



Lyocell/silver knitted fabrics for prospective diabetic foot ulcers treatment: Effect of knitting structure on bacteria and cell viability

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ABSTRACT

Diabetic foot ulcers (DFUs) are a serious complication of diabetes, often resulting in infections and further health deterioration. Thus, the development of an approach combining different therapies in just one formulation to treat DFUs remains very challenging. Silver-plated polyamide fibers offer antimicrobial properties, while lyocell provides biodegradability, biocompatibility and moisture management abilities. In this sense, the present study explores the potential of lyocell/silver-plated polyamide fabrics as part of advanced wound dressings designed to improve DFU treatment. The most common knitting structures, namely single jersey, “false” rib 1 × 1, single pique, and “false” interlock, were selected for combining the yarns and successfully processed using seamless technology. The knitted fabrics were then subjected to a comprehensive analysis of their physical, chemical, and thermomechanical properties, demonstrating that the samples met the criteria for effective wound dressing development. Their antimicrobial efficacy was evaluated against DFU-associated Gram-negative pathogens, *Escherichia coli* and *Pseudomonas aeruginosa*, showing strong antimicrobial activity for up to 24 h, with total inhibition in some cases (jersey, pique and interlock structures for *E. coli* and interlock structure for *P. aeruginosa*). Antioxidant testing revealed DPPH reduction of 61.7 ± 14.4 %. Biocompatibility was assessed using keratinocytes HaCaT cell lines, showing that knitted fabrics with up to 1.46 % silver content did not harm mammalian cells. In general, interlock structure revealed the most promising features, including mechanical performance, and air and water vapor permeability, for promoting optimal wound healing conditions.

1. Introduction

The field of medical textiles has been diversifying with new materials and innovative designs. Advances in fiber technology and manufacturing processes allowed the introduction of cost-effective, environmentally friendly and innovative products in the market [1,2]. Knitting, known for its versatile forming technology, is characterized by increased production efficiency, flexible structures and diverse patterns [3], being able to meet different application requirements [1]. The adoption of seamless knitting technology has seen a remarkable growth, enabling the efficient and flexible production of knitted fabrics with

desirable properties such as comfortable compression, air permeability, durability and easy care [4].

The use of polymers obtained from natural sources in areas such as biomedicine has had a huge impact in recent decades [5]. Lyocell is a relatively new fiber made from natural cellulose via a sustainable and environmentally friendly process. It is the only regenerated cellulosic fiber in which 99.8 % of its solvent, *N*-methylmorpholine *N*-oxide, is recycled [6]. This fiber has exceptional physical properties, such as high tensile strength and tenacity in wet state, good thermal resistance and shape stability, as well as high crystallinity and absorbency. Comfort and softness are also typical traits of this fiber. In addition to their non-toxic,

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non-polluting, and biodegradable nature, lyocell presents great adaptability and processability with other fibers [7]. Polyamide is a synthetic polymer with excellent mechanical features, namely high tenacity and tensile strength. Also, its surface structure possesses small cavities that can be used as chelation points for subsequent metal plating, making it a great substrate for metallization [8]. Silver is renowned for its numerous benefits, including its high antimicrobial, anti-odor, electrical conductivity and thermo-reflective properties [9]. Furthermore, it is one of the most suitable metals to incorporate into textile yarns, as they retain all their original properties after undergoing metallization. Currently, silver-plated polyamide fibers are being used in a wide range of functional textiles, particularly in the antimicrobial, antistatic, thermal conductive and electromagnetic fields [10]. In the medical sector, these yarns knitted into fabrics serve the purpose of treating burn victims and patients with open wounds [11]. Also, these fibers can be blended and processed with various natural or synthetic materials, increasing their functional range [8].

Worldwide, 10.5 % of the population suffers from diabetes, a disease responsible for imposing significant disruptions in the health care systems' resources, with annual costs of approximately 918.8 billion euros [12]. One of the most common problems arising from this illness is the development of DFUs, which affect 15–25 % of the diabetic population during their lifetime. DFUs significantly increase the risk of lower extremity amputation and/or patient mortality [13]. Current treatment strategies for DFUs vary depending on the lesion severity and may include debridement of non-viable tissue, infection control, revascularization, wound protection, and ulcer off-loading [14,15]. However, most of these interventions focus on addressing individualized barriers to wound healing without considering other potential events or factors that may also interfere with the healing process. Consequently, developing an integrated therapeutic approach in a single formulation remains a considerable challenge in the field. Here, the objective is to elucidate about the most suitable structural configuration that can significantly enhance the efficiency of knitted products, produced via seamless technology, in the context of chronic wound care. Therefore, to overcome the previously mentioned limitations of DFU treatments, the fabrication of a novel wound dressing from lyocell/silver-plated polyamide based yarns is proposed to generate a clean and moist environment around the injured site, absorb exudates and toxic components from the wound bed, allow gas exchange between the wound and environment, and protect the wound from the penetration of microorganisms and foreign particles. Physical, chemical, and thermomechanical analyses were conducted to confirm these characteristics. Additionally, the knitted fabrics were subjected to antibacterial tests against Gram-negative bacteria, namely *Escherichia coli* and *Pseudomonas aeruginosa*, which are amongst the most prevalent bacteria colonizing DFUs and pose significant challenges to wound healing [16]. Antioxidant and cytocompatibility evaluations, to establish the safety of the dressing systems against mammalian cells, were also conducted. To the authors' knowledge, this is the first report on the production of a knitted fabric combining lyocell with polyamide-silver plated, while also investigating the influence of structural variations on its potential for wound care applications.

2. MATERIAL AND METHODS

2.1. Materials

Lyocell spun yarns (149 dtex) were kindly provided by Risatel, Lda. (Portugal) and silver-plated polyamide 6 multifilament yarns (40 dtex) were purchased from Shieldex® (Germany). Sodium chloride (NaCl, Merck, Germany), potassium chloride (KCl, Merck), sodium phosphate dibasic (Na_2HPO_4 , Sigma-Aldrich, Germany) and monopotassium phosphate (KH_2PO_4 , Sigma-Aldrich) were used in the preparation of phosphate buffer saline solution (PBS at 0.01 M: 8.00 g/L of NaCl, 0.20 g/L of KCl, 1.44 g/L of Na_2HPO_4 and 0.24 g/L of KH_2PO_4 , adjusted

to physiological pH 7.4). Artificial wound exudates were prepared with NaCl (124×10^{-3} M), magnesium chloride (MgCl_2 , 831×10^{-6} M, Chem-Lab, Belgium), calcium chloride (CaCl_2 , 2.48×10^{-3} M, Chem-Lab), sodium bicarbonate (NaHCO_3 , 36.8×10^{-3} M, Sigma-Aldrich), glucose ($\text{C}_6\text{H}_{12}\text{O}_6$, 5×10^{-3} M, Sigma-Aldrich), albumin (150×10^{-6} M, VWR Chemicals, Portugal) and lactic acid 90 % ($\text{C}_3\text{H}_6\text{O}_3$ 10×10^{-6} M, Merck), at pH 6.72 and pH 9 [17]. The bacteria culture media trypticase soy broth (TSB), trypticase soy agar (TSA), nutrient broth (NB) and nutrient agar (NA) were obtained from VWR Chemicals, and Müeller-Hinton broth (MHB) was supplied by Condalab (Spain). Bacterial strains were provided by American Type Culture Collection (ATCC, USA): *E. coli* ATCC 25922 (grown in TSB/TSA) and *P. aeruginosa* ATCC 27853 (grown in NB/NA). 1,1-diphenyl-2-picrylhydrazyl (DPPH) and trypan blue were supplied by Merck, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was acquired from Fisher (India). The antibiotic-antimycotic solution (penicillin/streptomycin), trypsin-EDTA, dimethyl sulfoxide (DMSO) cell culture grade, and resazurin (Xpert blue cell viability assay) were purchased from GRISP (Portugal). Fetal bovine serum (FBS) and cell culture media Dulbecco's modified Eagle's medium (DMEM) were acquired from PAN-Biotech (Germany), DKFZ HaCaT adherent cell line (immortalized human keratinocytes) was obtained from Cell Lines Service (CLS, Germany) and provided by DKFZ (Germany). All reagents were used without further purification.

2.2. Production of lyocell/silver knitted fabrics

Lyocell spun yarns and silver-plated polyamide multifilament yarns were used to produce full *Jacquard* seamless weft fabrics via knitting machine Santoni model SM08-EVO4J (Sidónios Seamless Tech S.A), with a E28 gauge, diameter of 13" and 1152 latch needles. The fabrics were produced with varying percentages of silver content (w/w), specifically 0.00 % (sample 0), 0.31 % (sample 1), 0.65 % (sample 2), 1.03 % (sample 3), and 1.46 % (sample 4), and with four distinct structures, namely single jersey (sample J), "false" rib 1 × 1 (sample R), single pique (sample P), and "false" interlock (sample I). The silver content percentages were carefully selected to optimize processability and ensure structural integrity of the knitted fabrics; higher silver-containing yarn contents were avoided due to the resulting thin samples with excessively large pores, which were deemed unsuitable for the intended application.

Fabrics were washed three times in a 200 mL distilled water (dH_2O) bath at 50 rpm, 90 ± 5 °C for 30 min using an Ibelus C-720 machine (Portugal) equipped with infrared heating. The primary objective was to remove the surface treatment (desizing), specifically paraffin wax applied to the yarns.

2.3. Characterization

Lyocell/silver and lyocell (control) knitted fabrics were subjected to a comprehensive analysis of their physical, chemical, and thermomechanical properties. In specific examinations, the inherent structure of the knitted fabrics was considered inconsequential (did not influence the outcomes). In such scenarios, consideration was given to samples with a single jersey structure, as this represents the simplest structural arrangement from which the others derive; in those situations, jersey structure may be perceived as a control structure.

2.3.1. Reflectance test

The reflectance of the knitted fabrics was evaluated pre- and post-washing using a Shimadzu UV-VIS spectrophotometer UV-2600 (Japan) in the range between 400 and 700 nm. Data were expressed as percentage of reflectance.

2.3.2. Morphology

The morphology of the samples was observed by brightfield

microscopy using a Leica DM IL LED inverted microscope (Leica Microsystems, Germany). Additionally, images were taken using a Leica EZ4 D magnifier (Leica Microsystems) at a magnification of $30\times$, and these images were collected with a digital camera (OnePlus Nord N10 5 G).

2.3.3. Physical properties

The physical properties of the knitted fabrics were characterized by mass per unit area (digital scale), contexture (using a yarn-counting magnifying glass), loop length (based on 10 measurements of the length of the yarn, collected from 10 wales; Artialab Sodemat meter), thickness (10 measurements in random areas of the samples, using a Mitotoyo analog micrometer with a resolution of 0.01 mm, 10 mm of pressure area and 18 Pa of applied pressure), and porosity calculated using the following equation (Eq. 1):

$$\varepsilon (\%) = \left(1 - \frac{\rho_a}{\rho_b \times t}\right) \times 100 \quad (1)$$

where ρ_a (g/cm³) is the fabric density (weight per unit area), ρ_b (g/cm³) is the fiber density, and t (cm) is fabric thickness [18].

