

# Engineering Biohybrid Fibrous Materials: Silk Fibroin Textiles Combined with Decellularized Porcine Small Intestine for Wound-Healing Applications

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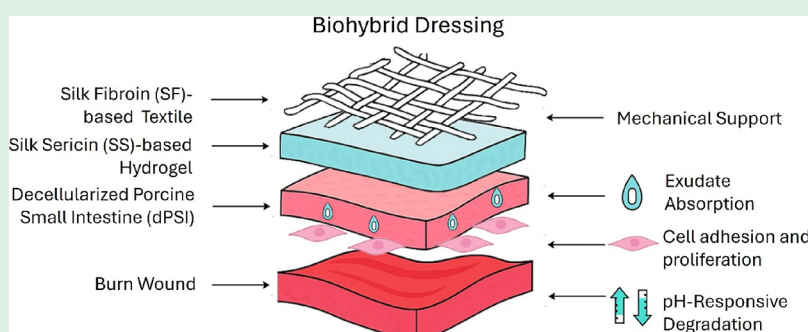
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**ABSTRACT:** Burn wound treatments remain a clinical challenge due to the complexity of skin regeneration and the need for advanced biomaterials that recapitulate the native extracellular matrix (ECM). In this study, a multilayer hybrid dressing is developed by integrating a silk fibroin (SF)-based textile with a decellularized porcine small intestine (dPSI) matrix, embedded within an enzymatically crosslinked silk sericin (SS) hydrogel. This composite construct combines mechanical reinforcement, bioactivity, and bioadhesion, offering a multifunctional platform with potential for wound dressing applications. The decellularization process effectively reduced cellular content while preserving essential ECM components. The incorporation of dPSI into SS-based hydrogel results in a stable and bioactive layer, reinforced by the SF-based textile for structural integrity. The hybrid dressings exhibit favorable swelling capacity for absorbing wound exudate and demonstrate a stable degradation profile across wound-relevant pH conditions in simulated wound fluid (SWF). Tunable mechanical properties are observed depending on the orientation of the dPSI (tunica serosa or mucosa) relative to the SF-based textile. *In vitro* studies with human dermal fibroblasts (hDFs) confirm high cell viability, adhesion, and metabolic activity over 10 days, with enhanced proliferation in the hybrid constructs compared to dPSI alone, indicating the positive contribution of SS hydrogel in supporting cell response. These findings demonstrate that the developed multilayer hybrid construct possesses promising structural, chemical, and biological properties for application as a dressing in wound treatment.

**KEYWORDS:** hybrid dressings, silk fibroin textile, silk sericin hydrogel, decellularized extracellular matrix, porcine small intestine, wound healing

## 1. INTRODUCTION

Human skin acts as a protective barrier against external factors and aggression playing essential physiological functions. Specific burn injuries remain one of the most common and traumatic wounds worldwide, being classified according to their depth and extent, factors that directly influence the type of treatment to be applied and clinical outcome.<sup>1</sup>

In the treatment of partial-thickness (extend through the epidermis and superficial dermis) and full-thickness (extend through the epidermis and deep dermis) burn wounds, the standard treatment is still the use of autografts, specifically split-thickness skin grafts (STSGs).<sup>1,2</sup> However, limited tissue

availability, donor-site morbidity, and impaired functionality of the regenerated tissue continue to motivate the development of alternative wound-healing strategies.<sup>3</sup> A wide range of biomaterials, both of natural and synthetic origin, have been identified to contribute to the treatment of these complex

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wounds, especially when the extent of the burned area is large and autologous solutions are not feasible. Hybrid dressings combine the advantages of different biomaterials and technologies to address multiple healing challenges, providing the opportunity of an optimal healing environment.<sup>4</sup> Commercial hybrid dressings designed for complex burn wounds include Integra (bovine collagen–GAG matrix with a protective silicone layer), Biobrane (silicone–nylon membrane with porcine collagen), Promogran Prisma (collagen and oxidized cellulose with silver for antimicrobial control), and Hyalomatrix (hyaluronic acid scaffold with a semi-permeable silicone membrane).<sup>5</sup> Although these hybrid dressings have demonstrated favorable outcomes by providing structural support and some biological activity, their moderate bioactivity and non-biodegradability limit their use as permanent scaffolds for full integration into host tissues.<sup>4</sup> To address this limitation, there is growing interest in fully natural-based scaffolds that mimic the native extracellular matrix (ECM) with controlled biodegradability, enabling seamless integration with host tissue and supporting long-term healing without the need for secondary removal procedures.<sup>6</sup>

Silk has been explored for different tissue engineering and regenerative medicine applications, including skin and wound-healing strategies.<sup>7,8</sup> Silk from *Bombyx mori* species has been the most relevant source to be used in biomedical applications. This fibrous material is composed of two main proteins, silk fibroin (SF, the inner core) and silk sericin (SS, the external protective layer). The highly stable SF possesses exceptional mechanical properties, competing with some synthetic fibers. By combining the outstanding mechanical performance with the well-established biocompatibility, biodegradation, and low immunogenicity, SF fibers can be converted in a suitable textile dressing for burn wound applications.<sup>9</sup> SS has poor mechanical properties. Nevertheless, it exhibits anti-inflammatory activity and supports cell growth and encapsulation, which makes it a valuable complement for the development of burn wound dressings.<sup>7,10</sup> Decellularized extracellular matrices (dECMs), derived from human and animal tissues, have been explored as a new cutting-edge solution for burn wound management. These matrices can retain essential structural, biochemical, and topographical cues of the native tissue that guide cell function and promote tissue regeneration.<sup>11</sup> dECMs from human cadaver skin such as AlloDerm, DermACELL, and GraftJacket® are commercial decellularized human dermal matrices used for wound healing and reconstructive skin surgery.<sup>12</sup> However, the ethical issues and limited availability of this biomaterial source is an unsolved challenge. Thus, decellularization of xenogeneic-origin tissues emerged as a sustainable alternative. Among them, porcine small intestine submucosa (SIS) has shown considerable success in wound-healing applications and forms the basis for the development of clinically available products, such as Oasis Wound Matrix and Oasis Ultra Tri-Layer Matrix (Cook Biotech, Inc.).<sup>13</sup> These are good examples of FDA-approved dECM-based scaffolds from porcine SIS with considerable success in the healing of deep chronic wounds. Although these matrices are based on the tunica submucosa of SI due to its advantageous biochemical and ultrastructural properties, the outermost tunics of SI, mucosa, and serosa also present significant benefits in terms of absorption and mechanical performance, respectively.<sup>14</sup> Moreover, due to its inherent adhesive properties within the abdominal cavity, both tunics can potentially improve the integration of a decellularized SI tissue as a wound dressing or for hybrid dressing

development.<sup>15</sup> For this reason, the present study aims to expand upon the design principles of currently available SIS-derived dressings, by creating an alternative hybrid strategy that combines the biological advantages of SI-derived matrix with additional structural and mechanical functionalities within a single construct. Specifically, a preserved decellularized porcine small intestine (dPSI) matrix containing all tissue tunics (mucosa, submucosa, serosa, and muscularis) that preserves key features of the native ECM architecture and biochemical composition will integrate an upper SF fabric that does not directly contact the wound but provides mechanical strength and structural stability, ensuring the integrity and durability of the dressing over time. An SS-based hydrogel is employed as an interface binder between the dPSI and the SF fabric layer, ensuring bioactivity and interfacial adhesion between layers, essential for dressing stability and optimal biological function.<sup>7,8</sup> By combining these components, the proposed construct aims to simultaneously support cell-material interactions, moisture management, biodegradability, and mechanical robustness, features that are not typically integrated within a single dECM-based wound dressing platform. Moreover, the use of dPSI, a byproduct of the agri-food industry, and SS, typically discarded during SF extraction, represents a resource valorization strategy that enables the use of biological byproducts in the development of hybrid wound dressings. To evaluate the influence of the wound-contacting surface, hybrid dressings were developed and evaluated using either the tunica mucosa (MuCW) or tunica serosa (SerCW) of the dPSI in contact with the wound. Mechanical testing confirmed that MuCW hybrid dressing exhibits superior tensile strength and flexibility, key properties for maintaining structural integrity and consistent wound coverage in dynamic wound environments. In contact with hDFs, the MuCW significantly enhanced cell proliferation and metabolic activity compared to plain dPSI, highlighting the synergistic potential of the proposed multilayer hybrid constructs as a promising platform for burn wound dressing applications.

## 2. EXPERIMENTAL SECTION

### 2.1. Materials and Reagents

Porcine small intestine tissue was obtained as a Category 3 animal byproduct from pigs slaughtered for commercial food production at Seara S.A. (establishment approval/registration no. PT D41CE), Vila Nova de Famalicão, Portugal. No animals were bred, handled, subjected to experimental procedures, or euthanized specifically for this study. Therefore, approval by an animal experimentation ethics committee was not required. The collection and use of the tissue for research purposes were authorized by the Portuguese Directorate-General for Food and Veterinary Affairs (DGAV) on February 7, 2023, in accordance with Article 17 of Regulation (EC) No. 1069/2009. Universidade Católica Portuguesa is registered by the DGAV as a user of Category 3 animal byproducts for research purposes (registration no. N.14.054.UDER). *Bombyx mori* silk cocoons were provided by the Portuguese Association of Parents and Friends of Mentally Disabled Citizens (APPACDM, Castelo Branco, Portugal). Silk-based textile was provided as taffeta fabric by CITEVE–Centro Tecnológico das Indústrias Têxtil e do Vestuário, Vila Nova de Famalicão, Portugal.

Reagents include dimethyl sulfoxide (DMSO), ethanol (EtOH), and monopotassium phosphate purchased from Honeywell (Charlotte, NC, USA); chloramphenicol from VWR (Radnor, PA, USA); and peracetic acid (PAA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) from Thermo Scientific (Waltham, MA, USA). All other reagents were purchased from Merck (Sigma-Aldrich, Rahway, NJ, USA).

## 2.2. Preparation of the Hybrid Dressings

The experimental setup applied for the development of the hybrid dressings is presented in Figure 1 and includes a step for the decellularization of porcine small intestine (PSI), the fabrication of the SF-based textile, the production of the silk sericin (SS)-based hydrogel solution, and the final assembly into layered constructs.

**2.2.1. Fabrication of Silk Fibroin-Based Textile.** A 100% commercial silk organza fabric with taffeta structure was used as the textile substrate in this study. The woven fabric had a grammage of 51 g/m<sup>2</sup> and was supplied by Atelier Companhia das Noivas (Portugal) to CITEVE. As shown in Figure 1a, the fabric consists of warp (vertical) and weft (horizontal) yarns interlaced in a one-over one-under pattern.<sup>16</sup> A double-fiber configuration was incorporated in the weft direction, increasing the yarn density along the axis.

Silk-based textiles were degummed in boiling deionized water for the full extraction of SS and washed in a 0.15% (w/v) natural soap aqueous solution to remove the remaining impurities resulting from the manufacturing process. The resulting SF-based textiles were dried at room temperature (RT) and kept until further use.

