

Research paper

From textile waste remediation to sustainable 3D cellular models and tissue regeneration

Silvia Buonvino^a, Leonardo Duranti^b, Elisabetta Di Bartolomeo^b, Sonia Melino^{c,d,*}

^a Departmental Faculty of Medicine, UniCamillus-Saint Camillus International University of Health and Medical Sciences, via di Sant'Alessandro 8, 00131-Rome, Italy

^b Department of Chemical Science and Technologies University of Rome Tor Vergata via della Ricerca Scientifica, 00133 Rome Italy

^c Department of Experimental Medicine, University of Rome Tor Vergata, via Montpellier, 00133 Rome Italy

^d NAST Center- University of Rome Tor Vergata, 00133 Rome, Italy

ARTICLE INFO

Keywords:

Textile waste
Circular economy
Silk fibroin
Tumors
Stem cells
Biomaterials
Tissue regeneration

ABSTRACT

Textile waste poses a serious environmental challenge, contributing significantly to landfill accumulation, water pollution, and resource depletion. Effective recycling and valorisation of these materials are critical to reducing environmental burdens and recovering high-value resources. Innovative approaches to textile waste disposal are required to ensure sustainability. New protocols were here developed for recovering polymers from textile waste, leading to the production of textile waste-derived plastic and textile silk fibroin (TSF). TSF was used to develop tunable hydrogels (TSFHy) for 3D hydrogel-based cell culture systems of triple-negative breast cancer cells and mesenchymal stem cells (MDA/cMSCTSF). 3D-cMSCTSF systems exhibited cell viability and proliferation comparable to that of 3D-cMSCSF systems, in which SF was extracted from cocoons. Moreover, the expression of muscle differentiation markers in cMSCTSF systems was observed after two weeks. Overall, we provide a sustainable strategy to transform textile waste into high-value bio-inspired products, offering environmental and economic benefits while advancing circular economy, biomedicine and future food production.

1. Introduction

Textile waste represents an inexpensive source of valuable bioproducts for the development of innovative sustainable biomaterials for biomedical applications, as well as a new research avenue for the reuse and renewal of waste products, thus helping to address the costly problem of waste disposal. Improved waste management could increase opportunities to exploit biowaste and reduce environmental impact, while also enhancing competitiveness through the production of new bioproducts at lower cost [1–3]. Developing sustainable processes to reuse textile waste as a source of basic materials (such as pigments and polymers) offers a promising strategy to reduce environmental impact and recover valuable resources [3,4].

Each year, >92 million tonnes of textile waste are generated worldwide, a staggering amount that reflects deep inefficiencies in current fashion and textile systems [5]. According to the Ellen MacArthur Foundation, this waste represents a lost economic value of approximately \$500 billion annually [6] largely due to the underuse of garments and the lack of effective recycling systems. The financial burden extends further: managing textile waste is also costly for

governments and municipalities. In Europe, disposing of textile waste can cost between €500 and €1000 per tonne, accounting for transportation, sorting, and treatment [7,8]. In the United States alone, around 11 million tonnes of textiles are sent to landfills annually, costing over \$3 billion in disposal [9].

Beyond financial costs, there are severe environmental impacts that translate into long-term economic risks. Textile production and waste contribute significantly to water pollution, mainly from the toxic chemicals used in dyes and finishes [10,11]. They also generate over 1.2 billion tonnes of CO₂ emissions annually and are linked to land degradation, particularly through resource-intensive crops such as cotton [12]. The majority of used clothing, approximately 87%, is either incinerated or sent to landfill, causing serious environmental pollution [13]. The United Nations has warned that if these issues remain unaddressed, they could lead to systemic economic losses in the trillions of dollars, especially as climate change and resource scarcity intensify. Despite the scale of the problem, recycling remains very limited: approximately 1% of materials used to make clothing are recycled into new garments [7]. However, there is substantial potential for improvement. A more circular economy in fashion could unlock over

* Corresponding author at: Department of Experimental Medicine, University of Rome Tor Vergata, via Montpellier 00133 Rome, Italy.

E-mail address: sonia.melino@uniroma2.it (S. Melino).

<https://doi.org/10.1016/j.rineng.2026.112173>

Received 9 October 2025; Received in revised form 17 July 2026; Accepted 23 July 2026

Available online 24 July 2026

2590-1230/© 2026 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

\$100 billion in savings annually, according to the Circular Fibres Initiative [14]. Consumer behaviour also plays a crucial role in addressing this challenge. In this context, the urgent need for new strategies to recycle raw materials from post-consumer textile waste is clear. A range of approaches, including mechanical, thermal, and chemical processes, can be employed to recycle discarded textiles [15–23]. Biotechnological methods have also been highlighted as highly valuable for the valorisation of textile waste [24–26]. Current management of textile waste enables the production of diverse high-value materials, including bioethanol, biogas, monomers, polymers, and biochemicals such as succinic acid and cellulase [27–31]. Additionally, textile waste can be repurposed for the fabrication of construction materials [32]. Recycling fibres into value-creating products such as bio-fuel, bioplastic, chemicals, carpets, enzymes, acids, synthetic fibres, and resins is now achievable [33,34]. A major gap in the field of textile waste recycling is the lack of strategies capable of converting dyed textile waste into functional biomaterials for biomedical applications, particularly for tissue regeneration, while also enabling dye recovery. Opening a new frontier in multidisciplinary sustainable research, we present a strategy based on the recovery of polymers and biopolymers from textile waste, with new protocols developed for the dye and polymers extraction, reuse, and revalorisation (see Graphical Abstract) of synthetic and natural polymers, such as silk fibroin (SF), for biomedical applications. SF has recently attracted considerable attention for the development of innovative protein hydrogels in biomedicine [35–37]. SF derived from textile waste (textile silk fibroin, TSF) was purified using an optimised protocol and employed for the production of hydrogels for 3D cell cultures. The recycling protocols developed here from post-consumer textiles could provide a sustainable strategy to recover high-value biopolymers and dyes, reducing the environmental and economic

impacts of the textile industry and lowering costs in biomedical research and therapy. In this context, this work contributes to filling this gap by demonstrating a reprocessing route to obtain regenerated fibroin-based materials that support stem cell growth, viability, and differentiation, thereby highlighting their functional potential in biomedicine.

2. Results

2.1. Textile waste for TPLAST production

Pre-treatment of textile waste with mechanical, chemical, or thermal methods is generally used with the aim to eliminate contaminants such as dyes and finishes while also altering the fibre structure. This step is essential for the efficient recovery of valuable fibres and for converting textile waste into high-quality, value-added products. Fig. 1 illustrates the protocol used for the production of textile waste-derived plastic (TPLAST) where polyester textile waste was pre-treated with 50% trichloroacetic acid (TCA) solution (w/v), dimethyl sulfoxide (DMSO), or hexane at room temperature for 1 h. Only treatment with 50% TCA resulted in the extraction of a blue dye, as shown in Fig. 1. Neither hexane nor DMSO led to dye release, even after several days of incubation. The UV–vis spectrum of the TCA extract shows (Figs. 1 and S1A) absorption bands at 610 nm and 570 nm, with a shoulder at 520 nm, which are consistent with the presence of indigo and a complex disperse azo dye [38,39], respectively. The TCA extract was then reused for fabric staining, demonstrating efficient recycling and reuse of this component. The reduction of the percentage of TCA in the extract determined a reduction of solubility of the hydrophobic molecules, such as pigments. Therefore, we used a dilution with dH_2O up-to 10% of TCA or even less to determine a precipitation of these molecules and an easy

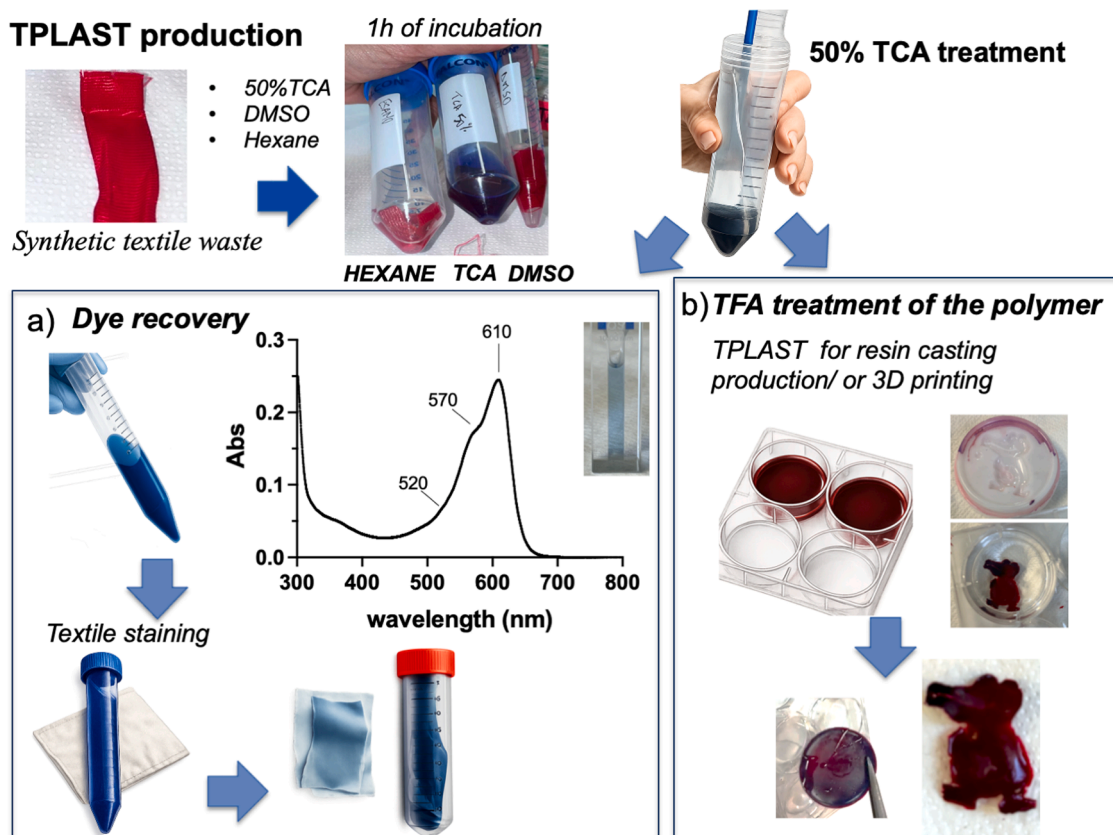


Fig. 1. Scheme of the experimental TPLAST production from synthetic textile waste. The synthetic textile waste was treated for 1 h with a solution of TCA 50% (w/v), DMSO or Hexane. Only the TCA treatment led to the dye recovery. a) Dye recovery, UV–vis spectrum of the TCA extract (1:19 dilution) in 50% TCA is shown and textile staining using the recovered dye; b) TFA treatment of the textile polymer. After drying the textile polymer at room temperature, the treatment with TFA was performed for 30 min (b). Then, the textile waste was completely dissolved and the mixture cast in the molds to obtain the TPLAST.

elimination of the TCA solution (as supernatant) from the pigments by centrifugation (5 min at 12,000 g) or sedimentation overnight at room temperature (see Figure S1B). The pigments present in the pellet can be freeze-dried or directly resuspended in organic solvents (such as ethanol or others solvents). We used this approach to recover the dye and analyse it by UV-vis spectroscopy in ethanol, as shown in the Figure S1. Furthermore, the TCA solution (as supernatant) can be freeze-dried to obtain TCA in crystalline form, which can then be reused in subsequent extraction processes, thereby improving the sustainability of the method.