2.3.4. Fourier-transform infrared spectroscopy in attenuated total reflectance mode (ATR-FTIR)

The chemical composition of lyocell/silver and lyocell knitted fabrics (single jersey structure) pre- and post-washing were analyzed by ATR-FTIR using an IRAffinity-1S, Shimadzu spectrophotometer (Japan), coupled with a HATR 10 accessory with a diamond crystal. Spectra were obtained over a range of 4000–400 cm⁻¹, with 200 scans being performed at a spectral resolution of 4 cm⁻¹.

2.3.5. Thermal gravimetric analysis (TGA)

The knitted fabrics (single jersey structure) thermal degradation behavior pre- and post-washing was assessed by weight loss monitoring on a STA 7200 Hitachi® (Japan), using aluminum crucibles. The assessment involved increasing the temperature within the range of 25–600 °C, at a heating rate of 10 °C/min under a dynamic nitrogen atmosphere at a flow rate of 200 mL/min to create an inert environment. Each sample had an initial mass of 6.7 ± 0.2 mg. Results were plotted as the percentage of weight loss vs. temperature.

2.3.6. Differential scanning calorimetry (DSC)

Thermal properties were also evaluated by DSC analyses using a power compensated DSC equipment PerkinElmer DSC 6000 (USA). Samples weighing 7.2 ± 0.6 mg were placed in an aluminum pan and exposed to a heating rate of 10 °C/min, from 0 to 445 °C, under a dynamic nitrogen atmosphere at 20 mL/min. DSC curves were plotted with heat flow vs. temperature.

2.3.7. Quasi-static mechanical testing

The mechanical properties of the knitted fabrics were measured using a uniaxial tensile test on the MTS™ Insight Electromechanical Testing Systems (USA) with a 10 kN load cell, following the ASTM D5035–11(2019) standard. The samples were prepared with dimensions of 2×7 cm, gauge length of 50 mm and analyzed at room temperature (RT). The tests were performed with a preload of 0.1 N and a speed of 10 mm/min until reaching the maximum elongation at break. Experiments were conducted on at least five replicates in each direction of the knitted fabric (course and wale). Young's modulus (MPa), total elongation (%) and ultimate tensile strength (MPa) were obtained from stress-strain curves.

2.3.8. Degree of swelling (DS)

The knitted fabrics DS was evaluated in dH₂O, PBS and artificial wound exudates (pH 6.72 and pH 9) solutions at 37 °C. To conduct the assessment, samples with a surface area of 2 cm² were weighed and

submerged in 2 mL of the respective medium for 1, 2, 4, 6, 24, 48, 72 and 168 h under static conditions. At each specified interval, the samples were collected, and their wet weight was measured. Gauze was used as reference. The DS was calculated using the following equation (Eq. 2):

$$DS (\%) = \frac{m_w - m_d}{m_d} \times 100 \quad (2)$$

where m_w (mg) is the weight of the sample after immersion and m_d (mg) is the weight of the dried sample before immersion [19].

2.3.9. Knitted fabric degradation profile

The degradation profile of knitted fabrics over time was assessed in dH₂O, PBS and artificial wound exudates (pH 6.72 and pH 9) solutions. Hydrated knitted fabrics, with a surface area of 2 cm², were weighed and incubated in 2 mL of each solution at 37 °C, up to 28 days. Experiments were conducted under static conditions, with solutions being exchanged every week. After 1, 3, 7, 14, 21, and 28 days of incubation, the samples were weighed. Gauze was used as reference. Degradation, determined by measuring mass loss, was calculated using the following equation (Eq. 3):

$$\text{mass loss } (\%) = \frac{m_{wi} - m_{wf}}{m_{wi}} \times 100 \quad (3)$$

where m_{wi} (mg) represents the weight of the hydrated knitted fabric at day 0, and m_{wf} (mg) corresponds to the weight of the sample after each incubation period [20].

2.3.10. Moisture and air permeabilities

To assess the knitted fabrics' capacity for moisture retention and air exchange, water/vapor and air permeabilities studies were conducted according to standards BS 7209:1990 and ASTM D 737–96, respectively. Gauze served as reference material in both tests. For the water vapor permeability (WVP) examination, samples (132.7 mm² area) were placed over cylindrical cups containing 46 mL of dH₂O for 24 h, utilizing an SDL Atlas M261 Shirley water vapor permeability tester (USA). The evaporation of water through the fabric under test was determined by weighing the cup before and after the testing period. An open cup was used as reference. Assessments were performed at 37 °C and 50 % of relative humidity within an Aralab FitoClima 150 EDTU chamber (Portugal). Measurements were conducted in triplicate. The water vapor transmission rate (WVTR) was determined using Eq. 4:

$$WVTR = \frac{24\Delta W}{A\Delta t} \quad (4)$$

where ΔW is the difference in the water weight (g) before and after the 24 h test, A is the inner area of the cup (m²), and Δt is the exposure time (h).

A FX 3300 air permeability tester III (Textest Instruments, Switzerland) was employed to apply an air pressure of 100 Pa on each sample at ten equidistant points of 20 cm².

2.4. Antimicrobial evaluation

To assess the antimicrobial efficacy of the knitted fabrics, time-kill kinetics tests were conducted using an adaptation of the ASTM E2149–01 standard. Here, only Gram-negative bacteria were examined due to the greater challenges associated with their effective elimination by antimicrobial wound dressings, compared to Gram-positive. Bacteria inoculums were prepared in TSB (*E. coli*) and NB (*P. aeruginosa*) and incubated overnight at 37 °C, 120 rpm. Then, bacteria suspensions were adjusted to a concentration of 1×10^5 colony forming units (CFUs)/mL in MHB. Samples with a surface area of 1 cm² were immersed in 1 mL of bacteria suspension and incubated at 37 °C and 120 rpm. At specific time intervals (1, 2, 4, 6 and 24 h), aliquots of the bacterial suspensions were collected and serially diluted in PBS (10^{-1} to 10^{-5}), plated on TSA/

NA, and further incubated at 37 °C for 24 h. Colonies of surviving bacteria were counted, and the results were expressed as log reduction by comparing the control suspensions (without sample) with those exposed to the knitted fabrics.

2.5. Antioxidant activity

The free radical scavenging activity of the knitted fabrics was determined using the DPPH assay. Samples with a surface area of 2 cm² were incubated in 2 mL of DPPH stock solution (100 μM, 100 % ethanol) at 37 °C and 120 rpm. At specific time intervals (1, 2, 4, 6, 24 and 48 h), aliquots were collected, and the absorbance was measured at 515 nm in a microplate reader EZ READ 2000 (Biochrom, UK). Trolox was used as a positive control, and DMSO was used as a negative control. The results were expressed in terms of the percentage of reduced DPPH at steady state (DPPHr) and were calculated following the Eq. 5:

$$\text{DPPHr (\%)} = \frac{A_c - A_s}{A_c} \times 100 \quad (5)$$

where A_c is the absorbance of control (without sample), and A_s is the absorbance of samples registered at steady state.

2.6. Cytocompatibility testing

The metabolic activity of human keratinocytes cell line (HaCaT) [21] was assessed through an indirect contact assay conducted on the knitted fabrics identified with the most effective structural characteristics for the foreseen purpose (based on the previous characterization testing). Samples with a surface area of 4 cm² were sterilized under ultraviolet light (10 min on each side) and submerged in 4 mL of DMEM medium for 24 h (conditioned media). Cells were thawed and sub-cultured in DMEM medium supplemented with 10 % (v/v) inactivated FBS and 1 % (v/v) penicillin-streptomycin. The cells were kept at 37 °C in a humidified atmosphere with 5 % CO₂ until reaching 80–90 % confluence. Thereafter, the cells were chemically detached from the culture flasks using 0.05 % (w/v) trypsin-EDTA solution. Cell counting, facilitated by a Neubauer Chamber and trypan blue (ratio 1:1 v/v) staining for dead cells exclusion, was conducted using a Leica DM IL LED Inverted Laboratory Microscope (Germany). Cells were then seeded in 96-well tissue culture polystyrene plates (TPP®, Switzerland) at a density of 1×10^4 cells per well and incubated at 37 °C and 5 % CO₂ for 24 h. The experiments were carried out using HaCaT in passages 5–11.

After 24 h, the conditioned media were diluted to concentrations of 50 %, 25 % and 10 % (v/v), and subsequently added to the cells for further incubation periods of 24 and 48 h. Following these intervals, 10 % (v/v) resazurin was added to the wells and incubated for 4 h at 37 °C and 5 % CO₂. Then, fluorescence levels were measured ($\lambda_{\text{ex}} = 560$ nm and $\lambda_{\text{em}} = 590$ nm) using a microplate reader (Synergy H1, BioTek Instruments, USA). To establish positive and negative controls, DMEM conditioned media (without samples) and 30 % (v/v) of DMSO were used, respectively. The percentage of cell metabolic activity, used as indicative of cell viability, was calculated using Eq. 6:

$$\text{Metabolic activity (\%)} = \frac{\text{Flu}_s}{\text{Flu}_{\text{pc}}} \times 100 \quad (6)$$

where Flu_s represents the fluorescence of the samples, and Flu_{pc} is the fluorescence of the positive control.

2.7. Statistical analysis

All experiments were conducted in triplicate unless otherwise referred to in the experimental sections. Numerical data were reported as mean $\pm$ standard deviation (SD). Data were treated using GraphPad Prism 8.0.1 Software (GraphPad Software Inc., USA). Normality analysis was performed, and results were analyzed using Tukey's test.

Statistically significant differences were considered at $p < 0.05$.

3. Results AND Discussion

3.1. Morphology and physical properties

The production of the knitted fabrics was highly successful, with no defects detected in any of the structures. After manufacturing, the fabrics underwent a series of washing procedures to eliminate potential paraffin residues from the yarns, and a decrease in water turbidity was observed after each wash. Reflectance tests were conducted to assess the effectiveness of these washes (Figure S1 in the Supporting Information file). Post-washing, the increase in reflectance values confirmed the successful elimination of paraffin, enhancing the surface reflectivity of the samples. Additionally, weighing the samples before and after washing revealed a mass loss of 3.65 ± 0.21 %. Brightfield microscopy examinations conducted after washing confirmed the integrity of the yarns, indicating that the fabrics remained intact and undamaged (Figure S2 in the Supporting Information file).

Fig. 1 shows the successful production of sample 4 with the desired knitted structures. This sample, which contained the highest percentage of silver-plated polyamide yarns (1.46 %), was selected as the representative sample to illustrate the structural organization of the knitted fabrics. To enhance visualization, loop diagram illustrations and brightfield microscopy images of each structure were included. The presence of the silver-plated yarns was evident by the increased shine and metallization observed. The images display both sides of the knitted fabrics, where the distinct appearance between them is noticeable. The use of seamless technology presents a significant advantage for the intended application, as it enhances the comfort and adaptability of the fabrics to the body, reducing friction and the risk of skin irritation, making it ideal for products where direct skin contact is constant. Additionally, it offers properties such as breathability, durability, and easy care [4]. The rib and interlock structures are classified as “false” because they are produced on a seamless knitting machine, resulting in differing appearances between sides. To the contrary, “true” rib and interlock structures appear identical on both sides.