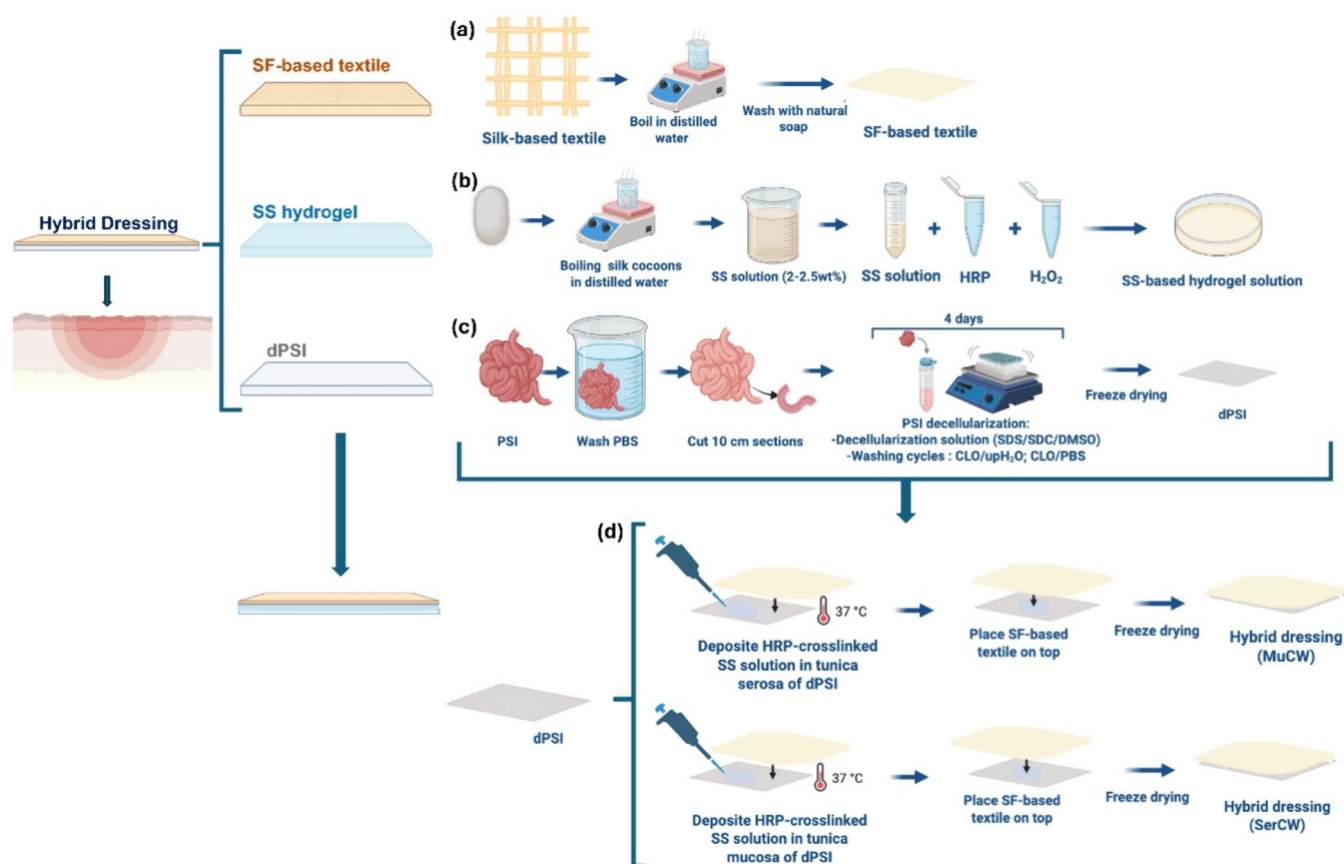
**2.2.2. Silk Sericin Purification and Hydrogel Formation.** Silk sericin (SS) was obtained following the extraction methodology previously reported by Baptista-Silva et al.,<sup>10</sup> in which the extracted sericin exhibited a molecular weight distribution ranging from approximately 40 to 440 kDa, with predominant fractions between 100 and 200 kDa.

The SS-based hydrogel was produced following a protocol previously described by Veiga et al.<sup>7</sup> (Figure 1b). Briefly, 5 g of silk cocoons was placed in 500 mL of boiling deionized water, until reaching a remaining volume of 100 mL of SS solution. The solution was stored at 4 °C until further use. Afterward, 20 mL of produced SS solution

was boiled until reaching 5 mL of solution, thus obtaining a final SS concentration of 2.0–2.5 wt % SS. Enzymatically crosslinked SS hydrogels were prepared by mixing 1 mL of SS solution (2.0–2.5 wt %), 50  $\mu$ L of horseradish peroxidase (HRP, 2 mg/mL), and 50  $\mu$ L of H<sub>2</sub>O<sub>2</sub> (0.3 wt %) in a 1.5 mL centrifuge tube (Eppendorf, Hamburg, Germany), with crosslinking occurring through the HRP/H<sub>2</sub>O<sub>2</sub> enzymatic reaction.<sup>7</sup>

**2.2.3. Decellularization of Porcine Small Intestine.** Porcine small intestine (PSI) tissue was obtained as an agri-food byproduct promptly following slaughter and transported to the Portuguese Catholic University of Porto according to pre-established conditions and transport guide. PSI tissue decellularization was adapted according to a protocol developed by Vasconcelos et al.<sup>17</sup> Briefly, samples were conveyed in a refrigerated box to inhibit protease activity. Prior storage, specimens were rinsed using a cold PBS solution, pH 7.4, and segmented into approximately 10 cm sections within a sterile laminar flow hood utilizing sterile scissors. To obtain a flat tissue configuration, each intestinal section was longitudinally opened along its tubular axis, exposing both the luminal (mucosa) and external (serosa) surfaces. After opening, the surface corresponding to the original luminal side was marked so that the mucosa and serosa tunics would remain identified throughout sample processing and hybrid dressings fabrication.

The specimens were then preserved at –80 °C and were used within a 3-month period from the time of collection. Decellularization of PSI was performed for 4 days (Figure 1c). Briefly, on day 1, the SI was cleaned by exposing the inner surface of the tubular tissue and removing the superficial fat tissue using sterile scissors and a clamp. Subsequent washing steps were performed for blood removal using chloramphenicol (CLO)/PBS solution (0.06 mg/mL), until the supernatant became clear (under cold conditions). Samples were then



**Figure 1.** Schematic illustration of the experimental setup applied for the development of the hybrid dressings. (a) Preparation of the silk fibroin (SF)-based textile. (b) Production of silk sericin (SS)-based hydrogel solution. (c) Decellularization process of porcine small intestine (PSI). (d) Methodology used for developing the hybrid dressings, combining the dPSI, SS-based hydrogel solution, and SF-based textile.

immersed in decellularization solution (0.5% (w/v) sodium dodecyl sulfate (SDS)/0.5% (w/v) sodium deoxycholate (SDC)/1 M dimethyl sulfoxide (DMSO)) for 4.5 h and washed in chloramphenicol/ultrapure water solution (CLO/upH<sub>2</sub>O) (0.06 mg/mL) overnight, under agitation (100 rpm, orbital shaker). On day 2, a new decellularization cycle was performed (0.5% (w/v) SDS/0.5% (w/v) SDC/1 M DMSO) for 4 h, followed by washing in chloramphenicol (CLO)/upH<sub>2</sub>O solution (0.06 mg/mL) overnight, under agitation (100 rpm, orbital shaker). On days 3 and 4, samples were subjected to washing cycles in chloramphenicol (CLO)/upH<sub>2</sub>O solution (0.06 mg/mL) and chloramphenicol (CLO)/PBS solution (0.06 mg/mL), respectively, to remove any trace of detergents and ensure thorough cleaning of the tissue (4 cycles, 2 h each). On day 5, samples were sterilized in a 0.188% (v/v)/4.8% (v/v) PAA/EtOH solution for 30 min, under agitation (100 rpm, orbital shaker). Afterward, PBS solution was used to wash the samples for additional 48 h and ensure the complete removal of the sterilization solution (under agitation, 100 rpm, orbital shaker). The resulting decellularized PSI (dPSI) was frozen at −80 °C, lyophilized for 72 h, and stored as off-the-shelf material. Native PSI tissue was used as control.

**2.2.4. Assembly of SF/SS/dPSI for Hybrid Dressings Construction.** The multilayer hybrid dressings were developed by embedding the off-the-shelf dPSI into a mixture of SS/HRP/H<sub>2</sub>O<sub>2</sub>, followed by the stacking of the SF-based textile on top. Two testing conditions were developed, one in which the tunica serosa gets in contact with the wound (serosa-contacting wound, SerCW) and other in which tunica mucosa gets in contact with the wound (mucosa-contacting wound, MuCW) (Figure 1d).

The structures were pressed and placed in the oven at 37 °C for 1 h to allow SS hydrogelation. Afterward, the layered structures were placed overnight at −80 °C and freeze-dried for at least 3 days to obtain off-the-shelf hybrid dressing materials. Plain SF-based textile and preserved dPSI were used as controls.

## 2.3. Decellularization Efficiency

**2.3.1. DNA Quantification.** Decellularization efficiency of the preserved dPSI was assessed through double-strained DNA (dsDNA) quantification and visualization of cell nuclei and matrix preservation by histological analysis. Untreated samples of PSI (native tissue) were used as control.

DNA extraction was achieved using the GRISPR Research Solution kit (Thermo Fisher) according to manufacturer's instructions. Briefly, dry specimens were minced using sterile microscissors until they became a fine powder. Afterward, 5 to 10 mg of sample dry weight was used for DNA extraction. An enzymatic digestion with Proteinase K was performed until a transparent sample solution was achieved. A BioTek Synergy HT microplate reader (BioTek Instruments, Winooski, VT, USA) was used to read the fluorescence intensity at 485/20 nm (excitation) and 530/20 nm (emission). The results were converted into ng mg<sup>−1</sup> sample dry weight.

Representative sections of native and decellularized tissues were processed for histological evaluation. Samples were transferred to histological cassettes and fixed with 10% (v/v) formalin solution (Thermo Scientific, USA). After paraffin embedding, samples were sectioned with 3 μm thickness by a microtome (Leica Microtome, RM2125RT, Portugal). Sections were stained with standard hematoxylin and eosin (H&E) to assess the presence of cell nuclei and ECM. Additional Masson's Trichrome (MT) staining was performed to assess the distribution of collagen-rich connective tissue and the preservation of the ECM architecture.

**2.3.2. Total Sulfated Glycosaminoglycans (GAGs) Quantification.** The sulfated glycosaminoglycan (sGAG) content of the preserved dPSI and untreated samples of PSI (Native tissue) was quantified using the Blyscan assay kit (Biocolor, UK), according to the manufacturer's instructions. Briefly, sGAGs were extracted by using 20 to 50 mg of sample dry weighed, using a 0.1 mg/mL papain solution. The samples were incubated overnight at 65 °C under agitation (100 rpm, orbital shaker). After extraction, 100 μL aliquots of each

sample were mixed with Blyscan reagent and incubated at RT for 30 min, under agitation (100 rpm, orbital shaker). The resulting dye-sGAG complex was pelleted, and the dye was eluted using an anionic surfactant. Absorbance of the eluted dye was measured at ds using a BioTek Synergy HT microplate reader (BioTek Instruments, Winooski, VT, USA). The total sGAG content was determined based on a calibration curve generated from known sGAG concentrations provided by the manufacturer.

**2.3.3. Collagen Quantification.** The collagen content of the preserved dPSI and native PSI tissue (control) was quantified using the Sircol-2.0 Soluble Collagen Assay Kit (Biocolor, UK) and Sircol™—Insoluble Collagen Assay kit (Biocolor, UK) following the manufacturer's instructions. To quantify soluble collagen, the samples were digested and solubilized in a 0.1 mg/mL pepsin solution prepared in 0.5 M acetic acid. The extracts were then incubated with Sirius red dye under agitation. To quantify the insoluble collagen, samples were treated with 50 μL of Fragmentation Reagent per 1 mg of tissue or residue and incubated at 65 °C for 3 h. Following incubation, the samples were mixed with Sircol Dye Reagent, allowing the formation of a collagen-dye pellet. Unbound dye was removed with ice-cold Acid-Salt Wash Reagent. Finally, the collagen-bound dye was dissolved using 1.0 mL of Alkali Reagent, enabling further quantification. Soluble and insoluble collagen absorbance was measured at 556 nm using a BioTek Synergy HT microplate reader (BioTek Instruments, Winooski, VT, USA) and determined using standard calibration curves, generated using known concentrations of collagen provided by the manufacturer.

## 2.4. Morphological and Physicochemical Characterization

**2.4.1. Optical Microscopy.** SF-based textile samples were first analyzed by using an Axio Vert.A1 Zeiss microscope with a 10× objective lens coupled with an AxioCam MRC Zeiss camera and Zen software.