Following TCA pre-treatment, the textile waste was washed in water and dried at 50 °C. The dried material was subsequently treated with trifluoroacetic acid (TFA, 5 mL) for acid cleavage. Within a few minutes, the textile transformed into a resinous mixture that was cast into moulds to yield, after air exposure, TPLAST.

2.2. Silk textile waste for TSF production

The revalorisation of silk textile waste was also investigated, and a new protocol for fibroin extraction was developed. Fig. 2 shows the protocol for fibroin recovery. Prior to protein extraction, the textile waste was treated with 50% TCA solution for dye recovery. The dried fabric was then dissolved completely after only 30 min at room temperature in 9.0 M LiBr. The solution was dialysed against water for 3 days and subsequently lyophilized. After dialysis the TSF was analyzed by XRD and compared to SF: the patterns in Fig. 2b show the presence of silk I ($2\theta = 12.2^\circ, 19.7^\circ, 24.7^\circ$ and 27.8°) and silk II characteristic peaks ($2\theta = 20.6^\circ$ and 24.7°) [40,41].

Gel filtration of the extracted TSF was performed to purify fibroin, assess the presence of other proteins, and compare retention times with

fibroin extracted from *Bombyx mori* cocoons (Fig. 3A). After gel filtration, complete dye removal was achieved, as shown in Fig. 2, where the lyophilized TSF product is presented. SDS-PAGE was also performed in order to assess the molecular weight of the purified TSF (about 200 kDa), thus demonstrating the absence of the fibroin degradation (see Figure 3Ac). A structural analysis of the TSF protein in water solution was performed by Circular Dichroism (CD) (see Fig. 3Ba). The CD spectrum of TSF exhibits a negative band at 218 nm and increased ellipticity below 210 nm, indicative of a predominantly β -sheet structure, which is also characteristic of the silk fibroin extracted by cocoon [42–44]. The SF and TSF CD spectra are also compared in the Figure S2B. The XRD pattern of the lyophilized TSF (Figure 3Bb) is characteristic of an amorphous state characterized by the broad featureless peak in XRD spanning from 12° to 32° , comparable to the SF purified by cocoon showed in the literature [40,41]. The microstructural analyses of the TSF before and after purification in gel filtration chromatography, were performed by scanning electron microscopy (SEM) and the micrographs are shown in the Fig. 3C. In both samples fibrose structures, characteristic of the fibroin polymerization, were observed, along with microfibers with diameters ranging from 0.2 to 10 μm . In the SEM micrographs of TSF not purified by gel filtration chromatography it is also possible to observe the presence of round particles (from 2 to 4 μm of diameter) on the material surface, not present in the TSF after gel filtration, probably ascribable to the pigments present in the not completely purified sample. The SEM micrographs of SF are also performed and shown in Figure S2.

2.3. TSF for 3D cell cultures

The extracted TSF was used to fabricate protein-poly(ethylene

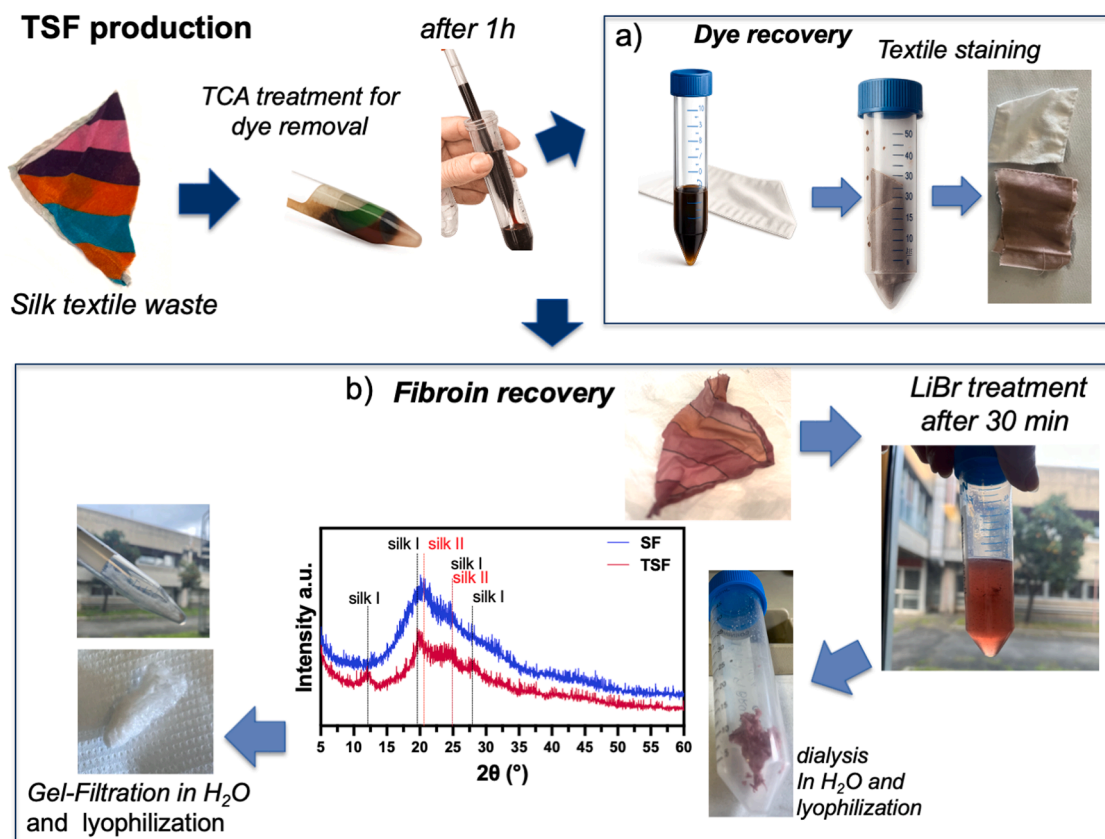


Fig. 2. Scheme of the extraction protocol for Textile silk fibroin (TSF) production from textile waste. Pretreatment with a solution of TCA 50% (w/v) for 1 h was performed for a) Dye and b) Fibroin recovery. Fibroin was obtained by treating the dried textile with 9.0 M of LiBr for 30 min at room temperature, dialysis against water and freeze-drying. XRD spectra of the TSF before the gel filtration and the SF are compared showing the presence of silk I and silk II characteristic peaks. Finally, the extracted TSF was purified by gel-filtration chromatography and freeze-dried.

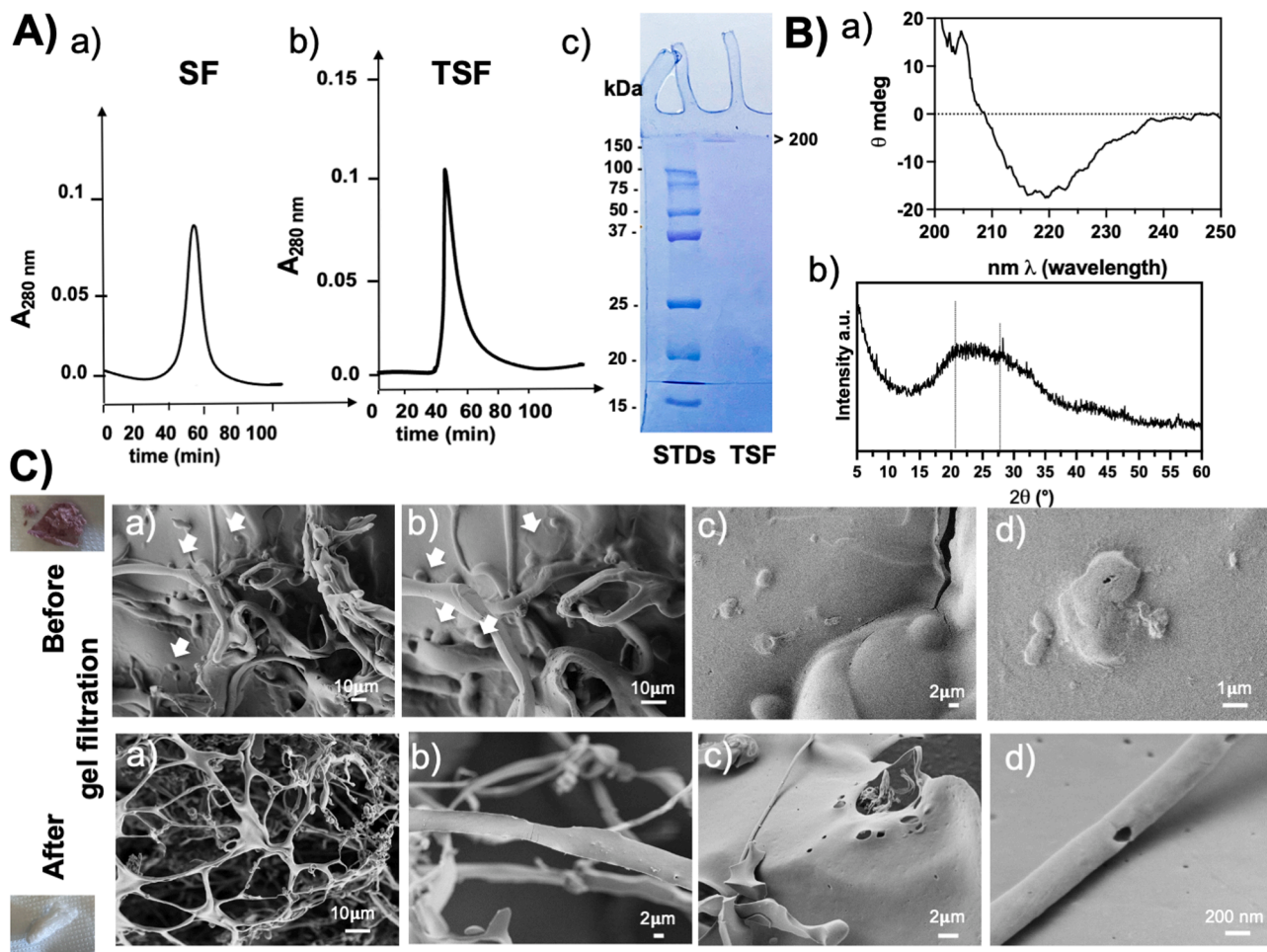


Fig. 3. TSF purification and characterization. **A)** Gel filtration chromatography of TSF (a) and SF (b) and SDS-PAGE (10% gel acrylamide) of TSF after gel filtration and molecular weight markers (STDs) (c); **B)** CD spectrum of about 10 μM of TSF in H_2O (a) and XDR spectrum of the lyophilized TSF (b); **C)** SEM micrographs of freeze-dried TSF before and after gel filtration were acquired using 2.0 kV and Mag. 500 X, 1, 2, 10, 20 and 50 KX. Scale bars were 200 nm and 1, 2 and 10 μm . The arrows in the TSF before purification by gel filtration micrographs indicate the presence of round particles likely associated to the presence of pigments, that are not present in the TSF after purification.

glycol) diacrylate (PEGDa) hydrogel-based 3D cell culture systems. Two types of 3D cellular constructs were produced: moulds (Fig. 4a) and spheres (Fig. 4b). Hydrogel stiffness was modulated by incorporating two different PEGDa concentrations (4.5% or 9% w/v) and evaluated by nano-indentation. Cell growth within the 3D systems was analysed using tunable hydrogels (TSFHy) of low (L; 2250 ± 51.96 Pa) and high (H; 8020 ± 91.09 Pa) stiffness (Fig. 4Ab).