Single jersey was the simplest and most fundamental knitted structure, serving as a basis for more complex patterns. Its technical face features vertical columns of knit stitches forming small V-shaped loops, while the technical back displays horizontal rows of purl stitches, which look like small horizontal loops or bumps (Fig. 1a). The “false” rib 1 $\times$ 1 structure exhibits a technical face with alternating columns of knit and purl stitches, and a technical back with more prominent rows of purl stitches, creating a smoother and less textured appearance (Fig. 1b). In the single pique structure, the technical face features small, raised bumps resembling a waffle-like pattern, created by tuck stitches that provide a three-dimensional look. The technical back is more uniform and smoother, with visible horizontal rows of purl stitches (Fig. 1c). Lastly, the “false” interlock structure has a technical face characterized by a smooth, flat surface created by the interlocking of knit stitches. The technical back is more textured, featuring a combination of knit and purl stitches that form a series of raised bumps or ridges (Fig. 1d) [22].

The physical properties of knitted fabrics are detailed in Table 1 (statistical analysis shown in Tables S1 and S2 in the Supporting Information file). Data from Table 1a indicates that an increase in the amount of silver-plated polyamide yarns correlates with a statistically significant decrease in mass per unit area. This observation aligns with expectations, as the dtex of these yarns is significantly smaller than that of the lyocell yarns, resulting in a reduced overall weight. Among the knitted fabric structures, interlock registered the largest weight. Regarding contexture (Table 1a), structures were generally similar, with the exception of the rib structure, which reported smaller values, particularly in the number of wales per cm ($p < 0.0001$). Additionally, this structure displayed greater loop length (Table 1a) and porosity (Table 1b), all indicatives of a more open knit [23]. Table 1b shows that

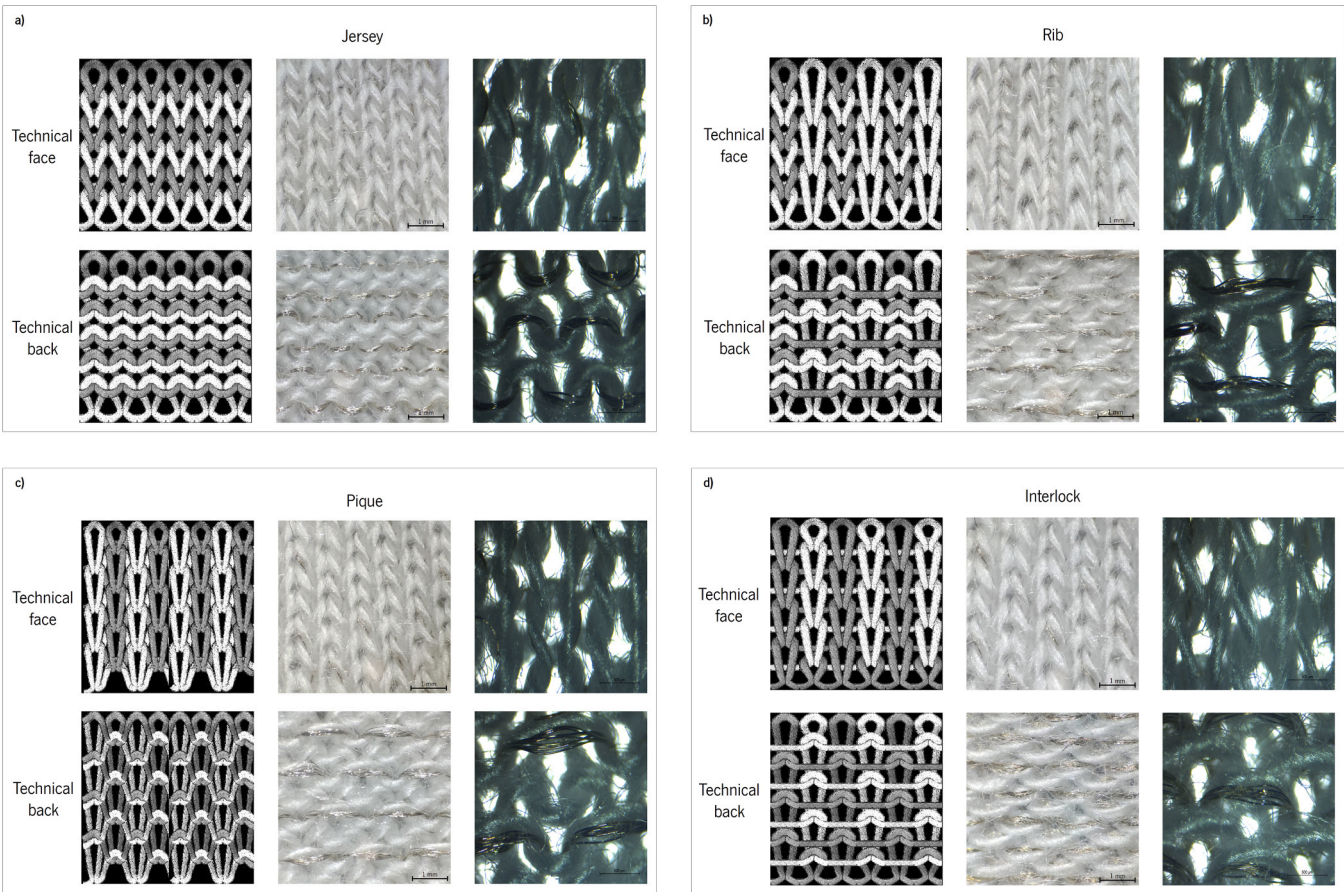


Fig. 1. Loop diagram, digital and brightfield microscopy images of the (a) jersey, (b) rib, (c) pique and (d) interlock structures, obtained from knitted fabrics containing the largest number of silver-plated yarns (sample 4 – 1.46 %). Scale bars represent 1 mm and 500 μm , for digital and brightfield microscopy images, respectively.

Table 1

Physical characterization of the knitted fabrics: (a) mass per unit area, contexture and loop length; (b) thickness and porosity (ϵ). Data are reported as mean $\pm$ SD ($n = 10$). Sample 0 corresponds to 100 % lyocell, while samples 1, 2, 3 and 4 correspond knitted fabrics with silver in their composition at 0.31 %, 0.65 %, 1.03 %, and 1.46 %, respectively.

a)												
Mass per unit area (g/m^2)					Contexture (courses/wales per cm)				Loop length (cm)			
Sample	Jersey	Rib	Pique	Interlock	Jersey	Rib	Pique	Interlock	Jersey	Rib	Pique	Interlock
0	208.6 $\pm$ 8.3	216.1 $\pm$ 5.0	219.4 $\pm$ 3.9	241.1 $\pm$ 6.6	13/16	13/11	13/15	13/15	33.8 $\pm$ 0.4	34.0 $\pm$ 0.2	21.8 $\pm$ 0.4	22.0 $\pm$ 0.3
1	198.8 $\pm$ 6.7	221.5 $\pm$ 4.3	217.0 $\pm$ 5.6	247.5 $\pm$ 6.0	13/16	12/11	13/16	13/15				
2	191.0 $\pm$ 7.2	210.0 $\pm$ 3.9	200.9 $\pm$ 5.7	222.5 $\pm$ 5.6	13/16	13/11	13/16	13/16				
3	170.9 $\pm$ 12.1	203.0 $\pm$ 3.9	196.8 $\pm$ 4.1	222.8 $\pm$ 5.3	13/16	13/11	13/17	14/15				
4	172.4 $\pm$ 11.8	182.3 $\pm$ 3.1	204.0 $\pm$ 8.1	216.3 $\pm$ 7.3	14/16	14/11	13/16	14/14				
b)												
Thickness (mm)					ϵ (%)							
Sample	Jersey	Rib	Pique	Interlock	Jersey	Rib	Pique	Interlock	Jersey	Rib	Pique	Interlock
0	0.58 $\pm$ 0.03	0.66 $\pm$ 0.04	0.62 $\pm$ 0.03	0.67 $\pm$ 0.02	68.5 $\pm$ 1.0	72.3 $\pm$ 1.7	69.1 $\pm$ 1.0		68.9 $\pm$ 0.8			
1	0.54 $\pm$ 0.03	0.63 $\pm$ 0.06	0.61 $\pm$ 0.03	0.66 $\pm$ 0.03	67.8 $\pm$ 1.4	69.6 $\pm$ 1.6	68.6 $\pm$ 0.6		67.0 $\pm$ 1.3			
2	0.56 $\pm$ 0.03	0.66 $\pm$ 0.06	0.61 $\pm$ 0.04	0.64 $\pm$ 0.05	70.5 $\pm$ 1.8	72.3 $\pm$ 0.9	71.5 $\pm$ 2.0		70.1 $\pm$ 0.9			
3	0.56 $\pm$ 0.02	0.63 $\pm$ 0.04	0.58 $\pm$ 0.03	0.66 $\pm$ 0.04	73.6 $\pm$ 2.2	72.4 $\pm$ 1.0	70.0 $\pm$ 1.5		71.0 $\pm$ 0.1			
4	0.56 $\pm$ 0.02	0.62 $\pm$ 0.04	0.55 $\pm$ 0.03	0.63 $\pm$ 0.03	73.6 $\pm$ 1.5	74.8 $\pm$ 2.4	72.8 $\pm$ 0.9		71.6 $\pm$ 0.5			

the thickness of the samples was relatively constant, regardless of the varying amounts of silver-plated polyamide yarns (not statistically significant, except R4 and P4). The interlock structure was found the thickest from the group, while the jersey was the thinnest ($p < 0.0001$). It was anticipated that the small dtex of the silver-plated polyamide yarns would result in a significant increase in porosity as their content augmented in the fabrics (Table 1b). Even though there was a slight increase between 2 % and 5 %, it is likely that the superior elasticity of

the silver-plated polyamide yarns (25.04 $\pm$ 5.11 %) compared to the lyocell yarns (8.04 $\pm$ 0.55 %) to have facilitated the adaptation of the first to the fabric construct, thus occupying the same spaces as lyocell yarns and slightly constricting their freedom. As can be seen by Fig. 1, silver-plated yarns appear to overlap with lyocell yarns.

3.2. Chemical analysis

ATR-FTIR analyses were performed on knitted fabrics exhibiting a jersey structure, both pre- and post-washing, with the aim of evaluating the efficacy of paraffin wax layer removal from the yarns (Fig. 2). In Fig. 2a, the presence of paraffin in the fabrics was observed through the detection of two distinct bands at 2916 and 2849 cm^{-1} , corresponding to the antisymmetric and symmetric stretching vibration of aliphatic hydrocarbons, respectively, which are main components of paraffin wax [24]. Post-washing (Fig. 2b), these paraffin-related bands disappeared, while a distinctive peak at 2896 cm^{-1} attributed to the CH alkane group in lyocell [25] became more pronounced. Furthermore, both spectra

displayed a peak at 3320 cm^{-1} corresponding to the -OH stretching band of cellulose, alongside notable peaks at 1018 and 895 cm^{-1} related to the P = O and P-O-C group, respectively [26].