**2.4.2. Scanning Electron Microscopy (SEM).** The ultrastructure preservation of the dPSI, SF-based textile morphology, and hybrid dressing assembling properties were evaluated by SEM, using a Phenom Pro G6 Desktop instrument (Thermo Fisher Scientific, Waltham, MA, USA). First, the hydrated samples were fixed in 2.5% (v/v) glutaraldehyde solution overnight at 4 °C. Then, a series of ethanol concentrations (30%, 50%, 70%, 85%, 95%, and 100% (v/v)) were used for sample dehydration, twice at each concentration for 30 min, followed by hexamethyldisilazane (HMDS) and overnight drying. Prior to SEM analysis, all samples were sputtered with gold palladium (Au/Pd) coating to allow electron conductivity (Polaron Sputter Coater, Grelly, UK).

**2.4.3. Fourier Transform Infrared Spectroscopy (FTIR).** The chemical structure and conformation of samples were analyzed by FTIR spectroscopy under an attenuated total reflectance (ATR) accessory with a germanium crystal. The measurements were performed on a Perkin-Elmer 1600 series spectrometer (CA, USA) coupled with an IRPrestige-21 ATR module (Shimadzu, Kyoto, Japan). ATR-FTIR spectra were acquired directly from the intact dPSI matrices and assembled hybrid constructs without prior separation of their constituents. Spectra were collected between 4500 and 800 cm<sup>−1</sup>, with a resolution of 4 cm<sup>−1</sup> with 32 scans. The analysis was conducted on three independent samples, using air as background reference.

**2.4.4. Mechanical Assessment.** The mechanical behavior of samples was analyzed by performing a tensile test using the TA.XT. plus Texture Analyzer (Stable Micro Systems, Surrey, UK) and the software Exponent Stable Micro Systems. Samples were cut into dumbbell shapes according to American Society for Testing and Materials (ASTM) D412 Type C standards and subsequently rehydrated in PBS with 0.02% (v/v) sodium azide for 24 h at 37 °C, under agitation (100 rpm, orbital shaker). Prior to testing, the dimensions of the gauge section (length, width, and height) were measured using a digital caliper. Tensile tests were performed using a 1000 N load cell, with pretest, test, and post-test speeds of 1.0 mm/s and trigger force of 0.005 N. Stress-strain curves were generated from the obtained force-displacement data, and

Young's modulus, ultimate tensile strength, and failure strain were subsequently determined.

The SF-textile samples were measured with the double-fibers aligned in both vertical and horizontal direction. The preserved dPSI and untreated PSI (Native tissue) were measured in longitudinal and transverse direction of the tissue, considering its native alignment and orientation of the structural components. Hybrid dressings were measured by matching the longitudinal and transverse direction of the tissue with the double-fiber alignment in the SF-based textile. Thus, four different conditions were tested, i.e., SerCW longitudinal (SerCW\_long), SerCW transverse (SerCW\_trans), MuCW longitudinal (MuCW\_long), and MuCW transverse (MuCW\_trans).

## 2.5. Evaluation of the Fluid Interaction

**2.5.1. Swelling Ratio.** The swelling ratio was tested in PBS solution. The initial dry weight of freeze-dried samples ( $0.5 \times 0.5$  cm) was measured, followed by immersion in 1 mL PBS solution. The analysis was performed at 37 °C under agitation (100 rpm, orbital shaker), for a time period ranging from 30 min to 72 h. After each time-point, the excess liquid from samples was removed with filter paper and the wet weight obtained. The water uptake was calculated following the equation:

$$\text{Water uptake (\%)} = \frac{m_w - m_i}{m_i} \times 100$$

$m_w$  corresponds to the weight of samples after each time-point and  $m_i$  the weight of freeze-dried samples.

**2.5.2. Degradation Profile.** The stability of samples was evaluated by enzymatic and oxidative degradation test. Protease XIV, derived from *Streptomyces griseus* (3.5 U/mg), was prepared at 1 U/mL in combination with 0.1% (v/v)  $\text{H}_2\text{O}_2$  and 10 mg/mL bovine serum albumin (BSA) for the development of a simulated wound fluid (SWF) solution. Three different pHs (5.5, 7.4, and 8.5) were tested to mimic the different stages of wound healing.<sup>18</sup> The selected enzyme concentration was also based on previous degradation studies of SF-based materials, being one of the most effective proteolytic enzymes for degrading  $\beta$ -sheet-rich silk protein structures.<sup>19</sup> As control condition, samples were incubated in PBS solution. The degradation profile was monitored using the same samples throughout the experimental period. The initial mass of samples corresponded to wet weight measured after sample hydration in PBS solution for 3 h at 37 °C. Samples were then immersed in 1 mL of SWF solution at different pHs, and the analysis was conducted at 37 °C, under agitation (100 rpm, orbital shaker), for a time period ranging from 1 to 21 days. The SWF solutions were changed every 3 days. After each time-point, the excess liquid from samples was removed with filter paper and the wet weight measured. The weight loss was determined from the relative mass variation of the hydrated samples according to the equation:

$$\text{Weight loss (\%)} = \frac{m_i - m_f}{m_i} \times 100$$

$m_i$  and  $m_f$  correspond to the initial and final (after degradation) wet weight of samples.

**2.5.3. Water-Vapor Transmission Rate.** To assess the water-vapor transmission rate, freeze-dried samples ( $d = 8$  mm) were secured on top of 2 mL glass bottles prefilled with deionized water and ensuring a 5 mm gap between the water surface and the sample. The initial weight of each sample system was determined and kept at 37 °C for 48 h. Weight measurements were taken at 24 and 48 h. The water vapor transmission rate was calculated using the following equation:

$$\text{Water vapor transmission rate} \left( \frac{\text{g}}{\text{m}^2 \times \text{h}} \right) = \frac{m_i - m_f}{A \times h}$$

$m_i$  and  $m_f$  correspond to the initial and final weight of after each time point.  $A$  and  $h$  correspond to the area of the samples and time in hour, respectively.

## 2.6. In Vitro Biological Assessment

**2.6.1. HDF Cell Culture, Expansion, and Seeding.** Human dermal fibroblasts (hDFs, Lonza, catalog #CC-2509) were selected as a representative cell type of dermal matrix and used to assess cell behavior in the hybrid dressings. Cells were expanded as monolayer in a standard Dulbecco's modified Eagle's medium (DMEM; Gibco DMEM, high glucose), supplemented with 10% (v/v) fetal bovine serum (FBS; BioWest, Nuaille, France) and 1% (v/v) penicillin–streptomycin solution (Lonza, Basel, Switzerland), at 37 °C and 5%  $\text{CO}_2$ . Cells were maintained until confluence, and the culture medium was renewed every 2–3 days.

Before cell seeding, the hybrid dressings (MuCW) and plain dPSI (control) were cut with  $5 \times 5$  mm and placed into 48-well suspension cell culture plates (Cellstar, Greiner Bio-One, Austria). Samples were hydrated in serum-free culture medium for at least 24 h and seeded with hDFs at a density of  $5 \times 10^4$  cells per 15  $\mu\text{L}$  cell suspension. The constructs were kept for 2 h under incubation and then 500  $\mu\text{L}$  of standard culture medium was added to each well. Samples were cultured under static culture conditions and collected after 3, 6 and 10 days. Culture medium was renewed every 2–3 days.

**2.6.2. Fluorescence Microscopy.** Cell-matrix interactions and organization within the matrices were observed by DAPI/Phalloidin fluorescence staining. After each time-point, constructs were fixed with 10% (v/v) paraformaldehyde solution overnight at 4 °C and stored in PBS solution until further use. Cells were permeabilized with (0.5% (v/v) Triton X-100) and rinsed three times with PBS. To block unspecific reactions, samples were incubated with 5% (v/v) FBS for 30 min at RT, followed by washing and the addition of the fluorescent markers. Flash Phalloidin™ Green 488, Biolegend (1:100), was added for 45 min, and DAPI solution, Biolegend (1:500), was added for 10 min, both prepared in PBS solution. Afterward, samples were washed twice with PBS solution and stored at 4 °C until observation under fluorescence microscopy in a laser confocal microscope (Leica TCS SP8; Leica, Wetzlar, Germany).

**2.6.3. SEM Analysis.** Cell adhesion and distribution into the matrices were observed by SEM. After each time-point, the constructs were washed with PBS solution and fixed overnight at 4 °C in a 2.5% (v/v) glutaraldehyde solution. Then, samples were dehydrated in a series of ethanol concentrations (30 to 100% (v/v)), followed by treatment with HMDS and left to dry overnight. Samples without seeded cells were used as control. Prior to the SEM observations, all constructs were sputter-coated with gold palladium (Au/Pd) (Section 2.4.2).

**2.6.4. Cell Metabolic Activity.** The metabolic activity of cells was quantified by performing a Resazurin assay. A stock resazurin solution (Resazurin sodium salt) was prepared at 0.1 mg/mL in PBS and sterilized using a 0.22  $\mu\text{m}$  filter (VWR, Radnor, PA, USA). After each time-point, a 10% (v/v) resazurin working solution was prepared in standard culture medium and added to samples in a 500  $\mu\text{L}$  vol %. The system was incubated in the dark for 3 h at 37 °C and 5%  $\text{CO}_2$  incubator. Supernatant fluorescence was obtained at an excitation wavelength of 560/25 nm and an emission wavelength of 590/35 nm using a BioTek Synergy HT microplate reader. Samples were carefully washed in PBS solution and kept in fresh culture medium until further analysis. Three samples of each group (MuCW\_long and dPSI) were tested at each time-point. Samples without seeded cells were used as blank control.

**2.6.5. Cell Proliferation.** Cell proliferation was assessed using a Proliferation ELISA, BrdU (colorimetric) assay (Roche, Basel, Switzerland), according to the manufacturer's instructions. Briefly, after the 2 h incubation in the cell seeding (Section 2.6.1), the 500  $\mu\text{L}$  standard culture medium was supplemented with a BrdU labeling solution (diluted 1000 $\times$  in complete cell culture medium – final concentration: 10  $\mu\text{M}$  BrdU). After each time-point, samples were fixed and DNA denatured by adding 200  $\mu\text{L}$ /well FixDenat solution, incubated for 1 h at RT. Afterward, samples were incubated in anti-BrdU-POD working solution (1:100 in antibody dilution solution) for 90 min at RT, binding to the BrdU incorporated in the newly formed cell DNA.

After washing in PBS, the reaction was carried out by adding 100  $\mu\text{L}$ /well substrate solution (ready-to-use), incubated at RT and protected from light for 30 min, and then stopped with 25  $\mu\text{L}$ /well of sulfuric acid (1 M,  $\text{H}_2\text{SO}_4$ ). Supernatant absorbance was measured at 450 nm in a BioTek Synergy HT microplate reader. Three samples of each group (MuCW and dPSI) were tested at each time-point. Samples without seeded cells were used as blank control.