The 3D cell cultures obtained embedding cells in TSFHy was compared to those obtained using SFHy, previously characterized for its cell-scaffolding properties [45,46]. Figure 5Aa,b shows breast cancer 3D cellular moulds generated using SF- and TSF-based PEG hydrogels of high stiffness, embedding human triple-negative breast cancer MDA-MB-231 cells. Fig. 5B presents a fluorescence Live/Dead cell viability assay after 7 days of culture. No differences in viability were observed between MDASF_H and MDATSF_H groups, confirming the suitability of TSF for producing 3D cancer cell models for cellular studies and drug discovery.

2.4. TSFHy as a scaffold for tissue regeneration

The production of 3D stem cell culture systems is highly relevant for studying the molecular mechanisms underlying cell growth, migration, and differentiation. These systems also hold applications in tissue regeneration and repair, as well as in cellular food and leather

production. TSFHy was therefore tested for 3D stem cell culture applications.

Fig. 6A and B show micrographs of cMSCSTF and cMSCSF spheres at low and high stiffness and their cell growth over time. Results demonstrated no significant differences in cell growth between the two hydrogel types (see also Figure S5). A proliferation reduction in cMSCSF/TSFH spheres at higher stiffness may reflect the onset of early differentiation processes. To investigate this, we assessed the expression of muscle differentiation markers by western blotting and immunofluorescence confocal microscopy (Fig. 6C and D).

After 7 days of culture, both cMSCSF_H and cMSCSTF_H exhibited ~ 10 -fold and ~ 20 -fold increases in α -smooth muscle actin (α -SMA) and smooth/skeletal myosin expression, respectively, compared to cells cultured on tissue culture plate (TCP). After 1 week, high expression of smooth/skeletal myosin was also confirmed by immunofluorescence microscopy (Fig. 6D). Although the sarcomeric α -actinin, specific to cardiac muscle differentiation, was absent after only 1 week of culture (Fig. 6C), it was observed in cMSCSTF_H molds after 2 weeks of culture by confocal microscopy, as shown in representative micrographs in the Fig. 7. These findings indicate commitment towards muscle differentiation of cMSCSTF systems.

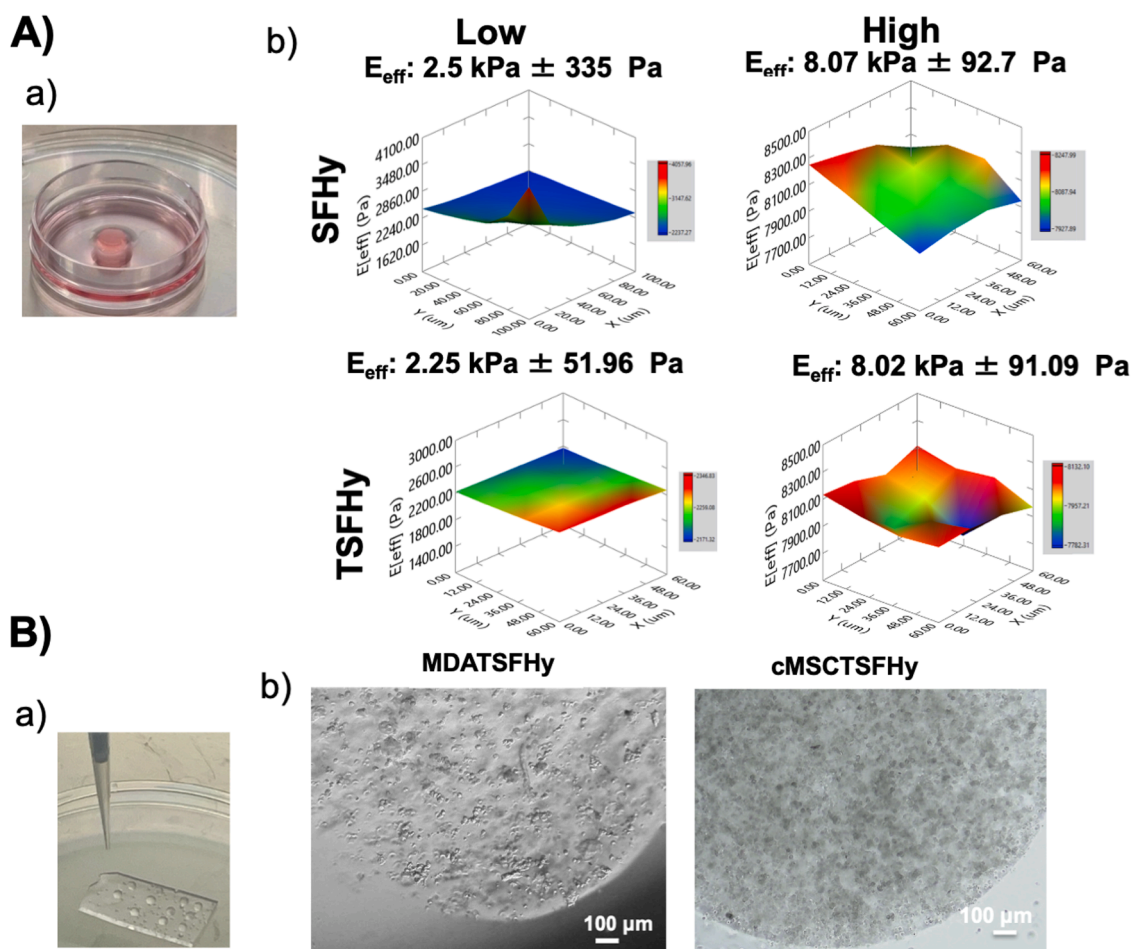


Fig. 4. TSFHy rheological characterization and 3D TSFHy-cell cultures production. **A)** a) digital image of TSFHy cell-loaded mold; b) 3D plot of the rheological analysis of SFHy and TSFHy at low and high stiffness with their effective Young's Modulus (E_{eff} , Pa) measured scanning a 4×4 matrix; **B)** a) TSFHy cell spheres dropped onto the nanostructured superhydrophobic surface using a cell density of 10^4 cells/ μL ; b) micrographs of MDATSF and cMSCTSF spheres. See also in Figure S4 the rheological analyses. Scale bars are of $100 \mu\text{m}$. All the data are the results of three or five independent experimental replicates.

3. Discussion

The textile industry in the EU generates approximately 16 billion kilograms of waste each year, yet only about one-quarter is recycled, with the remainder sent to landfills [47]. In recent years, companies have begun addressing this challenge by promoting recycled materials and adopting more sustainable production practices. These initiatives contribute to environmental protection and support the transition to a sustainable fashion industry. The Chinese government has set targets to increase textile waste recycling to 25% by 2025 and 30% by 2030 [48]. Textile waste is composed of both synthetic and natural polymers, making it a valuable source of raw materials after reprocessing. However, chemicals used in dyes, finishes, and additives pose a major obstacle to recycling, complicating the recovery process compared to less complex materials. Mechanical methods such as shredding and thermo-mechanical recycling are generally cost-effective but often yield lower-quality products than virgin fibres. In contrast, chemical recycling, though more expensive, has the advantage of preserving fibre quality. Sustainable chemistry can therefore play a central role in optimising textile waste recycling protocols.

Compared with conventional solvent-based reprocessing routes, the proposed protocol may offer potential sustainability advantages because it requires relatively small working volumes, potentially reducing solvent consumption, processing time, and energy demand per unit of recovered material; it is rapid, which may further improve process efficiency, lower operational costs, and support future scalability; and it

relies on an aqueous TCA system rather than on purely organic solvent-based routes, which may simplify processing and reduce some of the handling and environmental concerns typically associated with organic solvents. Conversely, the use of anhydrous TCA, which can be stored and handled as a solid and dissolved only at the point of use, may additionally facilitate transport, storage, and scale-up, contributing to reduced operational risks and more controlled process management. Additionally, we demonstrated the possible TCA and dye separation by simple water dilution (Fig. 1S) leading to a more sustainable approach. Our bench protocol shows the feasibility to recover the pigments from the TCA solution by a simple dilution and to reuse the TCA for other extraction, leading to a great sustainability of the process. Moreover, although the TCA is a strong acid, the use of TCA at concentrations up to 50–80% (w/v) has been also recently investigated in biomedical applications, including venous ulcer treatment and dermatological procedures [49,50], supporting its use as a manageable reagent under controlled conditions.

Acid cleavage of synthetic textile waste has previously been explored [51–54]. In this study, we developed a novel protocol employing acid cleavage with anhydrous TFA, a more sustainable option than those reported in the literature [55,56].

The synthetic polymers obtained using this method can be applied in the casting of TPLAST objects or optimised further for the production of printable TPLAST resins. Protocol optimisation may also enable the selective recovery of polymers from mixed-textile waste streams, potentially combined with dye recovery.