In contrast, no peaks corresponding to polyamide or silver were detected. The absence of polyamide peaks can be attributed to the low mass ratio of silver-plated polyamide yarns to lyocell yarns, which hinders their detection. Additionally, the silver coating presents challenges due to its high electrical conductivity, which limits its spectral response in the infrared range. FTIR is a technique that provides infrared spectral analysis of samples capable of absorbing infrared light. However, the free electrons within the silver's structure do not engage in the typical vibrational transitions observed in molecules during FTIR

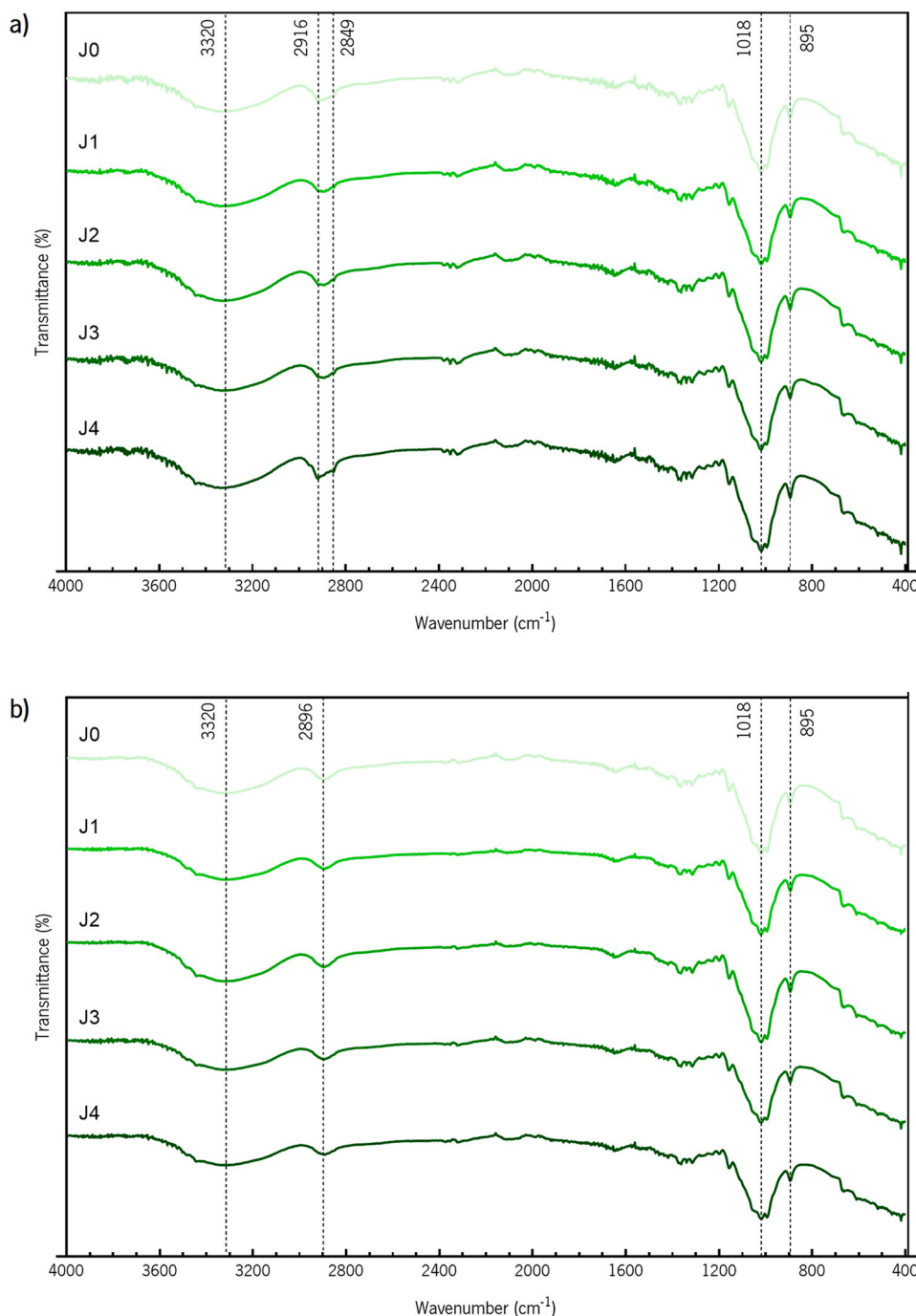


Fig. 2. ATR-FTIR spectra of lyocell and lyocell/silver knitted fabrics (a) pre- and (b) post-washing. Sample J corresponds to the jersey structure with 100 % of lyocell (sample 0), and 0.31 % (sample 1), 0.65 % (sample 2), 1.03 % (sample 3) and 1.46 % (sample 4) represent samples containing silver yarns at various ratios.

spectroscopy. Consequently, the absorption of infrared radiation, fundamental to FTIR spectroscopy, is not readily observed in metals such as silver [27].

3.3. Thermal stability

The thermal stability of the knitted fabrics (using the jersey structure as example) was evaluated using TGA (Figs. 3a, 3b and Table S3 in the Supporting Information file) and DSC (Figs. 3c, 3d and Table S3 in the Supporting Information file). The thermal degradation steps of the samples, both pre- and post-washing processes, were comprehensively analyzed using TGA. All samples showed similar thermal behavior. Initially, a distinctive degradation phase was observed up to around 112 °C, resulting in a mass loss of $\approx 8\%$ associated with the evaporation of water molecules bound to the lyocell structure. The process involves a sequence of depolymerization, hydrolysis, and dehydration reactions [26]. Subsequently, the fabrics maintained a thermal stability up to around 255 °C for the pre-washed samples and 280 °C for the post-washed samples. This discrepancy in thermal stability can be explained by the presence of paraffin in the unwashed samples, which volatilizes at low temperatures, primarily contributing to the earlier onset of degradation [28]. The start of the second degradation stage, extending up to around 368 °C, was marked by a substantial mass loss of $\approx 67\%$. This significant degradation stems predominantly from the decomposition of lyocell, which constitutes the primary component of the studied samples. During this stage, the breakdown of the crystalline structure of lyocell occurs, leading to the generation of L-glucose [26]. Following the extensive degradation of lyocell, a third weight loss stage was observed, characterized by a slight mass loss of $\approx 9\%$, attributed to the gradual decomposition of char residues originating from the lyocell yarns (residue from the prior degradation steps) [29]. Additionally, this stage may involve the thermal degradation of polyamide components present within the silver-containing knitted fabrics [30]. Thermal analysis using TGA highlights the thermal resistance of the fabrics, and

its main polymeric components, which exceed typical body temperatures. Thus, this thermal stability supports potential applications of these fabrics in wound dressings, guaranteeing their integrity and performance under physiological conditions.

Correlation between TGA and DSC thermograms facilitates the comprehension of the thermal phenomena occurring during the heat treatment of the samples. The initial broad endothermic event, featuring peaks within the temperature range of 100 – 116 °C with a high thermal energy of ≈ 1383.27 mJ, is attributed to the desorption of absorbed water molecules from cellulose [31]. Subsequent to moisture evaporation, a well-defined endothermic event, characterized by a peak at 258 – 261 °C, is exhibited only in samples containing silver-plated polyamide yarns; the peak area increases with the augment in number of silver-containing yarns within the samples. Upon further heating at 349 – 353 °C, another endothermic event becomes apparent due to structural transformations of cellulose, encompassing decomposition and depolymerization reactions, followed by an exothermic event associated with the subsequent decomposition of the resultant complex products [32]. The influence of paraffin was insignificant and was not detected by DSC, as its thermal decomposition occurs at around 60 – 70 °C [33], being likely masked by the significant water loss registered from lyocell yarns. This is corroborated by a small fabric weight loss observed after washing ($3.65 \pm 0.21\%$), thus indicating its minimal contribution to the samples. Similar results were observed in the interlock structure containing the largest number of silver-plated yarns (sample 4–1.46 %), thereby confirming that the findings are independent of the knitted fabric structure (Figure S3 in the Supporting Information file).

3.4. Mechanical performance

The mechanical resistance of the knitted fabrics was assessed in the course and wale directions, using parameters such as Young's modulus, elongation at break, and ultimate tensile strength (Fig. 4; statistical analysis shown in Table S4 in the Supporting Information file). Young's

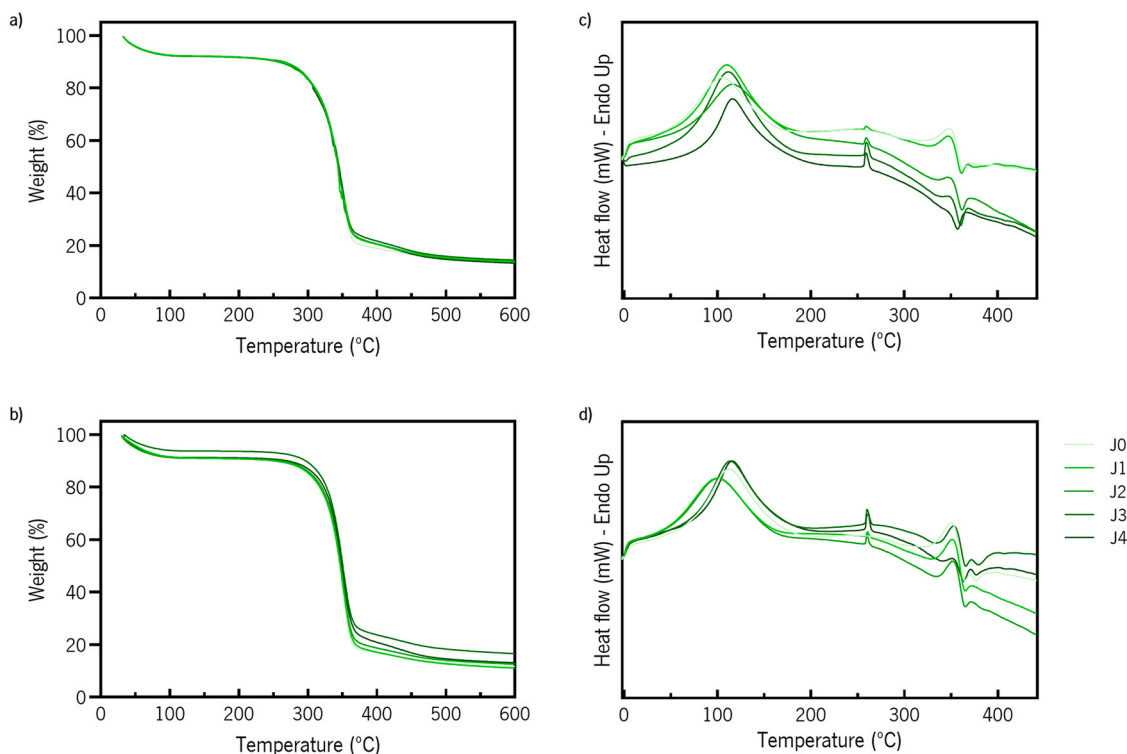


Fig. 3. TGA and DSC spectra of lyocell and lyocell/silver knitted fabrics (a and c) pre- and (b and d) post-washing, respectively. Sample J corresponds to the jersey structure with 100 % of lyocell (sample 0), and 0.31 % (sample 1), 0.65 % (sample 2), 1.03 % (sample 3) and 1.46 % (sample 4) represent samples containing silver yarns at various ratios.