## 2.7. Statistical Analysis

All numerical results are expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was conducted using GraphPad Prism 7.0 software (GraphPad Software, La Jolla, CA, USA). Initially, a Shapiro–Wilk normality test was performed to assess data normality. The results obtained from dsDNA quantification (Section 2.3.1), GAG quantification (Section 2.3.2), and collagen content (Section 2.3.3) were evaluated by means of a parametric unpaired *t* test. The results from mechanical analysis (Section 2.4.4) were analyzed by means of a non-parametric Kruskal–Wallis test followed by Dunn’s multiple-comparison test. For the *in vitro* quantification assays (Section 2.6.4 and Section 2.6.5), the differences between the experimental results were assessed using a non-parametric Kruskal–Wallis test followed by Dunn’s multiple-comparison test for comparisons involving more than two groups. For comparisons between two independent groups, a non-parametric Mann–Whitney test was used. The significance levels were set to  $^*p < 0.05$ ,  $^{**}p < 0.01$ ,  $^{***}p < 0.001$ ,  $^{****}p < 0.0001$ ,  $^{\&}p < 0.0001$ ;  $^{\$}p < 0.01$

## 3. RESULTS AND DISCUSSION

The healthy dermis is composed of multiple components, including proteoglycans, glycosaminoglycans, and collagen. However, when skin is severely burned, these vital components are destroyed.<sup>20</sup> In the field of burn wounds treatment, multi-layer hybrid dressings have been recognized for being capable of combining optimized moisture balance, improved protection, better fluid management, and faster tissue regeneration to accelerate healing.<sup>4</sup> The strategy presented in this study (Figure 1) integrates the structural and mechanical robustness of SF-based textiles;<sup>21</sup> the biocompatibility and bioactivity of emerging enzymatically crosslinked SS hydrogels;<sup>22</sup> and the native biochemical components, structural architecture, and bioactive cues of innovative preserved dPSI.<sup>7,20</sup> Together, these components are designed to provide cell-instructive signals that support cell–material interactions and cell proliferation.<sup>23</sup>

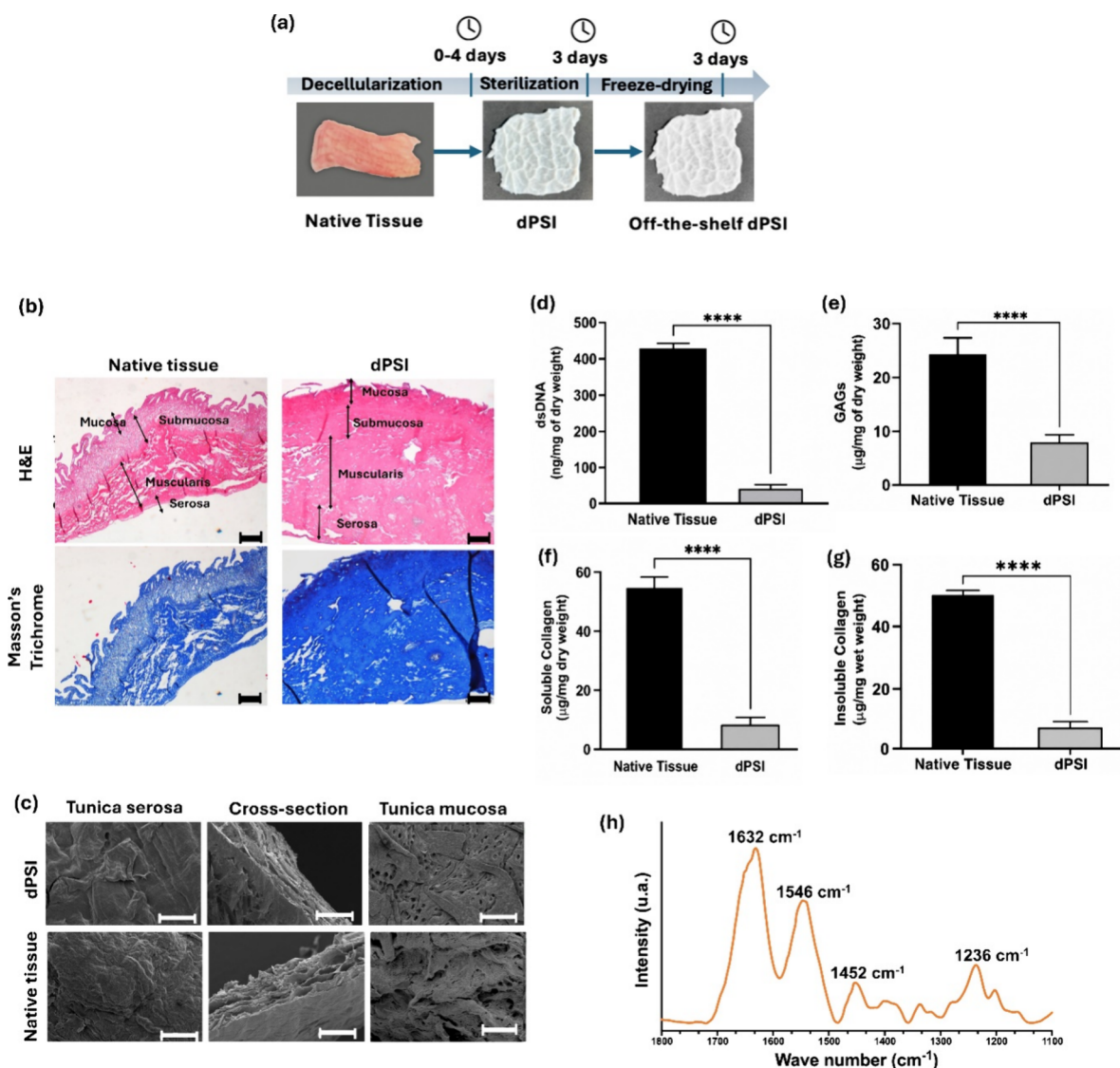
### 3.1. Decellularization Efficiency and ECM Characterization

To guarantee that the decellularization process was effective with minimal adverse effects on tissue integrity, a series of analyses were conducted to the dPSI (Figure 2) to be further integrated with the SS-based hydrogel and the SF-based textile for hybrid dressing construction.

After PSI decellularization, an off-the-shelf product was obtained (Figure 2a) as presented in the macroscopic images of the native tissue.

Hematoxylin-eosin (H&E) and Masson’s trichrome (MT) staining (Figure 2b) allowed to observe the cross-section of the tissue before and after decellularization. Native tissue exhibited well-defined tunica mucosa, submucosa, muscularis, and serosa with an intact structure and visible nuclei. Following decellularization, a marked reduction in nuclei was observed. H&E and MT staining demonstrated an overall collagen distribution throughout the different intestinal tunics, supporting the maintenance of the tissue architecture. Structural changes in the ECM were also evident, namely, a change in the compactness

and conformation of collagen fibers. This phenomenon may be attributed to the chemical decellularization process containing SDS, which has been reported to disrupt collagen fiber structure, especially when combined with extended decellularization periods. Such protocols may result in disorganization or damage to the collagen network and can also promote self-assembly or unintended physical crosslinking of residual collagen fibers.<sup>24</sup> Furthermore, an increase in tissue thickness was observed, mainly in the muscularis, which may be related to swelling effects induced during decellularization. The morphological properties of the dPSI analyzed by SEM (Figure 2c) reveal a smooth surface on both tunica serosa and mucosa of dPSI, compared to the rough native tissue tunics. In the mucosa, the villi and micropores typically exhibited in the inner intestinal wall become more perceptible post-decellularization. These topographical differences between the decellularized and native tissues are consistent with effective cell and debris removal, while preserving the overall tissue microarchitecture, as confirmed by H&E and MT staining (Figure 2b). Examining the cross-sections, it is possible to confirm that the different intestinal tunics remain preserved after decellularization, as well as the overall architecture of the tissue. The reduction of nuclei in the dPSI is corroborated by the dsDNA quantification (Figure 2d), which shows a significant decrease ( $^{****}p < 0.0001$ ) in dsDNA content, from  $\sim 400$  to  $\sim 40$  ng/mg dry weight after decellularization. This is below the threshold for effective decellularization ( $< 50$  ng/mg dry weight), confirming the success of the process.<sup>25</sup> GAG content also significantly decreased ( $^{****}p < 0.0001$ ) from  $\sim 24$  to  $\sim 8$   $\mu\text{g}/\text{mg}$  in dry weight after decellularization ( $\sim 33\%$  GAG retention) (Figure 2e). Similarly, soluble collagen decreased from  $\sim 54$  to  $\sim 8$   $\mu\text{g}/\text{mg}$  ( $\sim 15\%$  soluble collagen retention) (Figure 2f), while the insoluble collagen was reduced from  $\sim 49$  to  $\sim 7$   $\mu\text{g}/\text{mg}$  in wet weight ( $\sim 30\%$  insoluble collagen retention) (Figure 2g). Although the decellularization protocol effectively removed cellular material while preserving the overall tissue architecture (Figure 2c) and collagen distribution (Figure 2b), significant reductions in collagen and GAG content were observed. These results can be attributed to the use of harsh detergents (e.g., SDS) and an extended decellularization process, necessary to ensure the removal of the cellular content to acceptable decellularization levels.<sup>26</sup> Such losses are frequently reported for detergent-based decellularization protocols and reflect the inherent balance between efficient cell removal and preservation of ECM components.<sup>27</sup> Since collagen and GAGs contribute to tissue biomechanics, growth factor sequestration, and cell–matrix interactions, their quantitative reduction may influence the biological activity of the resulting scaffold. Some studies suggest that an effective decellularization process should preserve around 50%–80% of the native GAGs and soluble collagen, which are essential for cell attachment and bioactivity. Additionally, around 80%–90% of the native insoluble collagen are important for maintaining the biomechanical strength and structural integrity of the tissue.<sup>28</sup> However, this has not always been the case when trying to fall below 50 ng/mg of DNA per dry tissue. In a study by Chai et al.,<sup>24</sup> successful chemical decellularization of porcine small intestine submucosa (SIS) using SDS, alkali, and acid protocols also resulted in a significant loss of GAGs ( $> 80\%$ ) compared to fresh intestine. Despite this reduction, the ECM components and properties maintained after decellularization were essential for effective tissue remodeling after implantation in abdominal wall defects



**Figure 2.** Evaluation of PSI decellularization efficiency and impact on ECM components. (a) Schematic diagram illustrating the timeline for PSI decellularization and obtaining an off-the-shelf product with macroscopic images of the native tissue, dPSI, and off-the-shelf dPSI. (b) Representative H&E and MT images of the native tissue and dPSI cross-sections (scale bar: 200 μm). (c) SEM images dPSI (Scale bar: 300 μm). (d) dsDNA quantification of the native tissue and dPSI. Quantitative analysis of (e) sulfated GAGs, (f) soluble collagen, and (g) insoluble collagen at the native tissue and dPSI. Data are presented as the mean ± standard deviation (SD). Statistical analysis was performed using parametric unpaired t test (d–g). (h) ATR-FTIR of the dPSI.

of a rat model. These observations suggest that complete preservation of native ECM constituents may not be strictly required for a decellularized matrix to retain biologically relevant functionality. In fact, the biological performance of a decellularized scaffold is also determined by the total amount of retained ECM components, but also by residual structural proteins, preserved 3D architecture, topographical features, and remaining matrix-bound biochemical cues that collectively contribute for regulating cell adhesion and proliferation.<sup>11,25</sup> In our system, these retained ECM features, together with the bioactive properties of the SS-based hydrogel and the mechanical reinforcement provided by the SF-based textile, are expected to create a favorable microenvironment for cell–material interactions. Nevertheless, additional proteomic characterization of retained ECM-associated proteins and bioactive factors, such as fibronectin, laminin, growth factors, and cytokines, would

provide a more comprehensive understanding of the biological functions preserved following decellularization and their potential contribution to cell behavior.<sup>27</sup> In addition, a more detailed assessment of the type of collagen fibrillar organization, and molecular integrity would improve our understanding of how decellularization-induced structural alterations influence the mechanical and biological performance of the resulting scaffolds.

The complementary chemical analysis performed by ATR-FTIR confirms the retention of structural collagen and sulfated GAGs at the dPSI (Figure 2h). The obtained spectrum corresponds to the whole decellularized tissue rather than to isolated ECM components. Therefore, the detected absorption bands reflect the combined contribution of the ECM components retained after decellularization. Two strong absorbance peaks were identified at 1632 cm<sup>-1</sup> (amide-I) and 1546 cm<sup>-1</sup> (amide-II), corresponding to C=O stretching vibrations

characteristic of collagen in the amide-I band and N–H bending combined with C–N stretching vibrations in the amide-II band.<sup>29</sup> A smaller peak can also be identified at  $1452\text{ cm}^{-1}$ , typically assigned to  $\text{CH}_2$  bending vibrations of all types of collagens.<sup>30</sup> This peak is an indicator of the structural collagen within the highly crosslinked matrix.<sup>31</sup> As for the peak identified at  $1236\text{ cm}^{-1}$ , this can be relative to amide-III band (C–N stretching and N–H bending), which is also attributed to collagen. Nevertheless, the  $\text{S=O}$  asymmetric stretching of sulfate ( $-\text{SO}_3^-$ ) groups in sulfated GAGs, e.g., chondroitin sulfate, heparan sulfate, and dermatan sulfate, typically appear between  $1250$  and  $1220\text{ cm}^{-1}$ , suggesting that this peak can also be attributed to sulfated GAGs.<sup>32</sup> Considering that both GAGs and collagen are present in the dPSI (Figure 2d–f), and were not physically separated prior analysis, this peak can represent an overlapping of signals from both ECM components.

