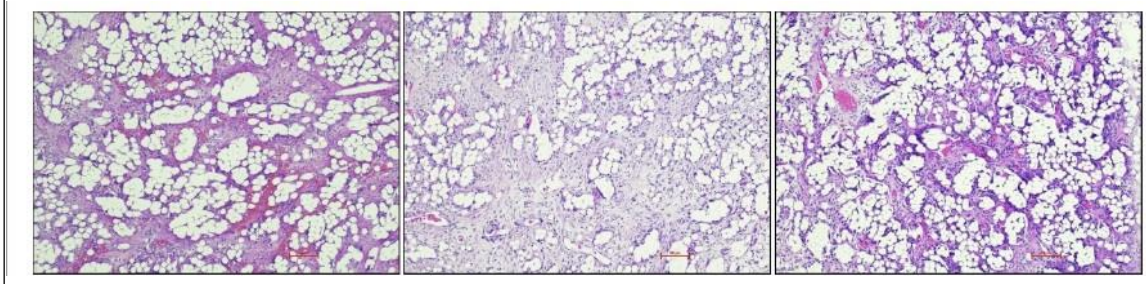

Figure 10. *Irzvivo* biocompatibility and cellular infiltration. Three 3DMF implants from 3 different rabbits are shown with rapid cellular infiltration between the fibers (cross-sectional cuts,fibers appear as rotmd emptyvacuoles) by H&E staining. E.'rtensive blood vessel ingrowth (crimson) is noted along with new dense collagenous depositions (magenta) arow1d the fibers with associated fibroblastic cells and occasional macrophages noted-l0X magnification for all images.

and an exceptional safety profile. The manufacturability of 3DMF presents compelling benefits in certain applications over other biotextile processes, such as electrospimung, which uses common poisonous and flammable solvents, or bybraiding, knitting, or weaving, which requires large, complex, and expensive equipment.

In basic biomaterial analysis for this pioneering 3DMF work, SEM imaging revealed highly organized structures formed by 3DMF, with anisotropic microfibers organized to mimic native anisotropic ligament and tendon microstructure. The collagen coating and bridging noted at highmagnification and the 14 µm diameter fiber size present a favorable cell attachment surface relative to synthetic polymer alone. SEM images of ethylene oxide-n·eated 3DMF constructs ftuther displayed characteristic waviness post-sterilization not seenin electron beam or unsterilized materials,which is presumed to be a result of the humidified hot air used in this process.

Intensile testing 3DMF conshucts, a remru:kable sn·ength of over 1300 N was exhibited for a conventional human implant-sized (**1** cm wide, 2 cm long, 0.2 mm thick) consn·uct, which maintained its sn·ength through 4 months of hydration in culture. This strength exceeds reports of electrospun collagen-based materials and lyophilized collagen by 3 orders of magnitude in our direct testing and the literature for collagen-lactide-based scaffolds (12], forther confirmed by our direct testing here. Remarkable, 3DMF implants held up to a 1,181% increase in sutme retention load and overall achieved 97% of native supraspinatus tissuestrength, making it a more biomimetic material for tendon and ligament repair. FFF-printed

PLA likewise peaked at 11150th the suture retention load of3DMF, at around 7-9 N, and closer in strength to conventional medical devices made by either lyophilized collagen or electrnsptm collagen/polymers. 3DMF implants also remarkably held 80% of their strength (maxi.mum sutme retention load) through 4 months in culture at 37°C, showing this an mmsuallystrong and stable material manufacturing method and fibrous product. Tensile testing for 'suture retention' strength is higher vs. gripping the implant directly, presmnably, as passing the suture through the loops of the 3DMF implant collectively shared the load, where grabbing the fibrous implant produced less even force. However, the 'suttu-e retention' mode is more relevant as it represents how such an implant would be used surgically in the clinical. In further tensile testing of 3DMF constructs, upon 50 N cyclic preloading of the implant during tensile testing, a hysteresis curve akin to native tendon tissue hysteresiswas noted in 3DMF implants. Elastic hysteresis in tendons describes dissipated energy from the materialviscosity and is important for efficient locomotion. While most materials usedin orthopedic tissue repair are rigid (e.g. plates, screws) or permanently deform under low load (e.g. electrospm1 materials, lyophilized collagen, etc.), 3DMF fibrous implants can be recoiled duringloading, potentially producing a more resilient implant.