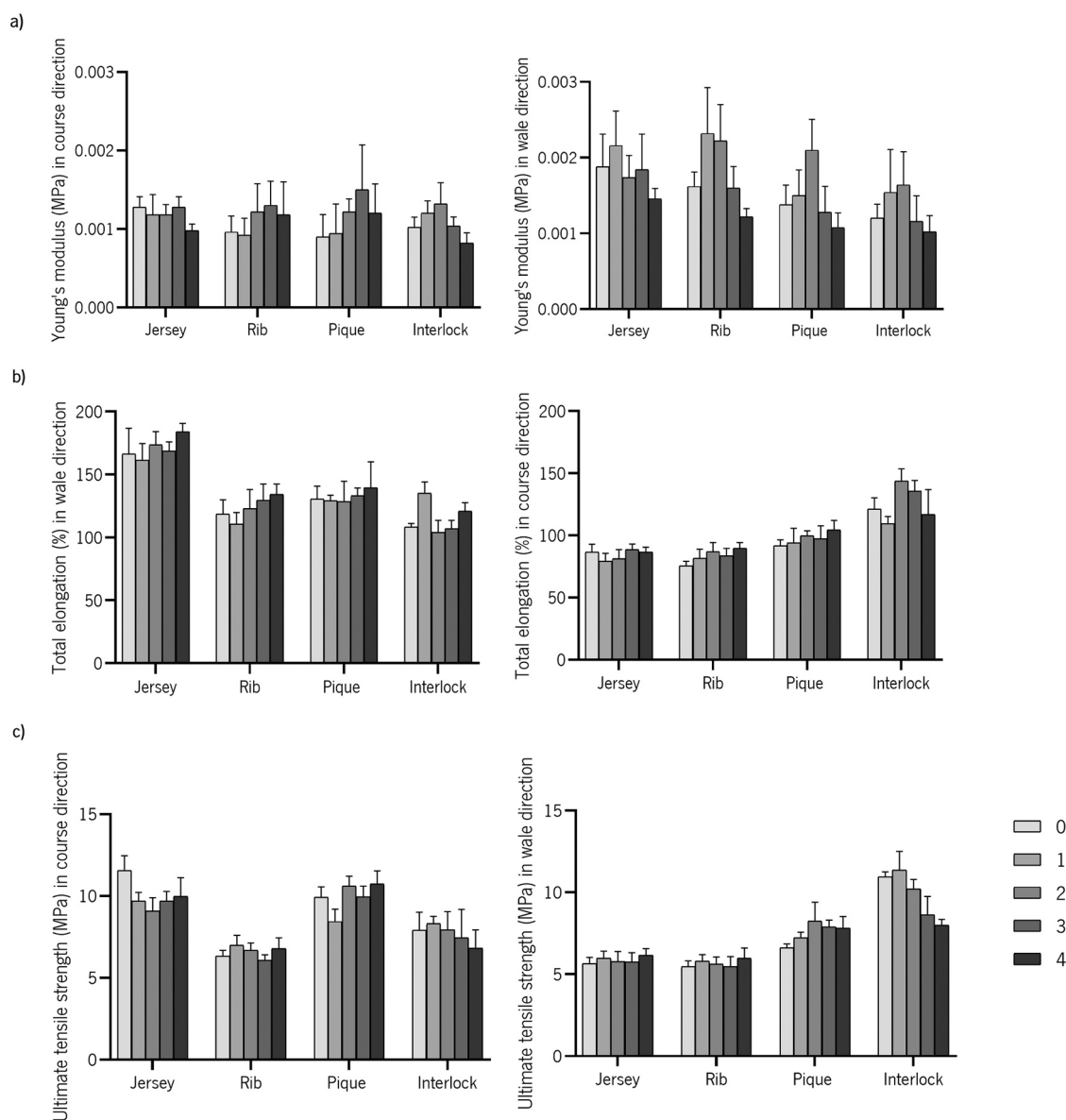


Fig. 4. (a) Young's modulus, (b) total elongation, and (c) ultimate tensile strength of the knitted fabrics in course and wale directions. Data are reported as mean $\pm$ SD ($n = 5$). Sample 0 corresponds to 100 % lyocell, while samples 1, 2, 3 and 4 correspond knitted fabrics with silver in their composition at 0.31 %, 0.65 %, 1.03 %, and 1.46 %, respectively.

modulus quantifies the relationship between stress (applied force) and strain (resultant deformation) in the linear region in the knitted fabrics. Elongation at break and tensile strength are particularly important for wound dressings: a high elongation at break indicates flexibility, aiding in skin application (adaptability to injured site), while high tensile strength ensures the fabric's integrity during wound placement or removal, minimizing patient discomfort and preventing damage to sensitive tissue [34,35]. Anisotropic behavior was observed in the sample's structures, characterized by distinct responses along the course and wale directions. Specifically, the wale direction exhibited higher Young's modulus and elongation at break for the jersey, rib, and pique structures, at the expense of a reduced strength. This suggests that these structures, while more flexible in this direction, may be more prone to rupture under excessive mechanical stress. Conversely, the interlock structure demonstrated a more uniform mechanical response between directions, with similar Young's modulus and elongation at break and a slightly higher tensile strength in the wale direction. These findings indicate that interlock structures may offer enhanced isotropy, which can be beneficial for applications requiring balanced mechanical

properties. For a clearer comparison between directions, representative stress-strain curves of tested structures, namely those composed of 100 % lyocell and the highest number of silver-plated yarns (sample 4), are provided in Figure S4 in the Supporting Information file. Analysis of these curves reveals that the wale direction generally exhibited a more abrupt rupture with lower overall deformation, indicating reduced ductility. This behavior suggests that, despite offering greater stiffness and elongation capacity, the wale direction may be more susceptible to sudden failure under tensile loading. These insights are crucial for optimizing the mechanical properties of knitted fabrics in biomedical applications, where a balance between flexibility and strength is essential for functional performance.

Overall, the samples displayed low Young's modulus (Fig. 4a), indicating a high degree of flexibility suitable for the intended purposes. The samples with the highest silver content (sample 4) exhibited the lowest stiffness in wale direction. All samples showed considerable deformability (Fig. 4b), with maximum elongation at break ranging from ≈ 75 –184 %, notably higher in the interlock structure in the wale direction ($p < 0.0001$) and in the jersey structure in the course direction

($p < 0.0001$). The rib structure demonstrated minimal deformation compared to other structures (Fig. 4c), and, consequently, the lowest strength ($p < 0.0001$). In general, the analyzed knitted fabrics possess mechanical properties that meet the criteria required for effective wound dressing development. However, the interlock structure can be deemed the most suitable for dressings intended for wounds in highly mobile body areas, such as DFUs, due to its lower anisotropic behavior, which may contribute to an improved conformability and mechanical stability in dynamic conditions.

3.5. Degree of swelling and degradation profile

Ensuring optimal wound healing requires the use of dressings with adequate moisture absorption and retention capabilities. This guarantees that the dressing not only absorbs excess exudates proficiently, preventing desiccation, but also maintains a moist environment in the wound, conducive to tissue repair and regeneration, essential for the progression of the healing process [36]. The swelling capacity of knitted fabrics under various conditions (dH₂O, PBS, and artificial wound exudates at pH 6.72 and pH 9) was monitored over a period of 7 days. Fig. 5 presents the measurements on the 7th day of incubation (statistical analysis shown in Tables S5 and S6 in the Supporting Information file), while the measurements monitored up until day 7 of incubation are illustrated in Figure S5 in the Supporting Information file. PBS was chosen due to its physiological similarity to human biological fluids in terms of osmotic and ionic conditions [37]. Artificial wound exudate solutions were prepared according to the characteristic environmental pH of chronic wounds, which typically falls within the range of 7.15–8.9 [38]. Additionally, dH₂O was included for comparative purposes, to assess the influence of the presence of salts in the media.

After the first hour, the knitted samples exhibited a significant DS, with an average weight increase ranging from ≈ 161 –236 %, while gauze, the control group, reached a DS above 328 %. This difference underscores the distinct absorption behaviors of knitted fabric compared to gauze, which possesses a more open and loosely packed structure, allowing for rapid liquid uptake. Similar trends were observed after 7

days of incubation, with DS values stabilizing in the range of ≈ 137 –227 %, demonstrating the sustained absorption capacity of the samples over time. A slight reduction in absorption capacity (≈ 36 %) was observed in the knitted fabrics when immersed in dH₂O after 6 h (jersey and rib structures) and 24 h (pique and interlock structures; Figure S5 in the Supporting Information file). This decrease can be attributed to the saturation of hydrogen bonding interactions between the water molecules and the hydroxyl groups of the lyocell celluloses [29]. Notably, a similar trend was observed in the presence of PBS, where a ≈ 27 % decrease in absorption capacity was recorded after 24 h (jersey and rib structures) and 48 h (pique and interlock structures). This reduction is possibly due to the competitive interaction of PBS salts with hydroxyl groups, altering the fabric's ability to retain free water molecules. The variation in saturation times between different structures is primarily related to their morphological characteristics; jersey, with its lower thickness values (Table 1b), saturates more quickly, while rib, with its denser architecture and compact arrangement, also reaches saturation sooner than pique and interlock structures (Fig. 1). When exposed to artificial wound exudates, all knitted structures exhibited comparable swelling behavior, maintaining an equilibrium DS (averaging 197 %) over the 7-day period at pH 6.72. On the other hand, exposure to alkaline exudates (pH 9) led to a moderate increase in absorption capacity (≈ 13 %) after 6 h (Figure S5 in the Supporting Information file).

The hydrophilicity of knitted fabrics is positively correlated with their absorption capacity, as higher hydrophilicity generally leads to increased fluid uptake. Despite multiple attempts, wettability data could not be obtained for any sample due to the extreme hydrophilicity of lyocell, facilitated by its high content of carboxylic groups [39].

An ideal wound dressing typically exhibits an absorbency range between 100 % and 900 % [40], positioning the engineered samples within the appropriate threshold for this application. Among the tested structures, the interlock exhibited the highest absorption ratings, on average, across all tested solutions. This enhanced performance can be attributed to its greater thickness (Table 1b), bulkier structure, and increased void space (Fig. 1), which collectively enabled higher liquid

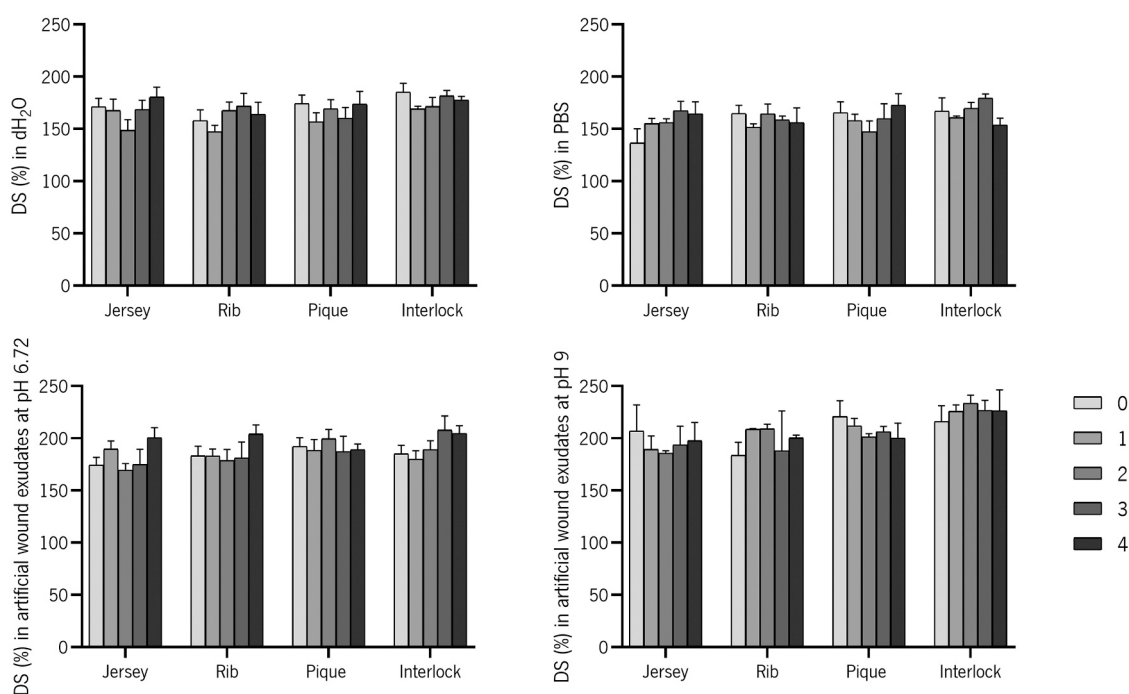


Fig. 5. Degree of swelling (DS) measurements of the knitted fabrics after 7 days of incubation in dH₂O, PBS, and artificial wound exudates at pH 6.72 and pH 9. Data are presented as average percentage of DS $\pm$ SD ($n = 3$). Sample 0 corresponds to 100 % lyocell, while samples 1, 2, 3 and 4 correspond knitted fabrics with silver in their composition at 0.31 %, 0.65 %, 1.03 %, and 1.46 %, respectively.

retention. Additionally, porosity and fiber orientation play crucial roles in dictating the absorption characteristics of these structures [41]. Overall, these findings highlight the effective absorption capabilities of the evaluated knitted fabric samples when in contact with a wound, underscoring their potential as viable options for wound dressing formulations.

