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New antibiotic-eluting mesh used for soft tissue reinforcement

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A B S T R A C T

The surgical implantation of prostheses for soft tissue repair may be followed by post-operative mesh-related infection, a significant and dramatic complication, that is treated by mesh removal. A new antibiotic-eluting mesh has been manufactured on pre-existing polypropylene prostheses using an airbrush spraying technology. Among the degradable polymers tested as coating agents and drug reservoirs, poly(ϵ -caprolactone) (PCL), which is deposited after heating, provides a homogeneous, regular and smooth shell around the polypropylene filaments of the mesh without dramatically altering the biomechanical properties of the new modified mesh. An anti-infective drug (e.g. ofloxacin) is incorporated into this polymeric coating giving a limited burst effect followed by sustained drug diffusion for several days. An ofloxacin-eluting mesh has demonstrated excellent antibacterial activity in vitro on *Escherichia coli* adherence, biofilm formation and inhibitory diameter, even with low drug loads. Although further in vivo investigations are required to draw conclusions on the anti-infective effectiveness of the coated mesh, the airbrush coating of ofloxacin-PCL on existing prostheses is already potentially appealing in an effort to decrease post-operative infection.

Keywords:

Mesh modification
Airbrush coating
Aliphatic polyesters
Biomechanical properties
Ofloxacin-eluting mesh

1. Introduction

Prosthetic materials are commonly used for abdominal hernia repair, pelvic organ prolapse or other soft tissue surgery. A metallic silver mesh was first introduced in the early 20th century, but prostheses have evolved greatly since in an effort to decrease post-operative complications and improve patient quality of life. Non-absorbable polymer materials were first introduced before the mid 20th century as nylon- and silicone-based prostheses. Changes made over the years have accelerated the trend to use synthetic prostheses made of polyesters (Dacron), polypropylene (PP), polytetrafluoroethylene (PTFE) and its expanded form (ePTFE) [1]. Of these, lightweight macroporous monofilament polypropylene type I, according to the Amid classification, is reported to possess many advantages over other materials [2–5].

PP materials, initially tested by Francis C. Usher in 1955 under the brand name Marlex, rapidly became one of the most popular prosthetic meshes implanted for soft tissue repair. Population aging and a trend to be over-weight have meant that soft tissue surgery is now the most common surgical procedure in western countries. Almost 1,000,000 hernias are repaired annually, and prolapse and incontinence disorders are expected to affect 10% of women in

the USA [6]. Even if PP meshes are nowadays described as the “gold standard” material, modifications are nevertheless under investigation to improve the short- and long-term results. The ideal prosthesis should be inert, induce a minimal inflammatory response, and promote vascular and fibroblastic colonization to avoid material encapsulation and erosion, limit the risk of infection and promote integration with the surrounding tissue [4,6]. However, the healing process may unfortunately be delayed by bacteria that are found at approximately 90% of all implantation sites immediately after surgery. Because mesh-related infections may cause severe complications that occur weeks to years after implantation, including final mesh removal [7], the conventional procedure is to administer intravenous antibiotics intra-operatively. Bacteria encountered at the implantation site originate from the local environment and gain direct access to the surgical site during mesh insertion, and despite prophylactic sterilization and clean surgical procedures, post-operative infection rates are still significant. Various studies have demonstrated that the presence of a foreign material, such as a surgical mesh or other implantable device, dramatically increases susceptibility to infection. It has also been reported that the number of bacteria required to induce an implant-related infection is dependent upon the macroscopic and microscopic structure of the device [8]. These materials may be colonized by microorganisms that form a biofilm consisting of a mono- or multi-layer of cells embedded in an extracellular matrix. Release of the microorganisms from the

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biofilm may cause an acute, disseminated infection. An anti-infective drug-eluting mesh that slowly delivers antibiotic around the surgical site could be used to avoid bacterial contamination of the prosthesis and subsequent biofilm formation. In addition, *in vitro* investigations of competition between bacteria and fibroblast growth on meshes have highlighted how infected materials show poorer tissue integration [9].

Fluoroquinolones, derived from nalidixic acid, are antibacterial drugs used as first line treatment for complicated urinary tract or abdominal infections [10,11]. Quinolone drugs penetrate the bacterial wall cell through porins and inhibit cell proliferation by interfering with DNA gyrase, a topoisomerase IV involved in transcription, chromosome segregation, and replication [10]. Moreover, these antibiotics have demonstrated the promising ability to penetrate and eradicate bacterial biofilms [12].

An effective drug and local delivery system would offer several advantages over systemic administration: greater efficacy, lower drug dose required, less toxicity due to the local release of the drug, extended activity, and last but not least, less likelihood of promoting antimicrobial resistance [13,14].

As a consequence, several drug delivery systems, such as antimicrobial catheters or sutures and drug-eluting stents have been developed, obtained FDA approval, and are now widely used [15–18].

Different authors have described how the prosthesis is coated by dipping into a solution containing the drug and a polymer agent [19–22]. This coating technology has several main drawbacks in that it is difficult to produce a multi-coated device, and the technique may modify the porosity or surface morphology of the material, doubtless having an impact on the biomechanical properties of the final medical device.