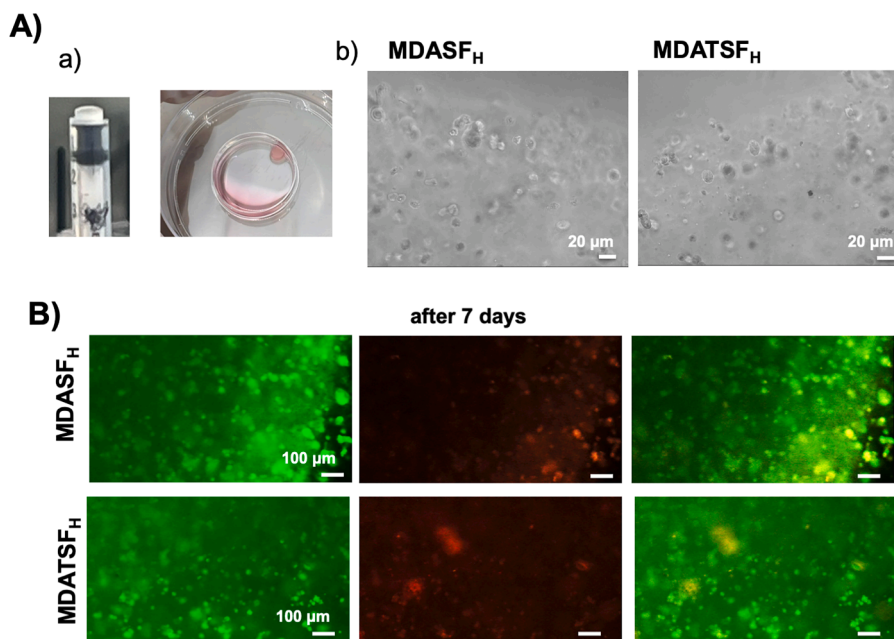


Fig. 5. TSFHy for 3D cancer cell culture. **A)** a) digital images of MDATSF molds production using a cell density of 10^4 cells/ μ L and culturing; b) brightfield micrographs of MDASF_H and MDATSF_H and **B)** LIVE/DEAD cell viability fluorescence assay after 7 days of cell growth. Scale bars are of 20 and 100 μ m. The results were obtained by 3 independent biological replicates.

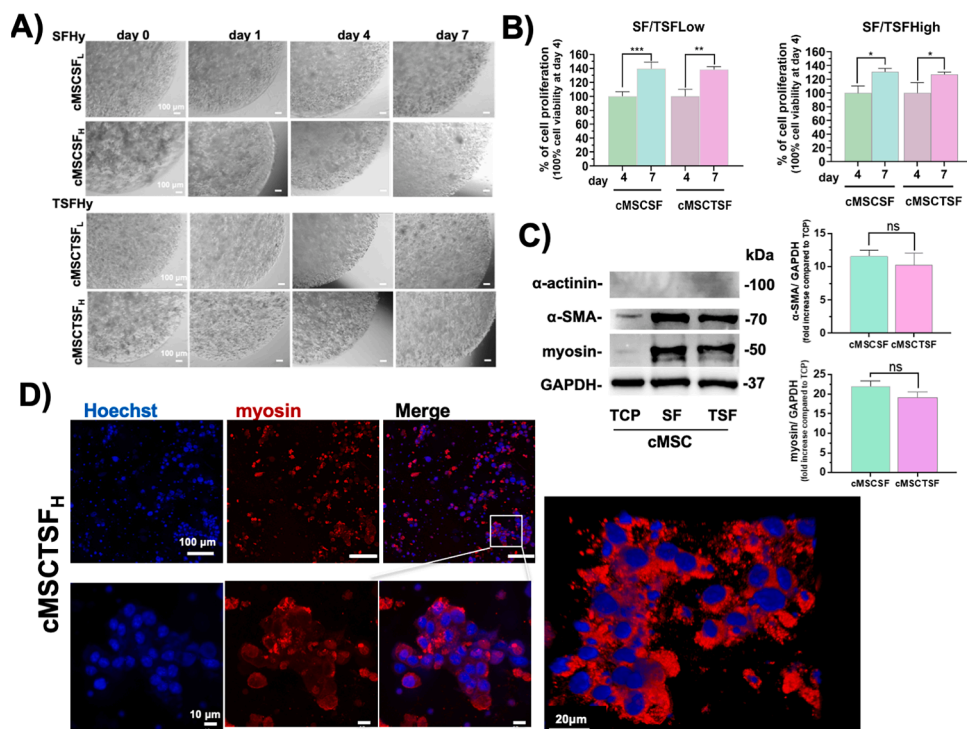


Fig. 6. 3D cMSCTSF cultures for in vitro muscle tissue production. **A)** Brightfield micrographs of cultured cMSCSF and cMSCTSF spheres (5 μ L with a cell density of 10^4 cell/ μ L) at high and low stiffness over time; **B)** WST-1 cell viability assay after 4 and 7 days of cell-growth; **C)** WB analysis of α -actinin, α -SMA and myosin expression in cMSCs grown in SF and TSF compared to TCP; **D)** Confocal microscopy of cMSCTSF_H after 1 week of cell growth, where nuclei of live cells are stained with Hoechst (in blue) and myosin recognized by Alexa Fluor- 546 nm is in red. Scale bars are of 20 and 100 μ m. The results were obtained by three independent biological replicates. Error bars indicate S.D. ns: non-significant; *p value < 0.05; ** p value $\leq$ 0.01; *** p value $\leq$ 0.005.

Moreover, for the first time, this study associated textile waste management with the production of SF hydrogels for tissue regeneration and 3D tumoroid fabrication. Although the use of LiBr involves economic and environmental concerns, the proposed protocol represents a

reprocessing route for existing silk textile waste rather than the production of a new material from virgin resources. Accordingly, despite the burden associated with LiBr use, this approach has the potential to offset a relevant share of the upstream impacts of virgin silk production

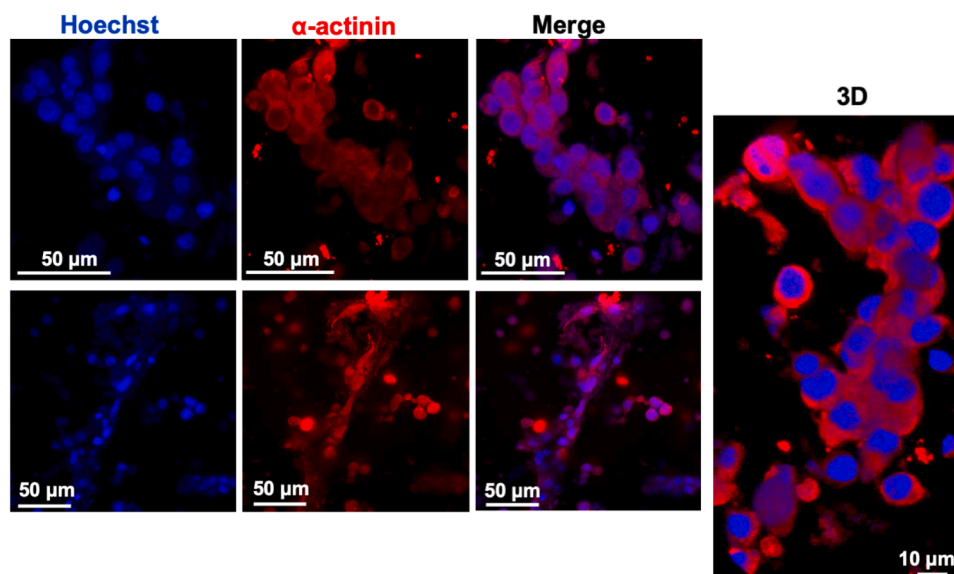


Fig. 7. Muscle differentiation of cMSCTSF system. Confocal microscopy of cMSCTSF_H (produced using a cell density of 10^4 cells/ μ L) after 2 weeks of culture, where nuclei of cells are stained with Hoechst (in blue) and α -actinin recognized by Alexa Fluor- 546 nm is in red. Scale bars are of 50 and 10 μ m. The results were obtained by three biological replicates.

by avoiding a substantial part of the inputs and processing steps associated with primary silk generation, particularly sericulture, cocoon production, and raw silk manufacturing. Combining textile waste reprocessing with SF production can enhance the sustainability of both processes. Owing to its superior biocompatibility, SF stands out from traditional synthetic polymers as a highly promising candidate for biomedical applications. SF derived from *B. mori* silkworms is a biocompatible, biodegradable protein with a unique structure, making it a key material for tissue repair [57]. In recent years, tunable protein-based hydrogels have been developed as promising biomaterials that mimic human tissues, support cell growth and repair, and can be adapted to damaged sites using minimally invasive techniques. In particular, SF-based hydrogels, obtained from *B. mori* cocoons, combine biocompatibility, biodegradability, and mechanical strength, making them attractive materials for tissue regeneration. SF hydrogels have also emerged as valuable scaffolds for bone tissue engineering. Implantation of SF hydrogels has been shown to significantly enhance bone regeneration [58], offering therapeutic potential in conditions such as bone defects, osteoporosis, osteoarthritis, dental tissue loss, intervertebral disc degeneration, chronic osteomyelitis, osteosarcoma, and tendon or ligament injuries [59–67]. Furthermore, because of their excellent biocompatibility, controlled immune activation, extracellular matrix (ECM)-mimicking properties, ability to provide sustained drug release, and modulation of cellular behaviour, SF hydrogels have been applied in the repair of bone and skin tissue and in anti-infective strategies. Additional applications include the repair of myocardial, neural, hepatic, uterine, corneal, and other tissues [68–72]. A key step in preparing SF for biomedical use is degumming, which removes sericin (SS) from silkworm silk to obtain regenerated SF. Incomplete separation can cause allergic reactions. Early studies showed that type I allergic responses to certain silk materials were linked to incomplete degumming, leaving residual SS on silk threads, and causing severe asthma, allergic pneumonitis, and elevated serum IgE levels [72–75]. This occurs because SS triggers T-cell-mediated allergic responses, whereas silk fibroin (SF) does not increase IgE levels [76,77]. This important and costly step is generally already performed during the preparation of textile silk. Therefore, in the protocol for TSF production, degumming was bypassed, making TSF production less expensive and faster than extraction from cocoons. Additives and dyes in the textile silk were removed by pre-treatment with TCA and, finally, by gel filtration, a step

also required in cocoon SF extraction, to obtain a pure protein. The TSF characterization by SDS-PAGE, SEM, XRD and CD analyses demonstrates the presence of not degraded fibroin able to form fibres in beta-sheet structure comparable to the SF. The functional properties of TSF as cellular scaffold were also here investigated. TSF promoted the myogenic commitment of cMSCs, as demonstrated by the increased expression of α -SMA and sarcomeric α -actinin, indicating its ability to drive stem cell differentiation without the addition of myogenic biochemical cues. The Young's modulus of skeletal muscle typically ranges from 10 to 15 kPa, and scaffolds with a stiffness of 8–17 kPa have been reported to induce the myogenic differentiation of MSCs [78–81]. Accordingly, our SFHy_H and TSFHy_H scaffolds can mimic the mechanical properties of skeletal muscle, as the effective Young's modulus of the acellular SF/TSFHy_H scaffolds was approximately 8 kPa. Furthermore, the presence of silk fibroin nanofibers and the β -sheet secondary structure in TSF may also provide physical cues that promote the spontaneous myogenic commitment of MSCs, in agreement with previous studies [82–84]. This result suggests possible applications of TSFHy not only in tissue repair and fabrication but also in the production of cell-based foods. Another relevant research field in recent years is the production of spheroids and tumoroids for pharmaceutical research and precision medicine [85,86]. TSF-based microspheres and moulds embedding cancer cells were produced and characterised to obtain low-cost, sustainable 3D cancer cell models. Preliminary results show that cell viability in MDATSF systems is comparable with that in MDASF systems.