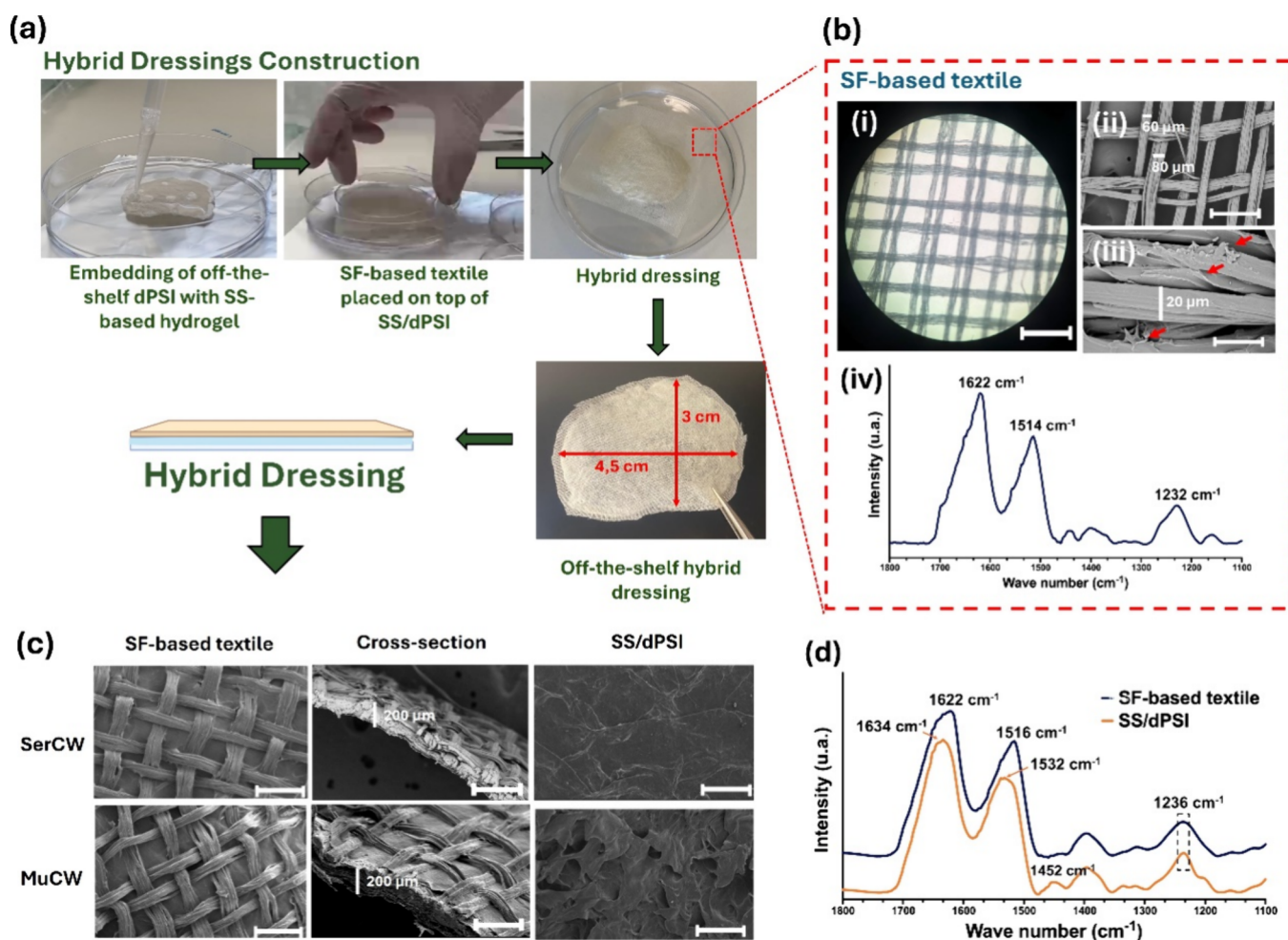
### 3.2. Morphology and Physicochemical Characterization

Figure 3a presents the hybrid dressings construction and characterization, which resulted in a multilayer off-the-shelf product.

Morphological analysis of the individual SF-based textile (Figure 3bi–iii) shows homogeneous plain-weave architecture composed of uniformly distributed fibers, indicative of a

controlled and reproducible manufacturing process. Optical microscopy and SEM observations showed yarn diameters ranging from  $60$  to  $80\text{ }\mu\text{m}$ , while individual SF filaments exhibited diameters of approximately  $20\text{ }\mu\text{m}$ . The surface appears relatively smooth with minor irregularities and slight fiber roughness, which may be attributed to the weaving process and degumming treatment. Complementary chemical analysis of the SF-based textile performed by ATR-FTIR (Figure 3biv) shows two strong absorbance peaks at  $1622$  and  $1514\text{ cm}^{-1}$  corresponding to the amide-I band (C=O stretching from the backbone) and amide-II band (N–H bending and C–H stretching), which can be attributed to the  $\beta$ -sheet crystalline conformation of SF.<sup>21,33</sup> The additional absorbance peak at  $1232\text{ cm}^{-1}$ , relative to the amide-III band (C–N stretching and N–H bending), is also associated to the high crystalline content ( $\beta$ -sheet conformation) on SF.<sup>34</sup>

The morphological analysis of the constructed hybrid dressings (Figure 3c) reveals a well-integrated multilayered structure. The ATR-FTIR spectra obtained for the hybrid dressings (Figure 3d) correspond to the assembled multilayered dressings reflecting the combined chemical contributions of the upper SF-based textile layer and the bottom SS/dPSI layer. Considering that all components are predominantly protein-based materials,



**Figure 3.** Ultrastructure and physicochemical composition of the hybrid dressings. (a) Representative photographs of the hybrids construction, indicating the dimensions of a representative off-the-shelf hybrid dressing. (b) SF-based textile characterization by (i) optical microscopy, (ii, iii) SEM and (iv) ATR-FTIR. Hybrid dressings characterization by (c) SEM and (d) ATR-FTIR. Scale bar:  $300\text{ }\mu\text{m}$ , except for the high-magnification image in panel (biii) ( $30\text{ }\mu\text{m}$ ). Red arrows indicate fiber surface roughness.

the overlapping of absorption bands is expected particularly within the amide-I, II, and III regions. The upper SF-based textile layer maintained the chemical signature of the plain SF-based textile (Figure 3biv), showing the typical amide-I ( $1622\text{ cm}^{-1}$ ), amide-II ( $1516\text{ cm}^{-1}$ ), and amide-III ( $1232\text{ cm}^{-1}$ ) peaks indicative of  $\beta$ -sheet conformation. The embedding of the SS-based hydrogel within the dPSI (SS/dPSI) is evidenced by the smoother decellularized surfaces and reduced microporosity, especially in SerCW, as compared to the plain dPSI tunics (Figure 2c). Similarly, the ATR-FTIR analysis of the underlying SS/dPSI layer confirms the integrity and integration of these components by the identification of the typical amide-I ( $1634\text{ cm}^{-1}$ ), amide-II ( $1532\text{ cm}^{-1}$ ), and amide-III ( $1236\text{ cm}^{-1}$ ) peaks, which can be simultaneously assigned to collagen ( $1630\text{--}1660\text{ cm}^{-1}$  for amide-I and  $1540\text{--}1550\text{ cm}^{-1}$  for amide-II),<sup>29</sup> GAGs ( $1250\text{--}1220\text{ cm}^{-1}$  for amide-III),<sup>35</sup> and to the secondary structure on SS with less  $\beta$ -sheet content compared to SF-based textile ( $1644\text{--}1649\text{ cm}^{-1}$  for amide-I,  $1520\text{--}1560\text{ cm}^{-1}$  for amide-II, and  $1233\text{--}1244\text{ cm}^{-1}$  for amide-III).<sup>7,36</sup> The small peak detected at  $1452\text{ cm}^{-1}$  also confirms the presence of collagen in the SS/dPSI layer.

### 3.3. Mechanical Assessment

The mechanical performance of burn wound dressings is extremely important for ensuring wound protection (covering joint regions) and stability during dynamic and exudative healing processes.<sup>37</sup> The optimal burn wound dressing profile must prevent tearing and avoid secondary trauma during movements.<sup>38</sup>

The mechanical behavior of SF-based textiles is presented in Figure 4. The representative stress–strain curve (Figure 4ai) shows that dry samples exhibit a significantly higher ( $****p < 0.0001$ ) Young's modulus (vertical:  $21.38 \pm 5.49\text{ MPa}$ , horizontal:  $23.22 \pm 2.45\text{ MPa}$ ), as compared to the same samples in hydrated state (vertical:  $7.92 \pm 0.68\text{ MPa}$ , horizontal:  $6.39 \pm 0.78\text{ MPa}$ ) (Figure 4aaii). This tendency is also observed for the ultimate tensile strength of samples, reaching a maximum of  $269.18 \pm 57.20\text{ MPa}$  in dry samples analyzed with the double-fibers aligned in vertical direction (Figure 4aiii). These results are consistent with previous studies,<sup>39</sup> which report a softening of the textile's properties upon hydration due to the disruption of the hydrogen bonds in the amorphous regions between  $\beta$ -sheets by water. The proposed SF-based textiles exhibit higher stiffness, strength, and fracture resistance when dried, but gain elongation and flexibility when hydrated.<sup>40</sup> When the double-fibers in the textile matrix are aligned with the applied force during mechanical testing, they exhibit higher resistance both in dry and hydrated states (Figure 4aiv). This anisotropic behavior is observed regardless of the hydration state and correlates well with the properties of native skin, which present higher mechanical properties along with the Langer lines.<sup>41</sup> Moreover, it can be attributed to its woven architecture, in which the orientation and density of the double-fiber are not equivalent in all directions. When the applied tensile load is aligned with the predominant fiber direction, stress is transferred more efficiently through the textile network, resulting in higher stiffness and tensile strength. Conversely, loading in the perpendicular direction promotes fiber reorientation and greater deformation before efficient load transfer is established.