While there was not a statistically significant decline in the ultimate strength of the 3DMF fibers post-sterilization when compared to m1sterilized 3DMF, there is an appai·ent decline in the slope of the load/strain cm-ve, indicating sterilization has made the fiber less stiff ai1d more elastic, which may be materially related to wavy structme. Still, this phenomenon would need to be studied further, along with exploring other potential sterilization methods for ensming desirable strength retention and not denatming and degrading tlie collagen, as electron beain and ganmia sterilization cai1 do to collagen. The native tendon displayed high stress associated ,-vi.th a smaller displacement level, indicating more energy loss ai1d less elasticity compared to the 3DMF samples,which havea tight coiloflarger displacement with lower stress, which is less energy loss ai1d greater elasticity [26].

In degradation testing, the implants retained most of their mass and strength through 4 months, past the ~3 month critical healing period for soft tissue repair [27, 30-32], suggesting an appropriate fit for soft tissue reinforcement ai1d regeneration in orthopedic soft tissue repair applications. For experimental design, samples were rinsed with DI water and dried to control the mass loss compai·isons because collagen-coated fibers may disperse collagen to promote healing. There were limitations in this setup, which may have affected 3DMF mass loss values at week 2, therefore affecting the statistical significance, given that there was no way to know how much media and or collagen was rinsed off beforedi-yingfor eachsample,so the post degradation mass was not perfectly controlled amongst samples. Nonetheless, the implants remained relatively stable through the 16 weeks with little relative mass loss, and most strength was retained throughout this study.

Further 3DMF material analyses had a high cytocompatibilitywith a musculoskeletalcell type,as anticipated for well-studied collagen and PLA microfiber feedstock materials. Future studies will investigate the constructs' cellular attachment, migration, and organization capabilities. Biological PRP wicking tests quantified that over 30 min, the absorption of biological fluids in 3DMF increased rapidly by 1 min and then began to plateau as it reached saturation at arom1d 5 min of wicking. The 3DMF implants had a biological fluid uptake with the absorption of *3x* their weight in PRP ai1d Sx their weight in biological fluid, an important biomaterial consideration to prospectively accelerated healing relative to the FFF PLA prints, which had just below lx their weight in PRP over the full 30 min. Many pro-healing biointegrative implants contain ingredients such as collagen or cai1 bind PRP to disperse into thewotmd siteai1d promote healing, which 3DMF implai1ts also exhibit. 3DMF implant functionalizationis further possiblewithbioglass or bioceraniic coatings. A freeze-dried collagen shell adds collagen to the outside to produce a 3DMF implant witli added collagen for biological integration to the patient tissue. Further, passages for suture passage to facilitate smgical placement and fixation are readily designed into the construct. As cunent technologies use cumbersome and expensive 'staples' to fix implants in place, the ability to additively manufacture 3DMF in1plantswith integral fixation features without staples or prospectively the need for added difficult-to-use and expensive tooling provides additional prospective smgical benefits.

While this work focuses on prospective ligainent and tendon repair, other musculoskeletal devices could be readily filament wom1d, notably boneanalogs, which could be wound with bioactive glass or other bioceraniics in the collagen bathto form long bone segments to repair segmental defects.

Beyond musculoskeletal indications for sheet-like construct additive manufacturing, the basic 3DMF round tubular manufacturing approach is also well suited to matching tubular anatomical structtu-es, such as the trachea, esophagus, intestines, ai1d blood vessels. Additionally, other biological components cai1 be discretely positioned on the fibers via the collagen or a secondai-y bath, including cells, growth factors, small molecules, ai1d other di·ugs to functionalize the implants further. Finally, alternative polymer fibers, both bioresorbable and permanent, either alone or in blended ratios ai1d assorted wind-

ing configurations,are amenable to thistechnology to target specific clinical indications. This rapid, reproducible, facile, functional 3DMF filament winding approach represents a new fibrous additive biomanufacturing scheme with great potential for medical device production. With PLA, PDO and collagen's successful history of use in medical devices, it is suggested that 3DMF implants will be safe for use *in vivo*. This accessible technology has the potential for fai·reachi.ng impact from basic research to biomedical manufacturing.

5. Conclusion

This researchis the first report ofbiocompatible polymer rn.icrofibers as rganized fibrous implants by filament winding with a collagen resin coating for medical device biomanufacturing. 3DMF can be formed with a multiaxial filament winding additive biomanufacturing process, resulting in fibrous orthobiologic implants si.m.il.u in size and strength to native tendons and ligaments and orders of magnitude stronger than conventional fibrous resorbable biomaterial manufacturing methods and currently marketed devices. This innovation presents a low-cost, accessible, highly scalable, h.igh-strength method to form microfibrous implants useful across myriad clinical indications and research use.

Data availability statements

The data cannot be made publicly available upon publication because they contain commercially sensitive information. The data that support the findings of this study a.re available upon reasonable request from the authors.

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Ethics statement

No htm1an subjects or animal studies were performed in this work.

Conflict ofinterest

Authors H A, A T, and M F a.re employed by Asante Bio.

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