The physical stability of the samples in contact with the same solutions (dH₂O, PBS, and artificial wound exudates at pH 6.72 and pH 9) was monitored over a 28-day incubation period. The degradation profile was assessed by tracking changes in mass over time. Fig. 6 presents the results of the last day of incubation (statistical analysis shown in Tables S7 and S8 in the Supporting Information file), while the measurements monitored throughout the 28-day incubation period are illustrated in Figure S6 in the Supporting Information file. Data revealed that the samples lost less than 20 % of their initial mass throughout the entire test duration, demonstrating significant physical stability over time regardless of knitting structure. Comparable results were observed with gauze. Figure S7 in the Supporting information file displays the samples' appearance after 28 days, showing no visible signs of degradation. However, submersion in aqueous solutions caused a change in fiber tension, leading to curling of the knitted structure. The minor mass losses observed may be attributed to the dissociation of carboxyl groups from the lyocell yarns and the release of silver ions (Ag⁺). However, mass fluctuations, particularly gains, were recorded during the test, likely resulting from interactions between the samples and salts present in PBS and artificial wound exudates, and possibly ions in water. Other factors influencing lyocell degradation have been documented. For instance, Dadashian and Wildin [42] showed that lyocell fibers lost near 3.3 % of their initial mass after 7 days of exposure to simulated sunlight. Royer *et al.* [43] exposed lyocell fabrics to real sea-surface conditions and observed complete degradation after 30 days. To further understand the degradation profile of these knitted structures, future research should include other testing conditions, such as extending the contact time with the tested solutions up to 90 days, applying a wider range of

temperatures and even evaluating their performance after prolonged sunlight exposure. These expanded conditions could provide further insights into the potential range of applications for the fabrics. Results suggest that these samples possess excellent stability for their intended application in wound dressings and may also be suitable for other long-term biomedical uses.

3.6. Permeabilities

As mentioned earlier, moisture management is a critical factor in facilitating proper wound healing, as it plays a vital role in promoting re-epithelialization. This process is mainly influenced by factors such as dressing absorption capacity, moisture vapor transmission, and air permeability [44]. Maintaining an appropriate moisture level at the wound site is imperative. Excessive moisture can lead to maceration of the wound bed, impairing the healing process and increasing susceptibility to infections. Conversely, inadequate moisture can result in desiccation, leading to delayed wound healing and increased pain [36]. Water vapor transmission rate (WVTR) refers to the rate at which water vapor permeates through a material to the atmosphere over a defined time period. It determines the dressing's efficacy in maintaining an optimal moisture balance at the wound site, a crucial aspect for facilitating proper healing [45]. Frequently, the WVTR for healthy skin falls between 200–300 g/m²/day, while for injured skin it can range from 800–1300 g/m²/day for a third-degree burn to 10000 g/m²/day for a granulating wound. It has been suggested that a rate of 5000 g/m²/day, falling within the mid-range of loss rates from injured skin, would offer an adequate moisture level without risking wound dehydration [46].

As shown in Fig. 7a, the WVTR of the knitted fabrics varied between 5194.3 ± 18.1 and 5702.8 ± 184.6 g/m²/day. In comparison, the control (open cup) showed a WVTR of 7760.1 ± 73.6 g/m²/day, and gauze exhibited a WVTR of 7082.5 ± 369.6 g/m²/day. Among the knitted structures, the jersey had the highest WVTR values, while interlock had the lowest. This is expected due to the configurations of the structures,

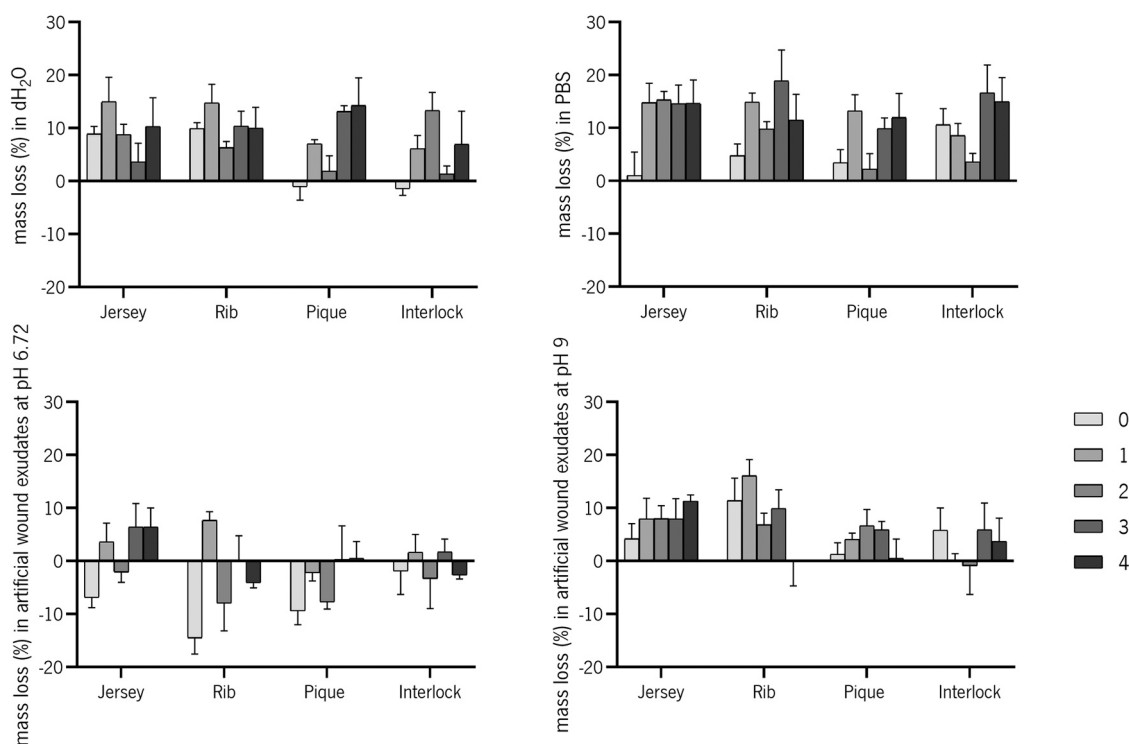


Fig. 6. Knitted fabrics mass loss after 28 days of incubation in dH₂O, PBS, and artificial wound exudates at pH 6.72 and pH 9. Data are presented as average percentage of mass loss $\pm$ SD ($n = 3$). Sample 0 corresponds to 100 % lyocell, while samples 1, 2, 3 and 4 correspond knitted fabrics with silver in their composition at 0.31 %, 0.65 %, 1.03 %, and 1.46 %, respectively.

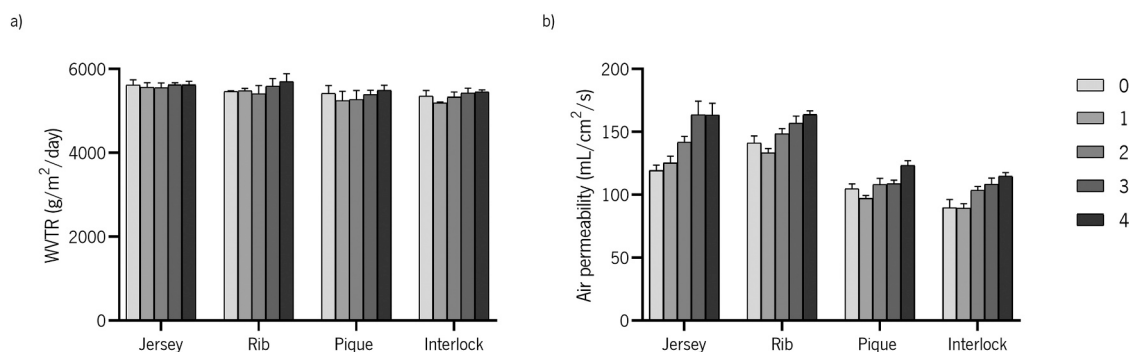


Fig. 7. (a) Water vapor transmission rate (WVTR) and (b) air permeability of lyocell/silver and lyocell knitted fabrics. Data were reported as mean $\pm$ SD ($n = 3$ – WVTR; $n = 10$ – air permeability). Sample 0 corresponds to 100 % lyocell, while samples 1, 2, 3 and 4 correspond knitted fabrics with silver in their composition at 0.31 %, 0.65 %, 1.03 %, and 1.46 %, respectively.

since jersey has an open structure and less thickness (Table 1b), allowing water vapor to pass through easily. The interlock, on the other hand, has a closed structure, greater thickness, and dense composition (Fig. 1), presenting greater resistance to water vapor transmission. However, no statistically significant differences were observed between fabric structures, as evidenced in Table S9 of the Supporting Information file. These high WVTR values make them suitable for treating highly exuding wounds such as heavily draining pressure ulcers or venous leg ulcers. This property prevents the accumulation of exudates, which could otherwise impede the healing process and elevate the risk of bacterial proliferation [47].

Air permeability is another crucial factor for effective wound healing. Ensuring adequate oxygenation of the injured area is essential for fostering optimal conditions conducive to fibroblast proliferation, re-epithelialization, and poly-morphonuclear cell functions [48]. This parameter is quantified by measuring the volume of air traversing a known area of the sample within a defined period of time under a specified pressure gradient [49]. Data from Fig. 7b (statistical analysis shown in Table S10 in the Supporting Information file) indicates an augment in air permeability with the increased number of silver-plated polyamide yarns in the knitted fabrics. However, in some cases, the control sample (0 – without silver yarns) exhibited greater air permeability than the samples with the smallest percentage of silver content (sample 1). This discrepancy is attributed to the greater elasticity of the silver-plated polyamide yarns, which relax and fill more empty spaces, thereby reducing air permeability. The maximum air permeability recorded for the samples was 163 ± 3.0 mL/cm²/s (sample R4), while gauze demonstrated significantly higher air permeability, measuring 375.1 ± 12.6 mL/cm²/s. Data suggests the importance of selecting materials with optimal air permeability to balance oxygenation while maintaining moisture levels conducive to wound healing. It was observed a consistent correlation between the thickness of the samples (Table 1b) and their air permeability across all structures. As anticipated, thicker materials tended to exhibit inferior air permeability. Particularly, the interlock structure demonstrated the lowest air permeability among the tested structures. This finding is mainly significant considering the high WVTR recorded in Fig. 7a. Excessive air permeability may predispose the wound to drying out, which can impede the healing progress [34]. It is therefore suggested that the interlock structure may offer the most promising solution for achieving optimal wound healing conditions. By balancing air permeability and moisture retention, this structure may offer a conducive environment for promoting effective wound healing.