All combined, hydration capacity and the adequate mechanical properties and anisotropic behavior of the structure are important requirements for textiles in applications where both

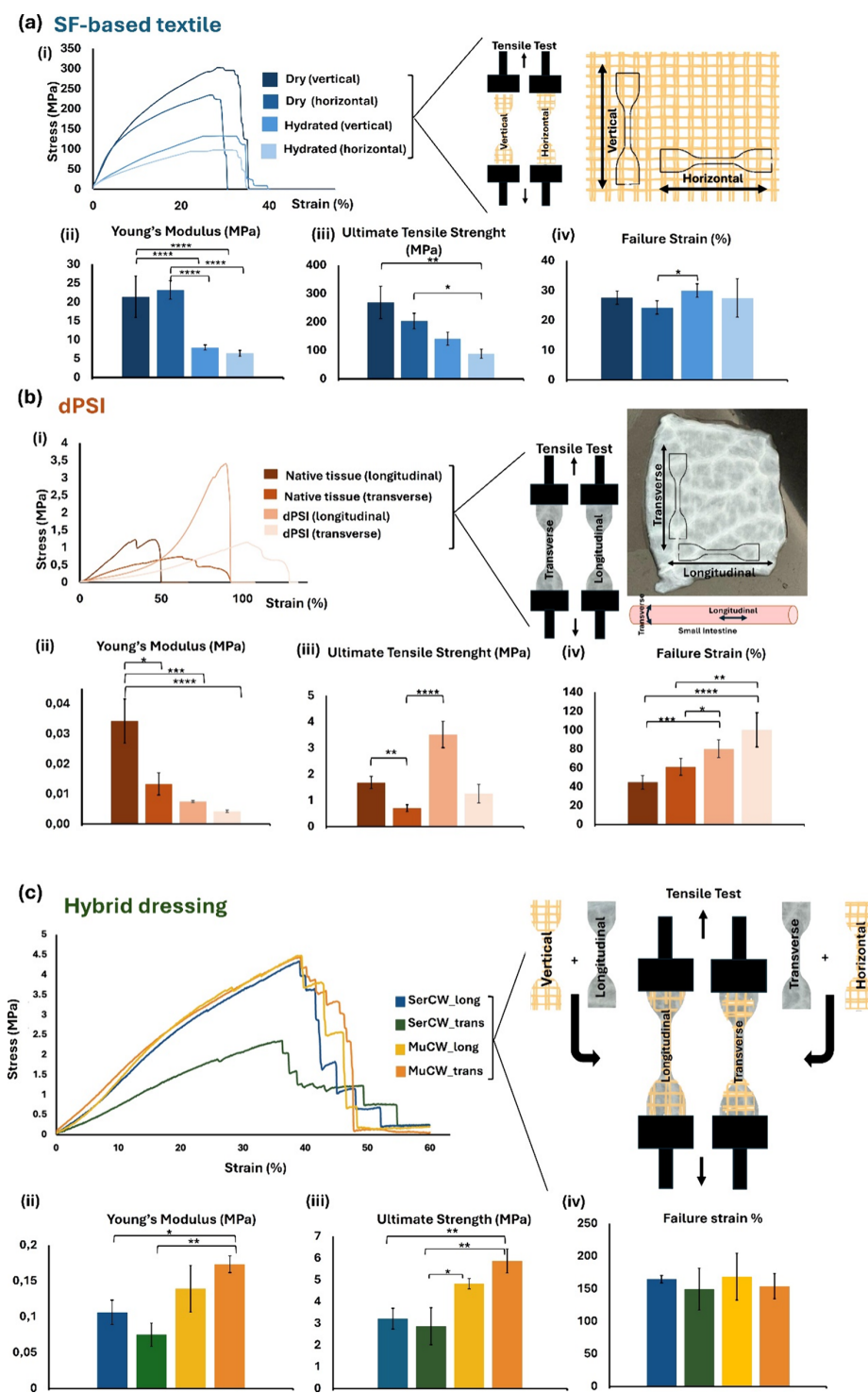
resistance and flexibility are necessary and different according to the direction, such as in wound dressing.<sup>35</sup> According to literature, the dSIS presents a broad range of mechanical properties influenced by factors such as porcine's age and gender, small intestine segment analyzed, and tissue orientation (longitudinal or transversal).<sup>15</sup> Figure 4b confirms that the analyses performed in the different PSI tissue orientations (longitudinal and transversal) influenced significantly the tensile properties of the native tissue and dPSI.

The longitudinal direction tends to exhibit superior mechanical properties. This behavior is consistent with the native organization of the SI, where collagen fibers, smooth muscle bundles, and ECM components exhibit preferential orientation along the longitudinal and circumferential axes of the tissue. Consequently, mechanical loads applied parallel to these structural elements are more efficiently resistant than loads applied perpendicularly, resulting in the observed directional dependence of tensile properties.<sup>42</sup>

Moreover, from representative stress–strain curves, it is evident that the decellularization process also affects the tensile properties of the tissue (Figure 4bi). A significant decrease in Young's modulus ( $***p < 0.001$ ) is observed when compared the native tissue ( $0.0343 \pm 0.0073\text{ MPa}$ ) with the dPSI ( $0.0075 \pm 0.0003\text{ MPa}$ ) in longitudinal direction (Figure 4bii). The same pattern was observed for these conditions analyzed in transversal direction (native tissue:  $0.0134 \pm 0.0037\text{ MPa}$ ; dPSI:  $0.0043 \pm 0.0004\text{ MPa}$ ). The ultimate tensile strength is enhanced in the dPSI conditions (longitudinal:  $3.52 \pm 0.51\text{ MPa}$  and transversal:  $1.26 \pm 0.35\text{ MPa}$ ), as compared to the native tissue (longitudinal:  $1.69 \pm 0.23\text{ MPa}$  and transversal:  $0.70 \pm 0.13\text{ MPa}$ ), potentially due to modifications of the collagen structure induced by the decellularization process, which leads to increased collagen fiber alignments, densification, and physical crosslinking (Figure 4biii).<sup>43</sup> Failure strain is also higher post-decellularization ( $**p < 0.01$ ,  $***p < 0.001$ ), exhibiting greater flexibility in the transverse direction and higher resistance in the longitudinal direction, which reflects the anisotropic nature of the SI tissue (Figure 4biv).

The stress–strain curves observed in Figure 4ci represent the contributions of both SF-based textile and SS/dPSI layers to hybrid dressings tensile properties. The mechanical behavior of the hybrid constructs likely reflects the combined contribution of two anisotropic components: the woven SF-based textile and the dPSI. The SS-based hydrogel acts as an interfacial layer that promotes structural integration between both components, allowing mechanical loads to be distributed throughout the multilayer construct. As a result, the anisotropic characteristics of the individual layers are partially transferred to the final dressing, contributing to its directional mechanical response. Our primary goal is to compare the differences in hybrid mechanical performance based on the different tunics of the dPSI (serosa and mucosa) in contact with the SF-based textile. MuCW conditions present significantly higher ( $**p < 0.01$ ) Young's modulus (MuCW\_long:  $0.14 \pm 0.03\text{ MPa}$ , MuCW\_trans:  $0.17 \pm 0.01\text{ MPa}$ ) and ultimate tensile strength (MuCW\_long:  $4.82 \pm 0.24\text{ MPa}$ , MuCW\_trans:  $5.86 \pm 0.55\text{ MPa}$ ), as compared to the Young's modulus (SerCW\_long:  $0.11 \pm 0.02\text{ MPa}$ , SerCW\_trans:  $0.07 \pm 0.02\text{ MPa}$ ) and ultimate tensile strength (SerCW\_long:  $3.21 \pm 0.47\text{ MPa}$ , SerCW\_trans:  $2.87 \pm 0.85\text{ MPa}$ ) of SerCW conditions.

One possible explanation for the superior tensile performance of MuCW is the orientation of the dPSI within the



**Figure 4.** Mechanical assessment of the hybrid dressings. (a) SF-based textile characterization in dry and hydrated conditions, both with the double-fiber aligned in vertical and horizontal direction: (i) representative stress/strain curves, (ii) Young's modulus (MPa), (iii) ultimate tensile strength (MPa) and failure strain (%). (b) dPSI characterization using native tissue as control and tests performed with tissues in longitudinal and transverse direction: (i) representative stress/strain curves, (ii) Young's modulus (MPa), and (iii) ultimate tensile strength (MPa) and failure strain (%). (c) Hybrid dressings (MuCW and SerCW) characterization in longitudinal and transverse direction of the tissue: (i) representative stress/strain curves, (ii) Young's modulus (MPa), and (iii) ultimate tensile strength (MPa) and failure strain (%). Data are presented as the mean  $\pm$  standard deviation (SD). Statistical analysis was performed using non-parametric Kruskal-Wallis test followed by Dunn's multiple-comparison test (a–c).

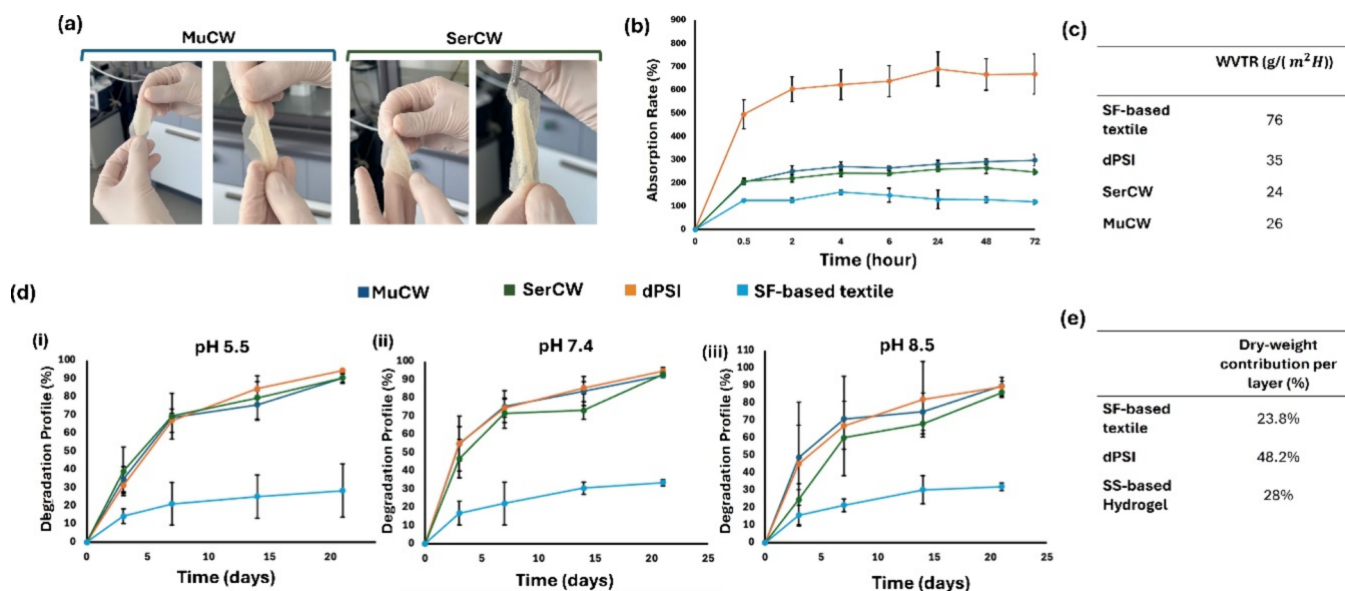
hybrid construct. In MuCW, the SF-based textile is positioned in direct contact with the tunica serosa, a collagen-rich connective tissue layer that naturally contributes to the mechanical support and flexibility of the SI to maintain its position in the abdominal cavity.<sup>14</sup> This configuration could potentially provide a more mechanically favorable interface for stress transmission between the biological matrix, the SS-based hydrogel, and the reinforcing textile. In contrast, in SerCW, the textile is positioned in contact to the tunica mucosa, whose physiological role is related to absorption rather than mechanical support.<sup>15</sup> Previous studies have demonstrated that the physical characteristics of SIS are location- and orientation-dependent, reflecting intrinsic differences in tissue microarchitecture that influence its physical and mechanical behavior.<sup>44</sup> Therefore, the orientation of the dPSI within the hybrid construct, together with the distinct structural organization and physiological roles of the serosal and mucosal surfaces, may have contributed to the superior tensile properties observed for MuCW. Cross-sectional SEM images (Figure 3c) confirmed the integration of all hybrid layers, with no evidence of delamination. However, these observations were comparable for both configurations and do not allow determination of the degree of hydrogel infiltration, interfacial adhesion, or local stress transfer that may have contributed to the improved tensile response of MuCW. Since these parameters were not specifically evaluated, further structural and interfacial characterization will be required to determine their contribution to the mechanical performance of the hybrid construct. Previous studies have reported that the Young's modulus of hybrid wound dressings can be as low as approximately 26.66 kPa, with ultimate tensile strength values ranging around 113 kPa, as demonstrated by Khalili et al.<sup>45</sup> and Pant and Wairkar,<sup>46</sup> respectively. Although the mechanical properties obtained in the present study are higher than those obtained in the reported studies, they remain within the range expected for wound dressings and demonstrate the enhanced mechanical reinforcement provided by the SF-based textile component. The higher Young's modulus and ultimate tensile strength indicate that the hybrid dressings are appropriate to better support mechanical stresses during wound coverage, while maintaining structural integrity. Despite the increased strength, no significant differences were observed in the failure strain of the hybrid dressings, which ranged from 31% to 40% (Figure 4civ). This indicates that regardless of the contacting layer or testing direction, the hybrids exhibit comparable resistance to deformation before failure. Importantly, no evidence of layer separation or delamination was observed throughout testing (Figure S1), which demonstrates that the SF-based textile and the dPSI layers remained integrated by the hydrogel component throughout deformation. The obtained values for failure strain are higher than 30%, suggesting that the hybrids are deformable and flexible, being beneficial for burn wound applications in which dressings need to be adaptable to body movements and irregular wound surfaces.<sup>47</sup> Skin tissue has a strain value that goes between 30 and 100%, which indicates that the proposed hybrids can mimic skin tissue mechanical properties to some extent.<sup>48</sup>