The development of 3D cellular cultures for biological studies, drug discovery, tissue regeneration, and cell-based food production is often limited by the high cost of biomaterials. Here, a sustainable process is proposed, where textile waste recycling is combined with biomaterial production to achieve high-value revalorisation of waste. This study demonstrates the feasibility of the proposed approach at laboratory scale, whereas further investigations will be necessary to assess its scalability and economic feasibility. Nevertheless, the proposed protocol is rapid, operationally simple, and potentially cost-effective compared with more complex multi-step recycling strategies. A further strength of this approach is its versatility, since a single dyed silk waste stream can generate multiple value-added products, including recovered dye, regenerated fibroin, and a residual fraction suitable for plastic-like materials. Importantly, the recovered fibroin-based hydrogel was shown to support stem cell growth, viability, and differentiation, highlighting the

potential of this waste-derived material for biomedical valorisation.

Although further studies are needed to clarify the potential applications of these recycled materials and their effects on stem cell growth and differentiation in food and biomedical contexts, our results indicate that silk recycling from post-consumer textiles may offer a sustainable strategy for recovering high-value biopolymers and reducing both the environmental and economic impact of the textile industry.

4. Conclusions

Addressing the problem of textile waste requires innovative, cross-disciplinary research exploring a range of biotechnologically advanced strategies. Textile waste management could play a pivotal role in advancing a sustainable bio- and circular economy. New approaches to textile waste valorisation and the development of sustainable biomaterials are essential for the bioeconomy. Our results demonstrate that textile waste could represent a valuable source of polymers for producing 3D cellular scaffolds to support the growth of both stem cells and cancer cells for tissue repair and anti-tumour drug discovery. Furthermore, this research may pave the way for the *in vitro* production of novel cell-based foods with reduced costs and enhanced sustainability. Several products with applications ranging from biomedicine to the food industry could be fabricated entirely from these versatile biomaterials, giving new life and value to abundantly produced waste.

5. Materials and methods

5.1. Dyes extraction protocol

The extraction of the pigments from the textile waste was performed using 50% of TCA solution (w/v) (Sigma-Aldrich, Milan, Italy). After only 1 h of incubation at room temperature a great amount of acid dyes was recovered from the textile waste. The UV-vis spectra of the TCA extracts (1:19 dilution in 50% TCA; 1:5 dilution in 10% TCA and in 80% ethanol acidic pH) were performed using UV-31 SCA ONDA spectrophotometer (Prodotti Gianni, Milan Italy) and a quartz cuvette of 1 cm optical path.

5.2. TPLAST preparation

After dye-recovery, the textile wastes were dried at room temperature and treated with TFA (Sigma-Aldrich, Milan, Italy). After 30 min the textile waste was completely dissolved and the mixture cast in the molds. The TPLAST was then washed with water several times for the pH neutralization.

5.3. SF and TSF extraction and purification

Silk fibroin (SF) solution was obtained using the previous protocol [46] and described in Figure S3 (see Supplemental Information). Briefly, silk *Bombyx mori* cocoon was cut into pieces and boiled, at bain-marie, in buffer 50 mM Tris-buffered saline (TBS), 8.0 pH, for 3 h. Then the cocoon was washed in TBS and incubated with trypsin (Sigma-Aldrich, Italy) (1:50 trypsin: cocoon ratio w/w), in TBS at 37 °C for 3 h. Supernatant was removed, the solid residue was treated with 9 M of LiBr (Sigma-Aldrich, Italy) solution and incubated at 37 °C overnight. The solution was then dialyzed against bi-distilled water at 4 °C, using a dialysis membrane with a cut-off of 12 kDa (Medicell International Ltd), for four days. Finally, the purification was performed by gel filtration chromatography in bi-distilled water/ or phosphate-buffered saline (PBS), 7.2 pH, monitoring the elution at a wavelength of 280 nm. The textile-derived silk fibroin (TSF) was obtained by the textile waste as follows. Briefly, the pretreatment with a solution of TCA 50% (w/v) for 1 h was performed for the dye recovery. Then, the dried textile was treated with 9 M of LiBr for 30 min at room temperature leading to complete dissolution of the fabric. The solution was then dialyzed as above.

Finally, the gel filtration of the extracted TSF was performed in bi-distilled water monitoring the absorbance at a wavelength of 280 nm.

The protein concentration of SF and TSF solutions were evaluated using the bicinchoninic acid (BCA) colorimetric assay (Sigma-Aldrich, Italy). The molecular weight of the purified TSF was assessed by SDS-PAGE analysis using a 10% (w/v) of polyacrylamide gel and the molecular weight markers (Precision Plus Protein standards Kaleidoscope -Bio-Rad, Milan, Italy).

5.4. Circular dichroism (CD) spectroscopy and X-ray diffraction (XRD)

Far-UV CD spectra of TSF and SF (about 5 µM in water solution) were recorded between 250 and 200 nm of wavelengths at 21 °C, using a Jasco 600 spectropolarimeter (Jasco, Tokyo, Japan) calibrated using camphorsulfonic acid. The used parameters were: 0.1 cm of path length, 1.0 s of time constant, 0.5 nm band width, 0.2 nm/ min of scan rate and 20 mdeg of sensitivity.

The crystallinity of the TSF before and after gel filtration and that of SF were measured via X-ray diffraction using a Philips PANalytical X'Pert Pro diffractometer, equipped with a Cu K α radiation (40 mA, 40 kV). The XRD patterns were recorded between $2\theta = 5^\circ$ and 60° , with a step size of 0.02° and 2 s time per step.

5.5. Scanning electron microscopy (SEM) analyses

The scanning electron microscopy micrographs of the TSF samples before and after gel filtration were acquired using a Zeiss Leo SupraTM 35 field emission gun scanning electron microscope (Carl Zeiss SMT, Oberkochen, Germany). The samples of TSF were prepared using freeze drying, placed on carbon tape and subjected to metallization with Au using an RF Emitech K550x sputter. Sputtering was performed for 3 min at 25 mA to obtain a homogeneous coating of about 20 nm. The micrographs were acquired using 2.0 kV and Magnification 500 X, 1, 2, 10, 20 and 50 KX.

5.6. SF and TSF hydrogels spheres and molds production

The SF or TSF hydrogels were obtained by photopolymerization of precursor solutions constituted by: 70 µl of 3 mg/ml SF or TSF in PBS buffer, 30 µl (high) or 15 µl (low) of 30% w/v PEGDa 10 kDa, 1% w/v of Irgacure 2959 for a total volume of 100 µl in PBS [36,46]. To produce the spheres the photopolymerization was performed on a nano-structured superhydrophobic surface obtained using polydimethylsiloxane (PDMS) and PVDF membrane following a procedure described by Patent LDO0252 [87]. The triple negative breast cancer cells of the MDA-MB-231 cell line (MDA) or Lin⁺ Sca-1⁺ human cardiac mesenchymal stem cells (cMSCs) were used for 3D cell systems production. cMSCs line was obtained as previously described [88,89]. Cells at a density of 10^4 cells/µl were resuspended in the gel precursor solution and spheres of 5 µl were deposited on the superhydrophobic surface and photopolymerized by exposure under UV at 365 nm (5 mW/cm²) for 2 min. Further, the precursor solutions with breast cancer cells or with stem cells (50 µl) were photopolymerized on a cut syringe for 5 min to obtain the mould hydrogels. These 3D culture preparations result in minimal variability of the cell number embedded within spheroids/moulds, owing to the photopolymerization process of the precursor solution at a defined cell density (10^4 cells/µl) [45,46]. 3D cell systems were incubated in culture medium at 37 °C with 5% CO₂ and monitored over time by microscopy using a Zeiss microscope (Primovert, Zeiss, Italy).

5.7. Rheological analysis

The rheological analyses of SF/TSFHy molds (without cells) were performed by nanoindentation using PIUMA nano-indenter equipment (Optics11 Life, Amsterdam, The Netherlands). The analyses were

conducted in PBS buffer using a probe having a 0.45 N/m cantilever stiffness and a 49.5 μm radius spherical tip. The effective Young's Modulus (E_{eff} , Pa) was obtained in peak load poking mode performing a matrix scan of 4×4 with a displacement on X and Y axes of 20 μm (max load 0.50 μN and piezo speed 30.0 $\mu\text{m/s}$). Data were analyzed setting an Hertzian contact model and obtaining a 3D plot of E_{eff} values across the matrix. In Figure S4 are reported the histograms of the collected E_{eff} values and a Load-Indentation curve. The rheological analysis was performed using three experimental replicates for each gel.

5.8. Cell cultures and cell viability analyses

Tumor cells of the MDA-MB-231 cell line and human cardiac Lin⁺ Sca-1⁺ mesenchymal stem cells (cMSCs), obtained as previously described [88,89] by extraction from auricular biopsies, during coronary artery bypass surgery with formal consent from patients, were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Italy), containing 10% (v/v) of Fetal Bovine Serum (FBS) (Gibco, Italy), 1% (v/v) of Pen/Strep/Glutamine solution (Gibco, Italy). The cell proliferation in the SF or TSF hydrogels after 4 and 7 days of cell growth was evaluated using the cell viability WST-1 assay (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolium]–1,3-benzene disulfonate (Cell Proliferation Reagent WST-1, Roche, Mannheim, Germany) [90]. The cell culture medium was replaced with fresh DMEM high glucose without phenol-red (GIBCO, Italy) with 5% (v/v) of tetrazolium salt WST-1. The cMSCSF/TSF spheres were cultured for 3 h and the absorbance of the 1:1 diluted medium using PBS was assessed at a wavelength of 450 nm using the microplate reader iMarkTM Microplate Absorbance Reader (Bio-Rad). All the experiments were performed in triplicate, and each sample containing 3 cell-spheres. The viability of MDASF/TSF molds was evaluated by LIVE/DEAD assay using a LIVE/DEAD[®] Cell Imaging Kit (488/570) (Invitrogen, Thermo Fisher Scientific, Milan, Italy) and the fluorescence microscopy using a Zeiss fluorescence microscope Axio Observer 7. The results were obtained by 3 independent biological replicates.