3.7. Bacteria time-kill kinetics evaluations

Bacterial wound infections can severely disrupt the healing process and, if not properly managed, may progress to sepsis, leading to

significant patient discomfort and complications. Consequently, dressings endowed with robust antimicrobial properties play a crucial role in preventing and combatting high-incidence bacterial infections, thereby fostering an efficient healing process [50].

Gram-negative bacteria growth inhibition induced by the knitted fabrics was examined after 1, 2, 4, 6 and 24 h of incubation at 37 °C. *E. coli* and *P. aeruginosa* bacteria were selected due to their prevalence and challenging inhibition/elimination from human skin wounds [34]. Results were expressed in log reduction (Fig. 8) and CFUs/mL (Figure S8 in the Supporting Information file).

As anticipated, sample 0 (100 % lyocell) showed no significant inhibitory effect against the tested bacteria. In contrast, all samples containing silver yarns exhibited antibacterial activity against *E. coli* (Fig. 8a) from the first hour of incubation, with bacterial counts decreasing over time. After 24 h, nearly all of these samples achieved complete bacterial elimination, reaching 100 % inhibition (log 7). Particularly, jersey and interlock structures reported complete inhibition even with the lowest silver content (sample 1 – 0.31 % silver). This behavior can be attributed to specific structural configurations. The jersey structure, being more open and less thick (Table 1b), facilitates the diffusion of Ag⁺ into the surrounding medium, enhancing their antimicrobial action. Conversely, while the interlock structure is denser and thicker (Table 1b), its configuration allows for increased exposure of silver-containing yarns at the surface (Fig. 1).

Against *P. aeruginosa* (Fig. 8b), the antibacterial activity of silver was less effective compared to its action against *E. coli*. However, total inhibition after 24 h was achieved exclusively in the interlock structure for samples containing over 0.65 % silver content, which also exhibited the highest antimicrobial activity from the earliest time point. Notably, unlike the results observed for *E. coli*, jersey structure registered the lowest efficacy. Given that *P. aeruginosa* is an opportunistic pathogen with intrinsic resistance mechanisms and a robust outer membrane that limits permeability [51], a more sustained and controlled release of Ag⁺ appears to be crucial for effective bacterial inhibition. In this context, denser and more compact structures, such as rib and pique (Fig. 1), which may promote a more gradual and prolonged ion diffusion, seem to provide enhanced antimicrobial performance, though they did not achieve total inhibition within the tested timeframe.

Similar studies have demonstrated effective antibacterial activity in lyocell blends with other metals such as copper [52] and zinc [53]. However, after 2 h of contact with *E. coli*, silver showed superior activity compared to zinc, with bacterial reductions of ≈ 97 % and ≈ 86 %, respectively [53].

Silver is known as a powerful antibacterial agent. In fact, its oligodynamic effect imparts high antibacterial activity even at low concentrations [54]. Its mode of action initiates with interaction at the bacterial cell envelope, destabilizing the membrane and resulting in the loss of potassium ions (K⁺) and a reduction in adenosine triphosphate (ATP)

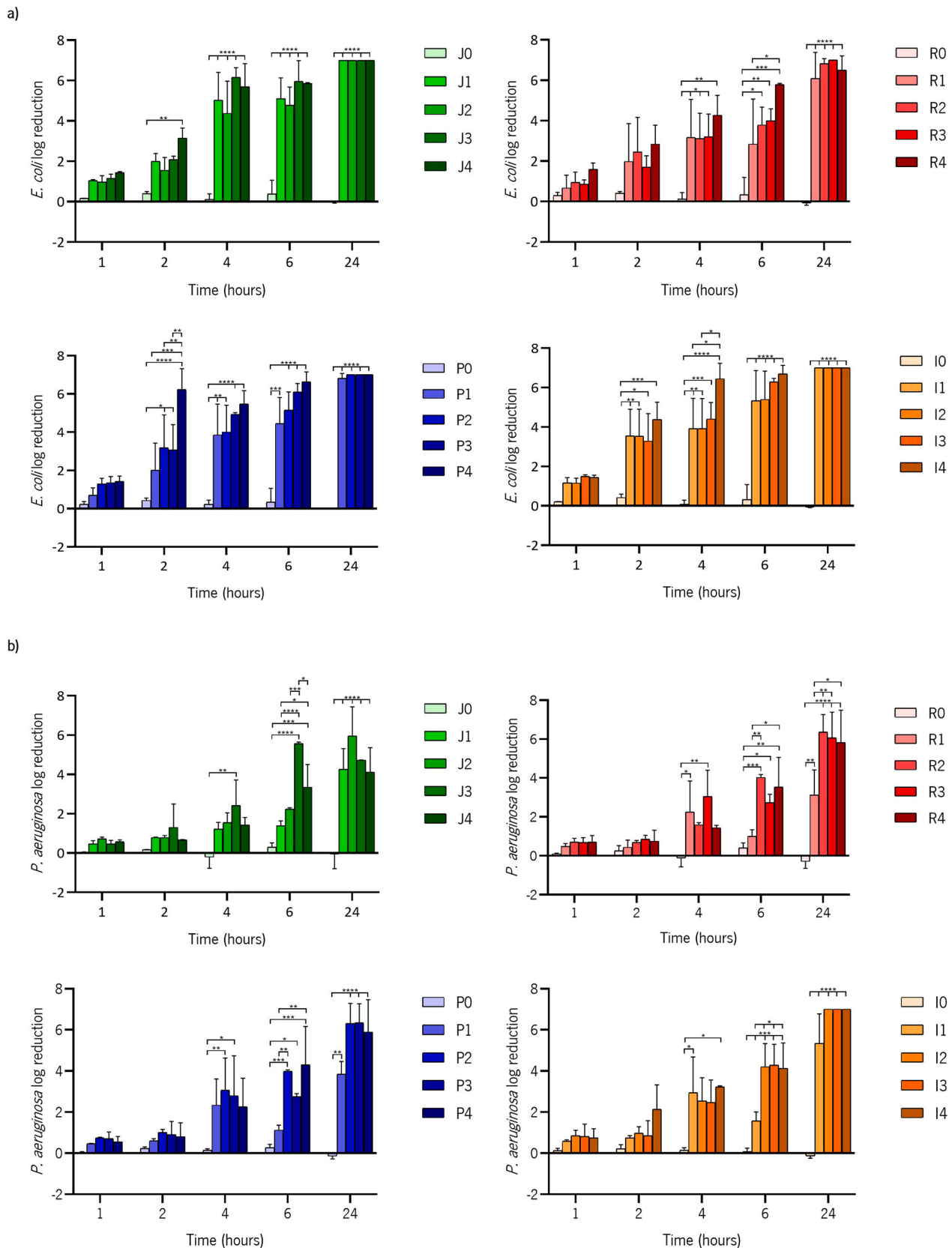


Fig. 8. (a) *E. coli* and (b) *P. aeruginosa* log reduction (calculated concerning bacteria suspensions growth without samples being present) in contact with knitted fabrics up to 24 h of culture. The absence of colonies (100 % bacterial reduction) was considered as log 7. Data are reported as mean $\pm$ SD (n = 3). Samples J, R, P and I correspond to the jersey, rib, pique and interlock structures, respectively, with 100 % of lyocell (sample 0), and 0.31 % (sample 1), 0.65 % (sample 2), 1.03 % (sample 3) and 1.46 % (sample 4) represent samples containing silver yarns at various ratios. Statistical significance was determined via Tukey's test applying multiple comparisons between the different samples in each period of time (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

levels. This process leads to the accumulation of Ag^+ , causing separation of the cytoplasmic membrane from the cell wall. Subsequently, Ag^+ interacts with intracellular molecules (nucleic acids and enzymes), inducing DNA damage through thiol group binding, thus incapacitating vital enzymes and subsequently affecting bacterial physiological processes [55,56]. Furthermore, the antibacterial actions of Ag^+ extends to the induction of reactive oxygen species (ROS) production, which triggers bacterial lysis and cytoplasmic leakage [57,58]. Previous studies have confirmed the rapid entry of Ag^+ into bacterial cells within 30 min after exposure, binding to cytoplasm components [59,60]. The results here obtained align with these findings, as bacterial counts were reduced by more than 50 % after just 1 h exposure.

Although the type of silver particles present in the polyamide yarn coating is not specified, since it is a commercial yarn, their release kinetics were investigated using UV-Visible spectroscopy. The analysis revealed a peak at 340.5 nm. Da Silva *et al.* [61] reported a similar peak for triangular prism-shaped silver nanoparticles, suggesting that the silver particles present in the current samples might share a comparable morphology. Given that the interlock structure exhibited superior antibacterial efficacy against both bacterial strains, only this structure was subjected to further analysis at 1, 2, 4, 6 and 24 h (Figure S9 in the Supporting Information file). The results demonstrated minimal variations in the absorbance values over time, indicating the high stability of the samples. It should be noted that this technique does not track the release of silver ions; therefore, the observations made earlier are still possible in light of the registered outcomes.

3.8. Antioxidant activity

Antioxidants play a crucial role in safeguarding molecules against oxidation, mitigating the detrimental effects of oxidative stress resulting from an imbalance between antioxidants and ROS, which can lead to cell death and damage [62]. The DPPH assay is a well-established method for assessing *in vitro* scavenging activity. DPPH, a stable organic free radical, undergoes a color change from purple to pale yellow and a decrease in absorbance when it reacts with a hydrogen and/or electron donor, such as an antioxidant. The extent of color disappearance correlates with the concentration of the antioxidant [63].

Here, only the interlock structure was evaluated, given its superior performance in preceding assays (mechanical performance, air and water vapor permeabilities, antibacterial activity against *P. aeruginosa*) aligning with the overarching goal of the research. No antioxidant activity was detected for the 100 % lyocell sample (0) across all tested time points. Among the other samples, the highest observed DPPH reduction was 61.7 ± 14.4 %, while positive control showed a maximum

reduction of 78.3 ± 3.0 % (Fig. 9 and Table S11 in the Supporting Information file). The observed elimination of DPPH free radicals scavenging was attributable to the presence of Ag^+ , which may neutralize the radicals through electron donation or transfer mechanisms [64]. The peak DPPH reduction was achieved within 24 h of contact, after which a decrease in antioxidant activity was noted. This decrease is potentially due to the quick release of Ag^+ during the initial immersion period, which subsequently stabilizes. These findings suggest that the tested samples can effectively reduce oxidative stress in wound environments during the critical early stages of wound healing.