### 3.4. Materials' Stability in Contact with Fluids

Dressings designed for direct contact with wounds require not only mechanical stability but also controlled biodegradability to maintain integrity during the different healing stages, in which pH variations may significantly accelerate or slow down

the degradation of susceptible polymers. Thus, hybrid dressings must be formulated with a degradation kinetic that accommodates dynamic wound pH changes, ensuring that they will remain functional and stable during early acidic stages and avoid premature degradation in late alkaline environments.<sup>49</sup> Moreover, the interlayer connectivity must be maintained to prevent delamination under swelling or degradation and sustain fluid management and barrier function for effective healing outcomes.<sup>50</sup> In this regard, the proposed SerCW and MuCW constructs demonstrated excellent performance, maintaining their structural cohesion after 21 days of immersion in PBS solution, simulating physiological conditions (pH 7.4, 37 °C). Notably, both dressings resisted layer detachment during repetitive stretch-shrink cycles to simulate body mechanical movements (Figure 5a and Video S1). The amino-, hydroxyl-, and carboxyl-rich functional groups of sericin may have contributed to hydrogen-bonding and electrostatic interactions with both the dPSI matrix and the SF-based textile, contributing to the adhesive and cohesive properties of the hybrid construct.

The swelling properties of the hybrid dressings are important parameters that imply the ability of fluid absorption in the wound-healing process. These are represented in Figure 5b, where the SF-based textile exhibits the lower absorption rate, reaching a maximum of 160% at the fourth hour. Although this swelling capacity is lower compared to dPSI, it is consistent with values reported in the literature, which typically are around 170%.<sup>51</sup> Conversely, the dPSI matrix demonstrates the highest absorption rate, peaking at 24 h and then stabilizing at approximately 667%. This can be attributed to the preserved content of hydrophilic GAGs and proteoglycans and to the open porous collagen network that enables matrix expansion under physiological hydration. The hybrid dressings achieved an absorption rate of 271%, attributed to the interaction between the less absorbent SF-based textile with the more wettable dPSI matrix. The integration of the SS-based hydrogel within the dPSI can form interpenetration networks reducing ECM porosity and its ability to expand by stabilizing the overall ultrastructure. It has been reported that dressings that absorb ~300% of its weight in exudate can be suitable for partial-thickness burns applications, in which a moderate amount of exudate is tendentially produced both in the emergent and acute phases of the infection.<sup>52</sup> The water-vapor permeability of a wound dressing plays an important role in preventing dehydration due to evaporative water loss.<sup>39</sup> Adding to these results, it was also evaluated the water-vapor transmission rate (WVTR) of the dressings, presented in Figure 5c. Second degree and third degree partial-thickness burn wounds have high values of WVTR at 178 g/(m<sup>2</sup> × h) and 143.2 g/(m<sup>2</sup> × h), respectively, comparing to healthy skin 8.5 g/(m<sup>2</sup> × h), leading to a slow regeneration.<sup>39,53</sup> SF-based textile, as expected, shows the highest WVTR of 76 g/(m<sup>2</sup> × h) due to the high porosity of the textile structure (Figure 3bii). The dPSI shows a lower WVTR of 35 g/(m<sup>2</sup> × h), explained by the matrix density, whereas SerCW and MuCW dressings present the lowest WVTR of 24 g/(m<sup>2</sup> × h) and 26 g/(m<sup>2</sup> × h), respectively, related to the integration of the SS-based hydrogel connecting the SF-based textile and the dPSI that increases the structural density of the hybrids. These results are similar with those obtained for other hybrid dressings proposed for wound healing, presenting WVTRs ranging from 22 to 130 g/(m<sup>2</sup> × h).<sup>54</sup> Minsart et al. reported moisture vapor transmission rate (MVTR) values ranging from ~40 g/(m<sup>2</sup> × 24 h) for DuoDERM ET to



**Figure 5.** Hybrid dressings stability. (a) Representative photographs and video in supplementary data showing the structural stability and integrity of MuCW and SerCW hybrid dressings after 21 days in PBS solution, at 37 °C under agitation (100 rpm, orbital shaker) and subjected to repetitive stretch-shrink cycles. (b) Swelling ratio of the hybrid dressings in PBS solution for a period of 21 days, (37 °C, 100 rpm). Data are presented as the mean  $\pm$  standard deviation (SD). (c) Water vapor transmission rate of the individual scaffolds and hybrid dressings. (d) Degradation profile of the hybrid dressings in the SWF solution at (i) pH 5.5, (ii) pH 7.4, and (iii) pH 8.5 for a period of 21 days (37 °C, 100 rpm). Data are presented as the mean  $\pm$  standard deviation (SD). (e) Contribution of each individual layer in the hybrid dressing dry weight.

930 g/(m<sup>2</sup>  $\times$  24 h) for Carbonet, with intermediate values for Hydrosorb ( $\sim$ 280 g/(m<sup>2</sup>  $\times$  24 h)), Mepilex Ag ( $\sim$ 510 g/(m<sup>2</sup>  $\times$  24 h)), and Mepilex ( $\sim$ 610 g/(m<sup>2</sup>  $\times$  24 h)).<sup>55</sup> Notably, the WVTR values of the developed hybrid dressings fall within a similar range to those of clinically used foam dressings such as Mepilex<sup>®</sup> and Mepilex Ag. This suggests that the proposed hybrid dressings can support a moist wound environment while still enabling controlled water-vapor transmission. These values suggest that the dressings may contribute to moisture retention while allowing controlled water-vapor exchange, which is considered beneficial for wound management. Although the WVTR values of the hybrid dressings fall within the range reported for commercially available wound dressings and hybrid dressings,<sup>56</sup> they are lower than those reported for partial-thickness burn wounds. However, extremely high WVTR values may promote dehydration and desiccation of the wound bed, whereas extremely low WVTR values may lead to exudate accumulation and peri-wound maceration. Therefore, achieving a balance between moisture retention and water-vapor exchange is considered critical for maintaining a favorable wound-healing environment.<sup>56</sup>

The degradation profile was evaluated in SWF solution containing protease XIV (Figure 5d), a broad-spectrum protease widely used as a standardized enzymatic model to assess the degradation of silk-based biomaterials.<sup>33,57</sup> Although protease XIV is not naturally present in the wound environment, its broad proteolytic activity enables accelerating the degradation of protein-based biomaterials, making it one of the most widely adopted enzymes for *in vitro* degradation studies. The BSA was included in the SWF to mimic the protein-rich composition of wound exudate, and H<sub>2</sub>O<sub>2</sub> was introduced as an oxidative component naturally produced in the wound environment and during the healing process.<sup>18,58</sup> To simulate the different pH levels associated with each phase of wound healing, pH 5.5 was used to represent the inflammation phase, pH 7.4

for the proliferation phase, and pH 8.5 for the maturation phase. Although this degradation model provides a standardized and physiologically relevant approach for comparing the degradation behavior of the produced scaffolds, it does not fully reproduce the complexity of the wound environment, where ECM degradation is regulated by multiple proteases, including metalloproteinases (MMPs), together with inflammatory and resident cells and other non-enzymatic factors. Therefore, the objective of this assay was not to replicate the *in vivo* degradation profile but provide a controlled and accelerated degradation model capable of confirming scaffold susceptibility to enzymatic degradation under standardized conditions. The results indicate that the degradation profile of both hybrid dressings and individual components was similar across the evaluated pH conditions. While previous studies have reported that an acidic environment (pH 5 and 6) tend to slow degradation by suppressing protease (e.g., MMPs) activity, whereas neutral (pH 7 and 8) to alkaline (pH 8–10) conditions may promote higher proteolytic activity and accelerate the degradation process;<sup>59,60</sup> only modest differences were observed among our tested conditions. These observations suggest that the developed dressings undergo gradual degradation while maintaining structural stability under wound-relevant conditions.<sup>61</sup>

The SF-based textile samples achieve a degradation rate between 30% and 40%, which is lower compared to the remaining tested conditions. This may be attributed to the SF secondary structure (silk-II) in its native state, as identified by ATR-FTIR (Figure 3biv), which confers a higher resistance to degradation.<sup>40</sup> This stability may also be attributed to the repetitive hydrophobic domains (GAGA) present in the SF heavy chains, which are resistant to degradation.<sup>62</sup> It is well known that the native fibrous SF exhibits slow biodegradability, taking months to even years to degrade.<sup>63</sup> In the context of wound healing, this can promote the initial mechanical support and

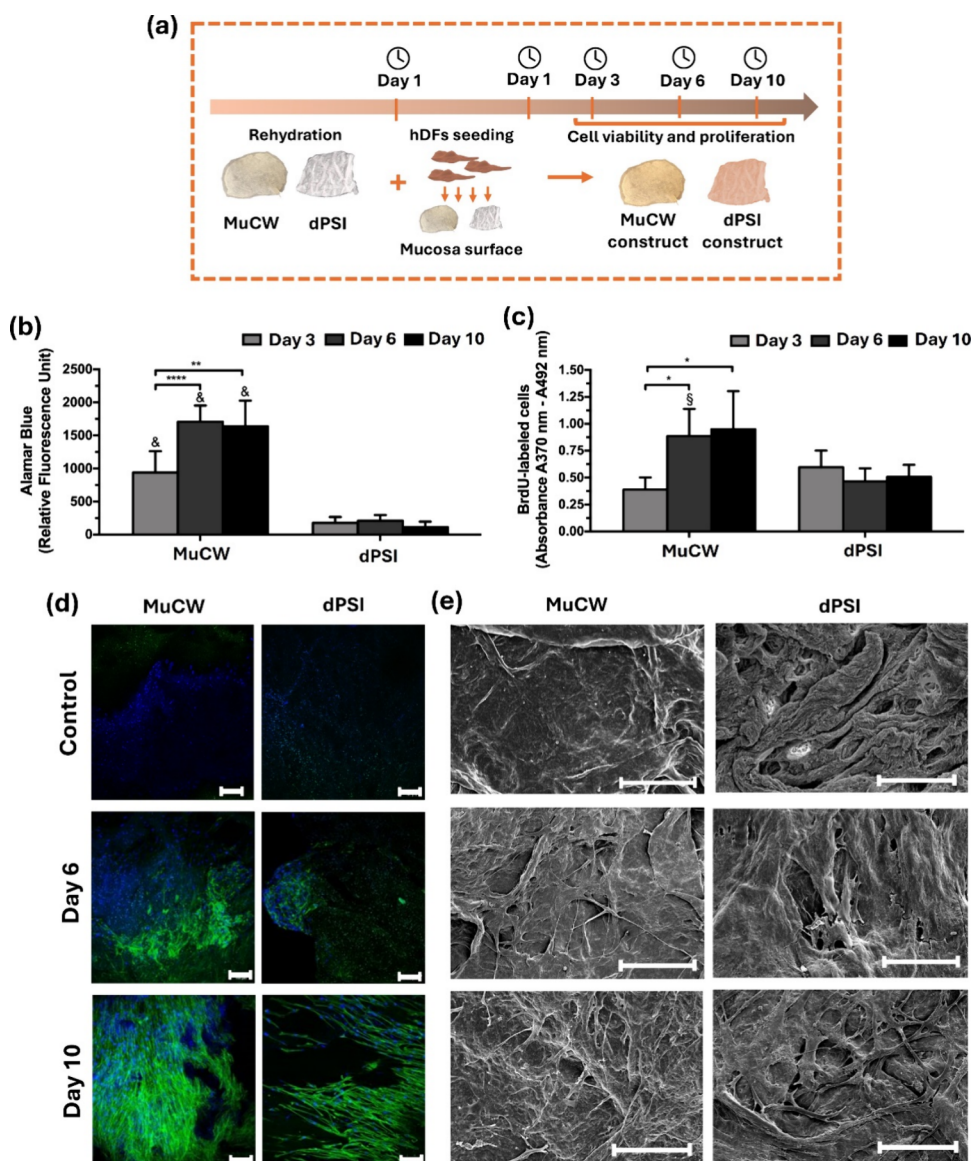
stability to the dressings and reduce the risk of infection while supporting a moist wound environment.<sup>62</sup> As expected from previous studies on native decellularized scaffolds, the dPSI matrix presents approximately a 90% degradation by the end of the 21 days. This degradation rate aligns with these reports that enzyme-sensitive dECMs derived from softer tissue sources undergo proteolytic degradation in physiological or enzyme-rich environments. In the case of the hybrid dressings, the integration of the dPSI matrix (covering both serosa and mucosa sides) with the SS-based hydrogel and SF-based textile did not significantly alter the overall degradation profile over time, with all hybrids maintaining a final degradation of approximately 90%. Considering that each layer has a distinct degradation rate and contributes differently to the hybrid dry weight (Figure 5e)—48.2% dPSI, 28.0% SS-based hydrogel, and 23.8% SF-based textile—the dPSI, as the predominant fraction, plays the most significant role in dictating the overall degradation kinetics of the dressing. Although SS-based hydrogel is more prone to enzymatic degradation due to its hydrophilic and globular conformation as compared with SF protein,<sup>23</sup> it was able to maintain its integrity and sustain both layers together during the degradation process.