5.9. Immunofluorescence microscopy analyses

Immunofluorescence analysis of cMSCSF_H spheres was obtained performing a live-cell fluorescent staining of the nuclei with Hoechst 33342 (Sigma-Aldrich, Italy) for four hours. After the spheres were fixed with 4% paraformaldehyde (PFA) in PBS for 30 min at room temperature, the cells were permeabilized using 0.3% Triton X-100 solution for 15 min and placed in a blocking buffer (10% of BSA (w/v), 0.1% of Triton X-100 (v/v) and 1% of glycine (w/v) in PBS) at 4 °C overnight. cMSCSF_H were then incubated overnight with a PBS solution with 20 mM of Gly and 1% (w/v) of BSA containing the primary antibody for myosin (smooth and skeletal) rabbit (M 7648, Sigma-Aldrich, Milan, Italy) or Ab- α -actinin sarcomeric mouse (A 7811, Sigma-Aldrich, Milan, Italy) followed by the rabbit or mouse Alexa fluorochrome-conjugated secondary antibody (546 nm, red) (Thermo Fisher Scientific, Invitrogen, USA). Confocal fluorescence microscopy was performed using a Stellaris Leica microscope platform. Immunofluorescence analyses were performed on three independent biological samples.

5.10. Protein expression analysis

The protein cellular extractions were performed by washing the cMSCSF/TSF spheres in PBS, resuspension in sample buffer for SDS-PAGE (50 μl) and boiling. For SDS-PAGE a 12% (w/v) of polyacrylamide gel was used. Polyvinylidene fluoride (PVDF) membranes (Bio-Rad, Italy) and a semi-dry Trans-Blot Turbo Transfer System (Bio-Rad, Italy) were used for electroblotting. After this, the membrane was incubated with blocking solution and probed with the following primary monoclonal antibodies: Ab- α -smooth muscle actin (α -SMA) mouse (A7607, Sigma-Aldrich, Milan, Italy), Ab- α -actinin sarcomeric mouse (A

7811, Sigma-Aldrich, Milan, Italy), Ab-myosin (skeletal and smooth) rabbit (M 7648, Sigma-Aldrich, Milan, Italy). The recognition of the primary antibodies was obtained incubating with the mouse or rabbit secondary antibody (dilution 1:3000) (7076#,7074#, Cell Signaling Technology, USA) for 4 h at room temperature. For controlling the protein loading the immunoblots were also probed with Ab-GAPDH rabbit (G9545, Sigma-Aldrich, Milan, Italy). An AmershamTM, ECL Select Western Blotting Detection Reagent kit (RPN2235, cytiva, UK) was used to visualize signals, followed by exposure to a FluorChem Imaging system (Alpha Innotech Corporation-Analitica De Mori, Milan, Italy). Three independent biological samples were used for each quantitative evaluation of protein expression and were 3 spheres for each 3D sample, which therefore is also inherently mediated.

5.11. Quantification and statistical analysis

The statistical data analysis was performed using GraphPad Prism version 8.0 for Windows (GraphPad Software, San Diego, CA, USA). Data were quantified and analysed for each variable using an independent Student *t*-test (Mann-Whitney test) and were obtained from three or more experimental independent biological replicates. A *p*-value < 0.05 was considered statistically significant. Data are presented as mean $\pm$ standard deviation (S.D.). ImageJ software was used for the densitometric analysis of the western blot protein bands and for analysing the fluorescence micrographs.

Fundings

The research leading to these results has received funding from the European Union – Next Generation EU through the Italian Ministry of University and Research under PNRR – M4C2-I1.3 Project PE_00000019 “HEAL ITALIA”. The views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them. The research was also partially supported by the Italian network of excellence for advanced diagnosis (INNOVA) PNC-E3–2022–23,683,266.

CRediT authorship contribution statement

Silvia Buonvino: Writing – review & editing, Investigation, Formal analysis, Data curation. **Leonardo Duranti:** Writing – review & editing, Formal analysis, Data curation. **Elisabetta Di Bartolomeo:** Writing – review & editing, Methodology, Formal analysis. **Sonia Melino:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation.

Declaration of competing interest

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

Acknowledgments

The authors would like to thank Gabriele Feltrin for the technical support in some of the experiments.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.rineng.2026.112173](https://doi.org/10.1016/j.rineng.2026.112173).

Data availability

Data will be made available on request.