3.9. Cytocompatibility

The cytotoxic nature of silver is well known and, hence, when formulating a wound dressing, it is particularly important to ensure that, after prolonged use of the product, silver is not released to an extent which could lead to a cytotoxic effect [34]. Therefore, to evaluate the safety of the knitted fabrics (interlock structure), cytocompatibility examinations through cell metabolic activity assay were conducted by indirect contact with HaCaT cell line. This cell line, representative of epidermal keratinocytes, was chosen for its important role in skin repair, thereby aligning the test conditions with real-life scenarios [65,66]. The cells were exposed to a conditioned culture medium in accordance with ISO 10993-5:2009 guidelines on the biological evaluation of medical devices [67]. Conditioned medium was obtained after 24 h of sample incubation. Then, HaCaT cells were subjected to varying ratios of that conditioned medium and of fresh medium (100:0, 50:50, 25:75 and 10:90 v/v, respectively) to assess the impact of their compositions on the cells' viability; hence, identifying potentially cytotoxic settings. According to the same ISO standard, a reduction in cell viability exceeding 20 % is indicative of cytotoxicity. Preliminary studies were conducted prior to the removal of paraffin and other contaminants from the samples to evaluate their effects on cells (Figure S10 in the Supporting Information file). For 100 % of conditioned medium, data revealed a cell viability of 75.2 ± 1.4 and 73.2 ± 0.9 % for the control sample (sample 0–100 % lyocell), and of 18.2 ± 0.4 and 12.5 ± 2.5 % for the sample with the highest silver content (sample 4 – 1.46 %) after 24 h and 48 h of contact, respectively. On the other hand, after washing, as shown in Fig. 10, for 100 % of the conditioned medium, the samples exhibited minimal impact on cell metabolic activity after 24 and 48 h of contact, with cell viability remaining above 97.4 ± 16.5 and 86.8 ± 24.6 %, respectively. Interestingly, these minimum viability values were observed in the samples with the lowest silver content (sample 1 – 0.31 %). These findings demonstrate that pre-washing the samples is essential to mitigate potential cell damage caused by the surface treatments applied to the yarns.

A slight increase in cell viability was observed after 48 h (Fig. 10), potentially indicating a reduction in the release of Ag^+ over time (as observed in Section 3.8) or their complexation with other medium components, which could mitigate their cytotoxic potential. Cells could also develop “immunity” or adaptation towards Ag^+ effect as time passes [68]. Although the mechanisms of action of silver against mammalian cells have not yet been fully elucidated, they are similar to the effect exerted on bacteria. It has been reported that Ag^+ penetration into cells triggers a cascade of intracellular reactions, disrupting specialized functions [69,70]. Additionally, other reports suggest that extracellular and intracellular generation of ROS have been implicated in proteins and nucleic acids damage, ultimately culminating in apoptosis and inhibition of cell proliferation [71–73]. However, this study disclosed the antioxidant activity of silver (Section 3.8), indicating that the silver content in the samples did not induce this reaction in cells, thereby not posing such a risk. Burd *et al.* [74] have explored the limit value of silver release from commercial medical dressings and established that its cytotoxicity is not only dependent on silver concentration, but also on factors such as the binding method to the dressing and its affinity to moisture. Medical dressings with greater hydrophilicity have been

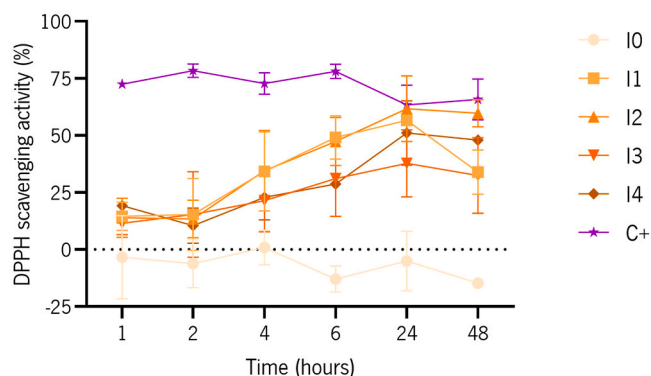


Fig. 9. DPPH scavenging activity of lyocell/silver and lyocell knitted fabrics (interlock structure). Data were reported as mean $\pm$ SD ($n = 3$). Sample I corresponds to the interlock structure with 100 % of lyocell (sample 0), and 0.31 % (sample 1), 0.65 % (sample 2), 1.03 % (sample 3) and 1.46 % (sample 4) represent samples containing silver yarns at various ratios. C+ corresponds to positive control (Trolox).

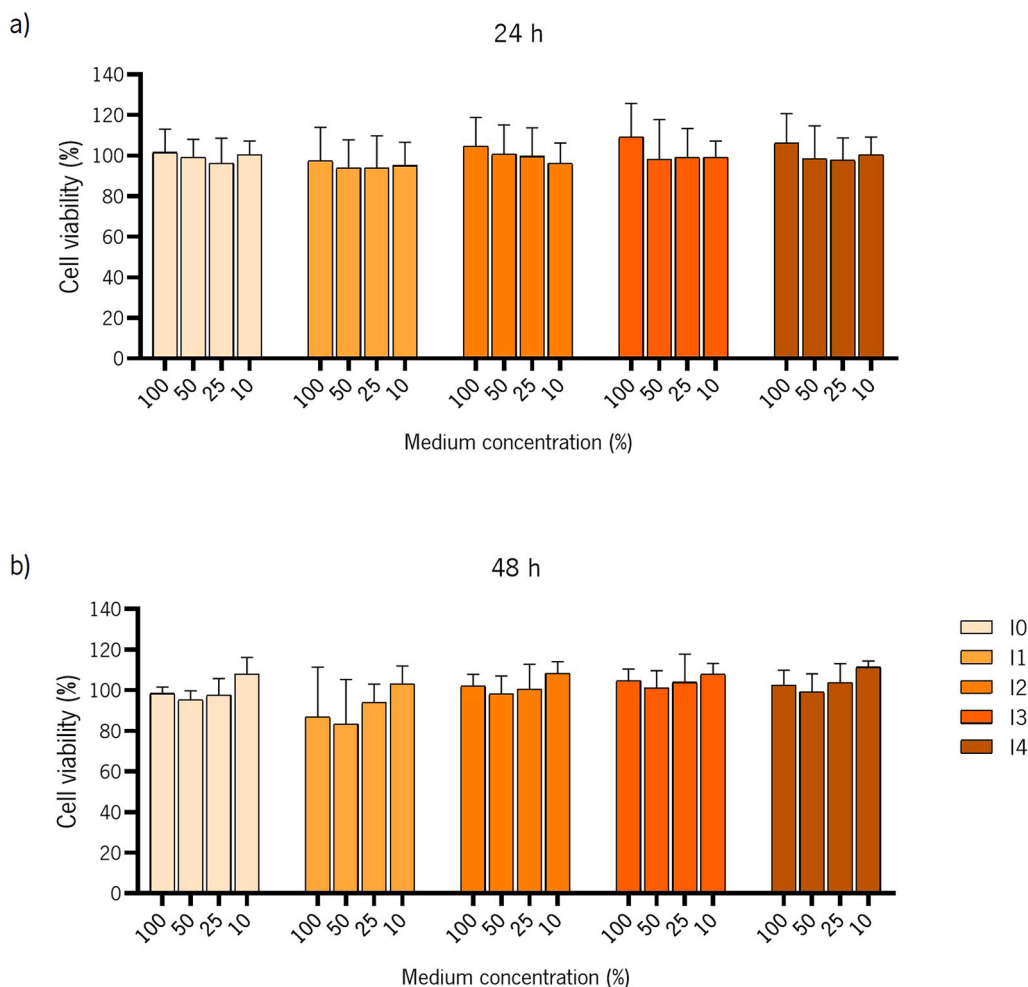


Fig. 10. Cell viability of human keratinocytes (HaCaT) cell lines measured via metabolic activity by resazurin assay after 24 h and 48 h of indirect contact with the knitted fabrics. Data are reported as mean $\pm$ SD ($n = 3$). Sample I corresponds to the interlock structure with 100 % of lyocell (sample 0), and 0.31 % (sample 1), 0.65 % (sample 2), 1.03 % (sample 3) and 1.46 % (sample 4) represent samples containing silver yarns at various ratios. Statistical significance was determined via Tukey's test applying multiple comparisons between the different samples in each medium concentration; no significant differences were registered.

shown to lead to a reduced silver release into wounds, thereby minimizing the potential cytotoxicity to human cells. Despite this reduction, the antibacterial efficacy is not compromised since the bacterial kill zone is primarily concentrated within the dressing's interior rather than the actual wound bed [74].

This study demonstrates that knitted fabrics containing up to 1.46 % silver exhibit potent antibacterial activity without causing harm to mammalian cells. These fabrics also show significant physical stability in dynamic environments, suggesting a promising therapeutic window for treating chronic wounds.

4. CONCLUSIONS

Different knitted fabrics based on lyocell/silver-plated polyamide yarns were successfully produced using seamless technology with the aim of improving DFUs treatment while maintaining the complete integrity of the materials. A pre-treatment was carried out to remove paraffin residues from all samples to mitigate the risks of cellular damage. Mechanical testing, combined with absorption and permeability evaluations, revealed that these fabrics are not only durable but also possess the functional properties necessary for effective wound dressing formulations. Among the tested structures, the interlock stood out in terms of mechanical performance and air and water vapor permeabilities, offering superior potential for wound dressing applications. Furthermore, antimicrobial tests showed promising bactericidal

performance, with more effective elimination of *E. coli* than *P. aeruginosa*. However, despite this variation in antimicrobial efficacy, both bacterial strains were significantly reduced, confirming the broad-spectrum potential of the knitted fabrics. Importantly, the fabrics demonstrated excellent cytocompatibility with keratinocytes cells, exhibiting efficient antimicrobial behavior. This synergistic approach take advantage, on one hand, the antibacterial and antioxidant role of silver, which controls ROS and prevents harmful oxidation mechanisms mitigating potential oxidative stress in wound environments, and on the other hand, of the advantageous thermomechanical swelling, permeability and degradation capacity behavior of lyocell to control structural integrity, adequate moisture and air exchange, helping to recover damaged tissues shortening healing time, and preventing infections. Moreover, this study is among the first to highlight the biocompatibility of lyocell, underscoring its potential for biomedical applications.

Even though the acquired data was highly encouraging, further research is necessary to enhance the dressing's performance and versatility. Specifically, functionalization with bioactive compounds that promote tissue regeneration or anti-inflammatory responses could significantly boost its therapeutic value. Additionally, the integration of multi-layered designs, each layer tailored for different stages of healing or wound management, could create an advanced dressing system capable of addressing the complex needs of chronic wounds such as DFUs.

CRediT authorship contribution statement

Antunes Joana C.: Writing – review & editing, Supervision, Funding acquisition. **Silva Carla:** Writing – review & editing, Supervision, Funding acquisition. **Begines Belén:** Writing – review & editing, Supervision. **Alcudia Ana:** Writing – review & editing, Supervision. **Rocha Diana:** Investigation. **Bengoechea Carlos:** Writing – review & editing, Supervision, Methodology. **Ribeiro Artur:** Writing – review & editing, Supervision, Methodology. **Tavares Tania D.:** Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Felgueiras Helena P.:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Helena P. Felgueiras reports financial support was provided by Foundation for Science and Technology. Tania D. Tavares reports financial support was provided by Foundation for Science and Technology. Artur Ribeiro reports financial support was provided by Foundation for Science and Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.mtcomm.2025.112389](https://doi.org/10.1016/j.mtcomm.2025.112389).

Data availability

No data was used for the research described in the article.

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