### 3.5. *In Vitro* Assessment of Cell Metabolic Activity, Proliferation, and Morphology

HDFs were direct seeded on the tunica mucosa of MuCW and dPSI and cultured up to 10 days for evaluating the *in vitro* cell morphology, metabolic activity, and proliferation (Figure 6a). The mucosa was selected for direct contact with cells, due to the presence of its native villi maintained even after hybrid construction, which are expected to enhance fibroblast activity by increasing the surface area available for cell attachment, spreading, and proliferation (Figure 3c). These are critical processes for new ECM deposition and tissue remodeling during burn wound healing.<sup>30</sup> The superior tensile strength and elasticity obtained for MuCW (Figure 4c) also make this hybrid dressing desirable for testing in contact with the hDFs as it is expected that they stimulate key fibroblast functions including adhesion, proliferation, and cytoskeleton organization. A mechanically stable and robust scaffold can “instruct” these cells to enhance focal adhesion, promoting organized cellular infiltration, limiting contraction, and supporting long-term regeneration of dermal tissue.<sup>64</sup> Although decellularization resulted in a significant reduction of GAGs and collagen content (Figure 2e–g), the preserved tissue microarchitecture and topography (Figure 2b,c), together with the enhanced mechanical performance of the selected MuCW (Figure 4c) and the incorporation of the SS-based hydrogel, were expected to provide complementary structural and bioactive cues capable of supporting hDF adhesion and proliferation. In addition, the residual ECM constituents may still provide biologically relevant cues that contribute to cell–material interactions. Alamar Blue assay shows a significant increase of cell metabolic activity on MuCW from day 3 to day 7 (\*\*\*\* $p < 0.0001$ ) and from day 7 to day 10 (\*\*\* $p < 0.001$ ), suggesting that the hybrid dressing provided a favorable microenvironment for fibroblast activity, likely associated with the combined contribution of preserved ECM architecture, the bioactive components provided by the SS-based hydrogel, and the mechanical properties of the hybrid construct (Figure 6b).<sup>11</sup> The metabolic activity of hDFs was significantly higher ( $p < 0.0001$ ) on MuCW compared to the dPSI in all tested culture periods, suggesting that the

superior mechanical properties achieved for MuCW (Figure 4c), together with the bioactive properties of SS, had a relevant impact on fibroblast performance.<sup>10</sup> The same pattern was obtained for BrdU-labeled cells indicative of cell proliferation (Figure 6c). A significant increase in cell proliferation on MuCW up to 10 days of culture ( $*p < 0.05$ ) is observed, as well as a significantly higher proliferation rate on MuCW ( $^{\$}p < 0.01$ ) after 6 days of culture compared to the dPSI at the same culture period. Previous studies have shown that dSIS-based scaffolds were able to support hDF activity and proliferation up to 14 days of culture,<sup>65</sup> which was enhanced when reinforced with nanofibers and additional bioactive compounds.<sup>66</sup> SS-based hydrogels have been recently proposed by our group for wound-healing applications, showing to provide the adequate environment for hDF adhesion, proliferation, and infiltration.<sup>7,8</sup> This is in good agreement with our results, in which the metabolic activity and cell proliferation are enhanced by the presence of the SS-based hydrogel integrating the dPSI layer of MuCW. Results from fluorescence analysis of cell nuclei and cytoskeleton (Figure 6d) confirm that MuCW constructs presented higher cell density as compared to the control dPSI. Cells presented a typical fibroblast-like morphology, as also observed from SEM micrographs (Figure 6d). Even after losing their relevant microporosity due to the integration of the SS-based hydrogel, the smooth surface of the hybrid bottom layer also allowed for a good cell adhesion and proliferation up to 10 days of culture, resulting in a dense cell layer and confirming the compatibility of the hybrid construct with dermal fibroblast adhesion and proliferation.

The role of dSIS in promoting wound healing has been highly investigated, and the beneficial effects of SIS-based materials have been associated with the preservation of ECM architecture, residual bioactive molecules, and tissue-specific structural properties capable of supporting dermal fibroblast adhesion, proliferation, and new ECM deposition.<sup>67</sup> In our study, this effect was further enhanced when the dPSI was integrated into the hybrid dressing combined with the SS-based hydrogel and the SF-based textile layer, suggesting that their combination created a multifunctional microenvironment capable of enhancing fibroblast response while contributing to the structural and mechanical performance of the hybrid construct. Each layer contributes with a different function and distinct bioactive properties, and their combination shows potential to improve wound healing. Although the decellularization process resulted in a significant reduction of collagen and GAGs, preservation of the overall tissue architecture and residual ECM constituents may still provide biologically relevant cues for cell–material interactions. However, the present results demonstrate only that the resulting hybrid construct supported fibroblast adhesion and proliferation, without establishing which retained ECM-associated proteins, matrix-bound bioactive factors, or structural features were primarily responsible for this response. Therefore, the reduction in ECM content should be considered a limitation of the current decellularization strategy, and future studies combining comprehensive proteomic characterization of the preserved matrisome with functional biological investigations in more physiologically relevant models will be required to determine how the degree of matrix preservation influences the biological performance of the hybrid construct. The effectiveness of a multilayer approach has been also observed in a previous work by our team,<sup>68</sup> where an electrospun silk fibroin mat was reinforced with decellularized bladder to improve



**Figure 6.** *In vitro* cell characterization. (a) Schematic illustration of the experimental setup proposed to evaluate hDF behavior on the hybrid construct exposing the mucosa layer (MuCW) and the native dPSI up to 10 days of culture. (b) Alamar blue assay analyzing the metabolic activity of cells and (c) BrdU labeling of cells quantifying cell proliferation. (d) DAPI/Phalloidin staining and (e) SEM micrographs showing cell morphology on seeded scaffolds over culture period and samples without cell seeding used as control. Scale bar 100  $\mu$ m. Data are presented as the mean  $\pm$  standard deviation (SD). Statistical analysis was performed using non-parametric Kruskal–Wallis test followed by Dunn’s multiple-comparison test for comparisons involving more than two groups and a non-parametric Mann–Whitney test to compare samples between independent groups (b, c).

tensile strength and suture retention, making them suitable for implantation. Interestingly, this system incorporated VEGF on the processed silk fibers, resulting in enhanced endothelial cell attachment and proliferation, toward better angiogenesis. This opens the possibility of using the hybrid constructs as carriers for bioactive molecules, which can be easily incorporated into the SS hydrogel to modulate cell–material interactions and biological performance.

Our results clearly show that the metabolic activity and cell proliferation of hDFs were significantly improved due to the integration of the SS-based hydrogel with the dPSI layer and the SF-based textile. The possibility to tune the thickness and mechanical performance of this multilayered structure is particularly attractive for the development of personalized systems adapted to dynamic body regions, such as joints, where

flexibility and durability are essential. This adaptability may also allow adjustment to wound contraction during healing and to skin differences associated with gender and ethnicity.

Taken together, the physicochemical, mechanical, and biological results of this study demonstrate that the incremental structural and functional benefits of silk-derived components incorporated into the dPSI matrix improved the biological characteristics of the hybrid matrix. Specifically, the SF-textile contributed to mechanical reinforcement, whereas the SS-based hydrogel enabled effective integration between the textile and biological matrix, resulting in a stable multilayer construct capable of maintaining its integrity under hydrated and mechanically dynamic conditions. These findings are particularly relevant when comparing with currently available SIS-derived wound matrices, such as OASIS Wound Matrix and OASIS Ultra

Tri-Layer Matrix, as well as other reported hybrid wound dressing strategies. While commercially available SIS-based products rely primarily on the biological activity of decellularized SIS, and many hybrid dressings combine biological and structural components through polymeric or hydrogel-based systems, the present strategy integrates a decellularized porcine small intestine containing all tissue tunics with a mechanically reinforcing SF-based textile and a bioactive SS-based hydrogel. This multifunctional architecture is designed to combine ECM-derived biological cues, mechanical reinforcement, and interface stabilization within a single construct. Although direct comparisons would require *in vivo* studies, the favorable swelling behavior, water vapor transmission properties, degradation profile, mechanical performance, and fibroblast compatibility observed in this study support the feasibility of this design for wound dressing applications.

#### 4. CONCLUSIONS

A multilayer hybrid dressing was developed for burn wound treatment, composed of a silk fibroin (SF)-based textile, an adhesive silk sericin (SS) hydrogel derived as a byproduct from fibroin extraction, and a decellularized porcine small intestine (dPSI) matrix obtained as an agri-food industry byproduct.

The combination of these biomaterials provides an optimal balance of mechanical strength, flexibility, and bioactivity, supporting cell adhesion, metabolic activity, and proliferation.

Mucosa-contacting wound (MuCW) outperforms serosa-contacting wound (SerCW) hybrids with respect to biomechanical performance, reflecting the intrinsic robustness of the mucosal tunic. Both hybrid conditions maintained structural cohesion after 21 days in PBS and shared similar degradation kinetics; however, MuCW displayed improved moisture retention and stability under physiological conditions. Biologically, MuCW supported human dermal fibroblast (hDF) metabolic activity and proliferation throughout all culture periods, attributed to the preserved mucosal villi that enhance cell attachment and to the synergistic interaction between the SS-based hydrogel and dPSI matrix.

Although further studies involving additional cell types and complementary *in vivo* studies are still required to further validate the performance of the hybrid dressing in clinically relevant wound-healing settings, the physicochemical stability, mechanical robustness, and favorable cellular responses observed in this study, particularly for MuCW, support its potential as a promising candidate for burn wound dressing applications. Moreover, the tunable architecture of this multilayered construct enables its adaptation to diverse mechanical demands, healing dynamics, and patient-specific skin characteristics, supporting its application as a personalized wound management platform. Importantly, the use of PSI and SS in its fabrication may contribute to resource valorization while supporting the development of biologically compatible hybrid wound dressings.

#### ■ ASSOCIATED CONTENT

##### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsabm.6c00846>.

Representative tensile testing demonstrating maintenance of structural integrity during mechanical loading (Figure S1) (DOC)

Structural stability of MuCW and SerCW hybrid dressings after 21 days in PBS (Video S1) (MP4)

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##### Notes

The authors declare that no other financial or non-financial competing interests exist.

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