References

- W. Leal Filho, D. Ellams, S. Han, D. Tyler, V.J. Boiten, A. Paço, H. Moora, A.-L. Balogun, A review of the socio-economic advantages of textile recycling, *J. Clean. Prod.* 218 (2019) 10–20, <https://doi.org/10.1016/j.jclepro.2019.01.210>.
- C.C. Okafor, C.N. Madu, C.C. Ajaero, J.C. Ibeke, C.A. Nzekwe, Sustainable management of textile and clothing, *Clean Technol. Recycl.* 1 (2021) 70–87, <https://doi.org/10.3934/ctr.2021004>.
- X. Sun, X. Wang, F. Sun, M. Tian, L. Qu, P. Perry, H. Owens, X. Liu, Textile waste Fiber regeneration via a green chemistry approach: a molecular strategy for sustainable fashion, *Adv. Mater.* 33 (2021) 2105174, <https://doi.org/10.1002/adma.202105174>.
- M. Shamsuzzaman, M. Islam, M.A.A. Mamun, R. Rayyaan, K. Sowrov, S. Islam, A.S. M. Sayem, Fashion and textile waste management in the circular economy: a systematic review, *Clean. Waste Syst.* 11 (2025) 100268, <https://doi.org/10.1016/j.clwas.2025.100268>.
- J. Kerr, Landry, Pulse of the Industry – GLOBAL FASHION AGENDA (accessed: Aug, <https://www.globalfashionagenda.com/publications-andpolicy/pulse-of-the-industry/#>, 2021 (accessed: Aug).
- G. Sandin, G.M. Peters, Environmental impact of textile reuse and recycling – a review, *J. Clean. Prod.* 184 (2018) 353–365, <https://doi.org/10.1016/j.jclepro.2018.02.266>.
- Ellen Macarthur Foundation, (2017). <https://www.ellenmacarthurfoundation.org/a-new-textiles-economy>.
- S. Hedrich, Scaling textile recycling in Europe—turning waste into value. <https://www.mckinsey.com/industries/retail/our-insights/scaling-textile-recycling-in-europe-turning-waste-into-value>, 2022.
- European Commission.Joint Research Centre, V. Romagnoli, T. Berlinghof, A. Parchomenko, J. Weissenbacher, L. Frattini, V. López Hernández, Y. Baron, H. Lörösch, K. Moch, K. Graulich, A. Stalmann, L. Asscherickx, T. Duhoux, M. Gama Caldas, V. Senatore, Study on the Destruction of Unsold Products, Publications Office of the European Union, LU, 2026. <https://doi.org/10.2760/3597352>.
- S. Dutta, Contamination of textile dyes in aquatic environment: adverse impacts on aquatic ecosystem and human health, and its management using bioremediation, *J. Env. Manage.* 353 (27) (2024) 120103.
- F. Chen, J. Zhu, Y. Yang, L. Wang, Assessing environmental impact of textile production with water alkalization footprint, *Sci. Total Environ.* 719 (2020) 137522, <https://doi.org/10.1016/j.scitotenv.2020.137522>.
- W. Leal Filho, P. Perry, H. Heim, M.A.P. Dinis, H. Moda, E. Ebbuoma, A. Paço, An overview of the contribution of the textiles sector to climate change, *Front. Env. Sci.* 10 (2022) 973102, <https://doi.org/10.3389/fenvs.2022.973102>.
- EPA, United States Environmental Protection Agency, (2026). <https://www.epa.gov/facts-and-figures-about-materials-waste-and-recycling/textiles-material-spec-ific-data>.
- Topics, European Parliament. <https://www.europarl.europa.eu/topics/en/article/20201208ST093327/the-impact-of-textile-production-and-waste-on-the-environment-infographics>, 2025 accessed 2025.
- EU, (2020). https://environment.ec.europa.eu/strategy/circular-economy_en?pre-flang=it.
- J. Ghosh, M.R. Repon, N.S. Rupanty, T.R. Asif, M.I. Tamjid, V. Reukov, Chemical valorization of textile waste: advancing sustainable recycling for a circular economy, *ACS. Omega* 10 (2025) 11697–11722, <https://doi.org/10.1021/acsomega.4c10616>.
- Ceo Water Mandate, n.d. <https://ceowatermandate.org/developer/circular-fibres-initiative>.
- A. Jeihanipour, C. Niklasson, M.J. Taherzadeh, Enhancement of solubilization rate of cellulose in anaerobic digestion and its drawbacks, *Process Biochem.* 46 (2011) 1509–1514, <https://doi.org/10.1016/j.procbio.2011.04.003>.
- S. Radhakrishnan, Application of biotechnology in the processing of textile fabrics. Roadmap to Sustainable Textiles and Clothing: Eco-Friendly Raw Materials, Technologies, and Processing Methods, Springer, 2014, pp. 277–325.
- N. Pensupa, S.-Y. Leu, Y. Hu, C. Du, H. Liu, H. Jing, H. Wang, C.S.K. Lin, Recent trends in sustainable textile waste recycling methods: current situation and future prospects, *Chem. Chem. Technol. Waste Valorization* 375 (2017) 189–228, <https://doi.org/10.1007/s41061-017-0165-0>.
- R.B. Baloyi, O.J. Gbadeyan, B. Sithole, V. Chumilall, Recent advances in recycling technologies for waste textile fabrics: a review, *Text. Res. J.* 94 (2024) 508–529, <https://doi.org/10.1177/004051752312102>.
- J. Singh, K. Sung, T. Cooper, K. West, O. Mont, Challenges and opportunities for scaling up upcycling businesses – The case of textile and wood upcycling businesses in the UK, *Resour. Conserv. Recycl.* 150 (2019) 104439, <https://doi.org/10.1016/j.resconrec.2019.104439>.
- A.K.M. Nayab-Ul-Hossain, S.K. Sela, S.B. Sadeque, Recycling of dyed fiber waste to minimize resistance and to prepare electro thermal conductive bar, *Results Eng.* 3 (2019) 100022, <https://doi.org/10.1016/j.rineng.2019.100022>.
- K. Lindström, F. Van Der Holst, L. Berglin, A. Persson, N. Kadi, Mechanical textile recycling efficiency: sample configuration, treatment effects and fibre opening assessment, *Results Eng.* 24 (2024) 103252, <https://doi.org/10.1016/j.rineng.2024.103252>.
- S.S. Chopra, L. Dong, G. Kaur, C. Len, C.S. Ki Lin, Sustainable process design for circular fashion: advances in sustainable chemistry for textile waste valorisation, *Curr. Opin. Green Sustain. Chem.* 39 (2023) 100747, <https://doi.org/10.1016/j.cogsc.2022.100747>.
- M.D. Stanescu, M. Fogorasi, M. Dochia, S. Mihuta, V.I. Lozinsky, Biotechnology for textile waste valorisation, *Rev. Chim.* 60 (1) (2009) 59–62. ISSN: 00347752.
- Y. Hu, C. Du, N. Pensupa, C.S.K. Lin, Optimisation of fungal cellulase production from textile waste using experimental design, *Process Saf. Environ. Prot.* 118 (2018) 133–142, <https://doi.org/10.1016/j.psep.2018.06.009>.
- F. Hermanutz, M.P. Vocht, N. Panzier, M.R. Buchmeiser, Processing of cellulose using ionic liquids, *Macromol. Mater. Eng.* 304 (2) (2019) 1800450, <https://doi.org/10.1002/mame.201800450>.
- P. Harmsen, M. Scheffer, H. Bos, Textiles for circular fashion: the logic behind recycling options, *Sustainability* 13 (2021) 9714, <https://doi.org/10.3390/su13179714>.
- J. Juanga-Labayen, K. Yanac, Q. Yuan, Effect of substrate-to-inoculum ratio on anaerobic digestion of treated and untreated cotton textile waste, *Int. J. Environ. Sci. Technol.* 18 (2021) 287–296, <https://doi.org/10.1007/s13762-020-02831-9>.
- I. Wojnowska-Baryla, K. Bernat, M. Zaborowska, Strategies of recovery and organic recycling used in textile waste management, *Int J. Env. Res Public Health* 19 (2022) 5859, <https://doi.org/10.3390/ijerph19105859>.
- S. Scheerer, R. Chudoba, M.P. Garibaldi, M. Curbach, Shells made of textile reinforced concrete – Applications in Germany, *J. Int. Assoc. Shell Spat. Struct.* 58 (1) (2017) 79–93, <https://doi.org/10.20898/j.iaass.2017.191.846>.
- K. Subramanian, M.K. Sarkar, H. Wang, Z.-H. Qin, S.S. Chopra, M. Jin, V. Kumar, C. Chen, C.-W. Tsang, C.S.K. Lin, An overview of cotton and polyester, and their blended waste textile valorisation to value-added products: a circular economy approach—research trends, opportunities and challenges, *Crit. Rev. Env. Sci. Technol.* 52 (2022) 3921–3942, <https://doi.org/10.1080/10643389.2021.1966254>.
- M. Suriani, R. Ilyas, M. Zuhri, A. Khalina, M. Sultan, S. Sapuan, C. Ruzaidi, F. N. Wan, F. Zulkifli, M. Harussani, Critical review of natural fiber reinforced hybrid composites: processing, properties, applications and cost, *Polym. (Basel)* 13 (2021) 3514, <https://doi.org/10.3390/polym13203514>.
- S. Buonvino, I. Arciero, E. Martinelli, D. Seliktar, S. Melino, Modelling the disease: h2S-sensitivity and drug-resistance of triple negative breast cancer cells can be modulated by embedding in isotropic micro-environment, *Mater. Today Bio.* 23 (2023) 100862, <https://doi.org/10.1016/j.mtbio.2023.100862>.
- S. Mantry, K. Silakabattini, P.K. Das, J. Sankaraiyah, C.S. Barik, S. Panda, S. Wahab, M. Khalid, Silk fibroin: an innovative protein macromolecule-based hydrogel/ scaffold revolutionizing breast cancer treatment and diagnosis – Mechanisms, advancements, and targeting capabilities, *Int. J. Biol. Macromol.* 309 (2025) 142870, <https://doi.org/10.1016/j.jbiomac.2025.142870>.
- D. Lin, M. Li, L. Wang, J. Cheng, Y. Yang, H. Wang, J. Ye, Y. Liu, Multifunctional hydrogel based on silk fibroin promotes tissue repair and regeneration, *Adv. Funct. Mater.* 34 (2024) 2405255, <https://doi.org/10.1002/adfm.202405255>.
- P. Sakong, W. Thosaikham, Revolutionizing indigo-Dyed fabric identification: a novel sulfonation approach for authenticity and quality assurance, *Trends. Sci.* 21 (2024), <https://doi.org/10.48048/tis.2024.8043>. Manuscript.
- M.H. Habibi, A. Hassanzadeh, A. Zeini-Isfahani, Spectroscopic studies of solophenyl red 3BL polyazo dye tautomerism in different solvents using UV–visible, 1H NMR and steady-state fluorescence techniques, *Dyes Pigments* 69 (2006) 93–101, <https://doi.org/10.1016/j.dyepig.2005.02.011>.
- P. Cebé, B.P. Partlow, D.L. Kaplan, A. Wurm, E. Zhuravlev, C. Schick, Silk I and Silk II studied by fast scanning calorimetry, *Acta. Biomater.* 55 (2017) 323–332, <https://doi.org/10.1016/j.actbio.2017.04.001>.
- N. Bhardwaj, S.C. Kundu, Chondrogenic differentiation of rat MSCs on porous scaffolds of silk fibroin/chitosan blends, *Biomaterials* 33 (2012) 2848–2857, <https://doi.org/10.1016/j.biomaterials.2011.12.028>.
- Y. Pei, X. Liu, S. Liu, Q. Lu, J. Liu, D.L. Kaplan, H. Zhu, A mild process to design silk scaffolds with reduced β -sheet structure and various topographies at the nanometer scale, *Acta Biomater.* 13 (2015) 168–176, <https://doi.org/10.1016/j.actbio.2014.11.016>.
- R.E. Marsh, R.B. Corey, L. Pauling, An investigation of the structure of silk fibroin, *Biochim. Biophys. Acta* 16 (1955) 1–34.
- G. Li, P. Zhou, Z. Shao, X. Xie, X. Chen, H.I. Wang, L. Chunyu, T.Yu the natural silk spinning process, *Eur. J. Biochem.* 268 (2001) 6600–6606.
- M. Ciocci, I. Cacciotti, D. Seliktar, S. Melino, Injectable silk fibroin hydrogels functionalized with microspheres as adult stem cells-carrier systems, *Int. J. Biol. Macromol.* 108 (2018) 960–971, <https://doi.org/10.1016/j.jbiomac.2017.11.013>.
- S. Buonvino, G. Paduano, V. Stefanizzi, H. Garay, S. Perea, B. Macchi, M. Venanzi, S. Melino, Microenvironment rheology modulates the effect of the anticancer peptide CIGB300 on 3D head and neck tumoroids, *IJMS* 27 (2026) 1973, <https://doi.org/10.3390/ijms27041973>.
- M.A. Bukhari, R. Carrasco-Gallego, E. Ponce-Cueto, Developing a national programme for textiles and clothing recovery, *Waste Manage Res.* 36 (2018) 321–331, <https://doi.org/10.1177/0734242X18759190>.
- Textile Technology. <https://www.textiletechnology.net/technology/news/china-recycling-of-25-of-all-textile-waste-by-2025>, 2022.
- L. Zenjari, F. Hali, S. Chiheb, Trichloroacetic acid (50%) in the treatment of venous leg ulcers, *JMV-J. Med. Vasc.* 46 (2021) 139–143, <https://doi.org/10.1016/j.jdmv.2021.03.006>.
- I.B.S. Sitohang, L. Legiawati, L.S. Suseno, F.D. Safira, Trichloroacetic acid peeling for treating photoaging: a systematic review, *Dermatol. Res. Pr.* 2021 (2021) 3085670, <https://doi.org/10.1155/2021/3085670>, 2021.

- [51] A. Ouchi, T. Toida, S. Kumaresan, W. Ando, J. Kato, A new methodology to recycle polyester from fabric blends with cellulose, *Cellulose* 17 (2010) 215–222, <https://doi.org/10.1007/s10570-009-9358-1>.
- [52] E. Ruuth, M. Sanchis-Sebastiá, P.T. Larsson, A. Teleman, A. Jiménez-Quero, S. Delestig, V. Sahlberg, P. Salén, M. Sanchez Ortiz, S. Vadher, Reclaiming the value of cotton waste textiles: a new improved method to recycle cotton waste textiles via acid hydrolysis, *Recycling* 7 (2022) 57, <https://doi.org/10.3390/recycling7040057>.
- [53] L. Lu, W. Fan, X. Meng, L. Xue, S. Ge, C. Wang, S.Y. Foong, C.S. Tan, C. Sonne, M. Aghbashlo, Current recycling strategies and high-value utilization of waste cotton, *Sci. Total Environ.* 856 (2023) 158798, [10.1016/j.scitotenv.2022.158798](https://doi.org/10.1016/j.scitotenv.2022.158798).
- [54] L. Villar, M. Pita, B. González, P.B. Sánchez, Hydrolytic-assisted fractionation of textile waste containing cotton and polyester, *Fibers Polym.* 25 (2024) 2763–2772, <https://doi.org/10.1007/s12221-024-00602-8>.
- [55] I.S. Bayer, S. Guzman-Puyol, J.A. Heredia-Guerrero, L. Ceseracci, F. Pignatelli, R. Ruffilli, R. Cingolani, A. Athanassiou, Direct transformation of edible vegetable waste into bioplastics, *Macromolecules* 47 (2014) 5135–5143, <https://doi.org/10.1021/ma5008557>.
- [56] S. Buonvino, M. Ciocci, F. Nanni, I. Cacciotti, S. Melino, New vegetable-waste biomaterials by Lupin albus L. as cellular scaffolds for applications in biomedicine and food, *Biomaterials* 293 (2023) 121984, <https://doi.org/10.1016/j.biomaterials.2022.121984>.
- [57] J.K. Sahoo, O. Hasturk, T. Falcucci, D.L. Kaplan, Silk chemistry and biomedical material designs, *Nat. Rev. Chem.* 7 (2023) 302–318, <https://doi.org/10.1038/s41570-023-00486-x>.
- [58] K. Zhou, T. Yuan, S. Wang, F. Hu, L. Luo, L. Chen, L. Yang, Beyond natural silk: bioengineered silk fibroin for bone regeneration, *Mater. Today Bio.* 33 (2025) 102014, <https://doi.org/10.1016/j.mtbio.2025.102014>.
- [59] L. Meng, C. Shao, C. Cui, F. Xu, J. Lei, J. Yang, Autonomous self-healing silk fibroin injectable hydrogels formed via surfactant-free hydrophobic association, *ACS Appl. Mater. Interfaces* 12 (2020) 1628–1639, <https://doi.org/10.1021/acsami.9b19415>.
- [60] P. Chuysinuan, P. Nooeaid, T. Thanyacharoen, S. Techasakul, P. Pavasant, K. Kanjanamekanant, Injectable eggshell-derived hydroxyapatite-incorporated fibroin-alginate composite hydrogel for bone tissue engineering, *Int. J. Biol. Macromol.* 193 (2021) 799–808, <https://doi.org/10.1016/j.ijbiomac.2021.10.132>.
- [61] L. Pierantoni, V.P. Ribeiro, L. Costa, S. Pina, A. da Silva Morais, J. Silva-Correia, S. C. Kundu, A. Motta, R.L. Reis, J.M. Oliveira, Horseradish peroxidase-crosslinked calcium-containing silk fibroin hydrogels as artificial matrices for bone cancer research, *Macromol. Biosci.* 21 (2020) 2000425, <https://doi.org/10.1002/mabi.202000425>.
- [62] Z. Yan, W. Chen, W. Jin, Y. Sun, J. Cai, K. Gu, R. Mi, N. Chen, S. Chen, Z. Shao, An interference screw made using a silk fibroin-based bulk material with high content of hydroxyapatite for anterior cruciate ligament reconstruction in a rabbit model, *J. Mater. Chem. B* 9 (2021) 5352–5364, <https://doi.org/10.1039/D1TB01006A>.
- [63] M. Li, Y. Wu, H. Li, C. Tan, S. Ma, J. Gong, L. Dong, W. Huang, X. Li, H. Deng, Nanofiber reinforced alginate hydrogel for leak-proof delivery and higher stress loading in nucleus pulposus, *Carbohydr. Polym.* 299 (2023) 120193, [10.1016/j.carbpol.2022.120193](https://doi.org/10.1016/j.carbpol.2022.120193).
- [64] Z. Lv, T. Hu, Y. Bian, G. Wang, Z. Wu, H. Li, X. Liu, S. Yang, C. Tan, R. Liang, A MgFe-LDH nanosheet-incorporated smart thermo-responsive hydrogel with controllable growth factor releasing capability for bone regeneration, *Adv. Mater.* 35 (2023) 2206545, <https://doi.org/10.1002/adma.202206545>.
- [65] K. Rui, X. Tang, Z. Shen, C. Jiang, Q. Zhu, S. Liu, N. Che, J. Tian, J. Ling, Y. Yang, Exosome inspired photo-triggered gelation hydrogel composite on modulating immune pathogenesis for treating rheumatoid arthritis, *J. Nanobiotechnology* 21 (2023) 111, <https://doi.org/10.1186/s12951-023-01865-8>.
- [66] P. Zhang, Y. Sun, H. Yang, D. Liu, F. Zhang, Y. Zhang, W. Zhong, B. Zuo, Z. Zhou, Vancomycin-loaded silk fibroin microspheres in an injectable hydrogel for chronic osteomyelitis therapy, *Front. Bioeng. Biotechnol.* 11 (2023) 1163933, <https://doi.org/10.3389/fbioe.2023.1163933>.
- [67] A. Zheng, X. Wang, X. Xin, L. Peng, T. Su, L. Cao, X. Jiang, Promoting lacunar bone regeneration with an injectable hydrogel adaptive to the microenvironment, *Bioact. Mater.* 21 (2023) 403–421, <https://doi.org/10.1016/j.bioactmat.2022.08.031>.
- [68] S. Chen, S. Liu, L. Zhang, Q. Han, H. Liu, J. Shen, G. Li, L. Zhang, Y. Yang, Construction of injectable silk fibroin/polydopamine hydrogel for treatment of spinal cord injury, *Chem. Eng. J.* 399 (2020) 125795, [10.1016/j.cej.2020.125795](https://doi.org/10.1016/j.cej.2020.125795).
- [69] Y. Wu, T. Chang, W. Chen, X. Wang, J. Li, Y. Chen, Y. Yu, Z. Shen, Q. Yu, Y. Zhang, Release of VEGF and BMP9 from injectable alginate based composite hydrogel for treatment of myocardial infarction, *Bioact. Mater.* 6 (2021) 520–528, <https://doi.org/10.1016/j.bioactmat.2020.08.031>.
- [70] I.A. Barroso, K. Man, T.J. Hall, T.E. Robinson, S.E. Louth, S.C. Cox, A.K. Ghag, Photocurable antimicrobial silk-based hydrogels for corneal repair, *J. Biomed. Mater. Res. A* 110 (2022) 1401–1415, <https://doi.org/10.1002/jbm.a.37381>.
- [71] D. Lin, M. Li, L. Wang, J. Cheng, Y. Yang, H. Wang, J. Ye, Y. Liu, Multifunctional hydrogel based on silk fibroin promotes tissue repair and regeneration, *Adv. Funct. Mater.* 34 (2024) 2405255, <https://doi.org/10.1002/adfm.202405255>.
- [72] L. Meinel, S. Hofmann, V. Karageorgiou, C. Kirker-Head, J. McCool, G. Gronowicz, L. Zichner, R. Langer, G. Vunjak-Novakovic, D.L. Kaplan, The inflammatory responses to silk films in vitro and in vivo, *Biomaterials* 26 (2005) 147–155, <https://doi.org/10.1016/j.biomaterials.2004.02.047>.
- [73] H. Liu, S.G. Wise, J. Rnjak-Kovacic, D.L. Kaplan, M.M.M. Bilek, A.S. Weiss, J. Fei, S. Bao, Biocompatibility of silk-tropoelastin protein polymers, *Biomaterials* 35 (2014) 5138–5147, <https://doi.org/10.1016/j.biomaterials.2014.03.024>.
- [74] S.H. Kim, H. Hong, O. Ajiteru, M.T. Sultan, Y.J. Lee, J.S. Lee, O.J. Lee, H. Lee, H. S. Park, K.Y. Choi, 3D bioprinted silk fibroin hydrogels for tissue engineering, *Nat. Protoc.* 16 (2021) 5484–5532, <https://doi.org/10.1038/s41596-021-00622-1>.
- [75] Y. Wang, Y. Liang, J. Huang, Y. Gao, Z. Xu, X. Ni, Y. Yang, X. Yang, Y. Zhao, Proteomic analysis of silk fibroin reveals diverse biological function of different degumming processing from different origin, *Front. Bioeng. Biotechnol.* 9 (2022) 777320, <https://doi.org/10.3389/fbioe.2021.777320>.
- [76] W. Zaoming, R. Codina, E. Fernández-Caldas, R.F. Lockey, Partial characterization of the silk allergens in mulberry silk extract, *J. Investig. Allergol. Clin. Immunol.* 6 (4) (1996) 237–241, PMID: 8844500.
- [77] S. Kurosaki, H. Otsuka, M. Kunitomo, M. Koyama, R. Pawankar, K. Matumoto, Fibroin allergy IgE mediated hypersensitivity to silk suture materials, *J. Nippon Med. Sch.* 66 (1999) 41–44, <https://doi.org/10.1272/jnms.66.41>.
- [78] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, *Cell* 126 (2006) 677–689, <https://doi.org/10.1016/j.cell.2006.06.044>.
- [79] J.S. Park, J.S. Chu, A.D. Tsou, R. Diop, Z. Tang, A. Wang, S. Li, The effect of matrix stiffness on the differentiation of mesenchymal stem cells in response to TGF- β , *Biomaterials* 32 (2011) 3921–3930, <https://doi.org/10.1016/j.biomaterials.2011.02.019>.
- [80] A.J. Steward, D.J. Kelly, Mechanical regulation of mesenchymal stem cell differentiation, *J. Anat.* 227 (2015) 717–731, <https://doi.org/10.1111/joa.12243>.
- [81] A.B. Mathur, A.M. Collinsworth, W.M. Reichert, W.E. Kraus, G.A. Truskey, Endothelial, cardiac muscle and skeletal muscle exhibit different viscous and elastic properties as determined by atomic force microscopy, *J. Biomech.* 34 (2001) 1545–1553, [https://doi.org/10.1016/S0021-9290\(01\)00149-X](https://doi.org/10.1016/S0021-9290(01)00149-X).
- [82] H. Han, H. Ning, S. Liu, Q. Lu, Z. Fan, H. Lu, G. Lu, D.L. Kaplan, Silk biomaterials with vascularization capacity, *Adv. Funct. Mater.* 26 (2016) 421–432, <https://doi.org/10.1002/adfm.201504160>.
- [83] X. Wang, Z. Ding, C. Wang, X. Chen, H. Xu, Q. Lu, D.L. Kaplan, Bioactive silk hydrogels with tunable mechanical properties, *J. Mater. Chem. B* 6 (2018) 2739–2746, <https://doi.org/10.1039/C8TB00607E>.
- [84] D. Baruffaldi, G. Palmara, C. Pirri, F. Frascella, 3D Cell culture: recent development in materials with tunable stiffness, *ACS Appl. Bio. Mater.* 4 (2021) 2233–2250, <https://doi.org/10.1021/acsabm.0c01472>.
- [85] Y.-H. Lo, K. Karlsson, C.J. Kuo, Applications of organoids for cancer biology and precision medicine, *Nat. Cancer* 1 (2020) 761–773, <https://doi.org/10.1038/s43018-020-0102-y>.
- [86] W. Li, Z. Zhou, X. Zhou, B.L. Khoo, R. Gunawan, Y.R. Chin, L. Zhang, C. Yi, X. Guan, M. Yang, 3D Biomimetic models to reconstitute tumor microenvironment In vitro: spheroids, organoids, and tumor-on-a-chip, *Adv. Heal. Mater.* 12 (2023) 2202609, <https://doi.org/10.1002/adhm.202202609>.
- [87] Leonardo S.p.A. Procedimento per la preparazione di un film superidrofoico IPC – B08B17/06 B29C39/14 | B29C39/148. (2009).
- [88] G. Forte, S. Pietronave, G. Nardone, A. Zamperone, E. Magnani, S. Pagliari, F. Pagliari, C. Giacinti, C. Nicoletti, A. Musaró, M. Rinaldi, M. Ribezzo, C. Comoglio, E. Traversa, T. Okano, M. Minieri, M. Prat, P. Di Nardo, Human cardiac progenitor cell grafts as unrestricted source of supernumerary cardiac cells in healthy murine hearts, *Stem Cells* 29 (2011) 2051–2061, <https://doi.org/10.1002/stem.763>.
- [89] E. Di Giovanni, S. Buonvino, I. Amelio, S. Melino, Glutathione-Allylsulfur conjugates as mesenchymal stem cells stimulating agents for potential applications in tissue repair, *IJMS* 21 (2020) 1638, <https://doi.org/10.3390/ijms21051638>.
- [90] M. Koyanagi, S. Kawakabe, Y. Arimura, A comparative study of colorimetric cell proliferation assays in immune cells, *Cytotechnology* 68 (2016) 1489–1498, <https://doi.org/10.1007/s10616-015-9909-2>.