The purpose of this study was to create a new antibiotic-eluting system on a pre-existing type I PP mesh used for the treatment of genital prolapse without affecting the intrinsic properties of the material. We investigated a spray coating system that uses an airbrush to produce a homogeneous and easily standardizable coating across the entire surface of the native PP mesh. Two different artificial degradable and biocompatible polyesters, poly(D,L-lactic acid) (PLA₅₀) and poly(ε-caprolactone) (PCL), were investigated as coating agents and drug delivery systems. The biomechanical properties of the modified prostheses were compared with those of the native PP type I mesh. Finally, we evaluated the antimicrobial activity of the drug-loaded mesh and used increasing quantities of ofloxacin to determine *in vitro* the lowest drug dose with anti-adherence and anti-biofilm effectiveness.

2. Experimental procedures

2.1. Formulation of modified meshes

Knitted PP meshes were kindly donated by Covidien (Trevoux, France). These macroporous monofilament meshes are commonly used in clinical practice for abdominal wall hernia, incontinence or prolapse surgery (large pore size >1 mm, density 35 g m⁻²). We evaluated two different biocompatible aliphatic polyesters that we used to coat the meshes: PCL (M_n 40,000 g mol⁻¹) was purchased from Sigma (Saint-Quentin Fallavier, France), while PLA₅₀ (M_n 75,000 g mol⁻¹) was a homemade product synthesized by a common ring opening polymerization method with D,L-lactide obtained from Purac (Gorinchem, The Netherlands), the process being catalyzed by zinc lactate from Sigma (Saint-Quentin Fallavier, France) [23].

The thermal characteristics of PCL and PLA were determined by differential scanning calorimetry (DSC) with measurements obtained under nitrogen and using a 10 °C min⁻¹ heating rate in a Perkin-Elmer DSC6 Thermal Analyser (Waltham, MA).

The mesh was modified by spray coating using an Infinity model airbrush supplied by Harder & Steenbeck (Oststeinbek, Germany). Polymer solutions (1% w/v in acetone) were sprayed directly at a pressure of 3 bar onto the surface of the native PP meshes from a distance of 5 cm. After drying under vacuum overnight the meshes were weighed to determine the amount of polymer coating. The morphology of the coated meshes was characterized by environmental scanning electron microscopy (ESEM) at an accelerating voltage of 10 keV (Philips XL30, FEI, Hillsboro, WA) and by optical microscopy (Leica MZ6 stereomicroscope connected to an EC3 digital camera, Nanterre, France). Meshes were coated with PCL or PLA with or without heating (termed PCL or PLA Cold coating compared with Hot coating). The meshes were heated directly using a hair dryer for 10 s.

Coating homogeneity and thickness on the mesh filament were evaluated by incorporation of a fluorescent probe (Nile Red, Sigma) into the polymer solution prior to spraying. Coated meshes were embedded in OCT (Miles, Zoeterwoude, The Netherlands) and snap frozen in liquid nitrogen vapor. Sections of mesh (6 μm) were obtained using a cryomicrotome (Leica, Wetzlar, Germany) and observed under a TE300 microscope fitted with a DMX1200 digital camera (Nikon, Tokyo, Japan).

2.2. Coating impact on biomechanical properties

The effect of the coating on the mechanical properties of the meshes was determined by several mechanical tests on native and modified meshes. Each mechanical test was conducted on three samples of a standardized size and in two directions. The first direction consisted of the soft orientation and the other was perpendicular to the mesh knit.

Stress–strain experiments on 15 mm width mesh strips (clamp to clamp distance 10 mm) were performed using a computer-assisted tensiometer (Instron 4444 series, Norwood, MA) at an elongation rate of 1 mm min⁻¹ until the mesh was loaded to failure. Load to failure in MPa was related to percentage mesh elongation. The elastic modulus of the mesh (E_I) was calculated over the first 15% elongation and the plastic modulus (E_{II}) was calculated from about 40% to 100% elongation.

Biomimetic tests were also conducted by loading measurements of cyclic displacement. The tensile stress of 10 20% elongations followed by 10 50% elongations of mesh length was recorded. The reduction in tensile strength was then calculated between the first and the last displacement to evaluate coating preservation. The surface integrity of the coating was also inspected by ESEM after the cyclic stress test, which provided information on the compatibility and functionality of the coated meshes for clinical applications.

A test using a Handle-O-Meter (Thwing-Albert, Berlin, Germany) instrument was performed to determine the bending rigidity properties of the different meshes. This measures the combined effects of resistance due to surface friction and flexibility of the material. Strips of meshes (2 × 10 cm) coated with PLA or PCL were compared, after heating, with native meshes in triplicate in the perpendicular direction to the mesh knit and at three points in each sample.

Briefly, meshes were placed over a 15 mm span and a penetrator engaged the sample at 159 mm min⁻¹ forcing it into the span. The apparatus measured the resistance encountered by the penetrator blade as it moved into the span (expressed in grams).

2.3. Antibiotic-eluting mesh formulation

Ofloxacin ((*RS*)-9-fluoro-3-methyl-10(4-methylpiperazin-1-yl)-7-oxo-2,3 dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carbox-

ylic acid) powder was purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France).

The mesh was modified using the airbrush (previously described) to spray on, first, a solution of polymer/ofloxacin (80/20 wt.%) dissolved in acetone then, after solvent evaporation, a second drug-free polymer solution (1% w/v polymer in acetone). In order to evaluate the effect of polymer matrix composition and the heating process on drug release rate four different meshes were formulated with ofloxacin loaded onto a PLA or PCL matrix with or without heating. Each 3 × 3 cm native mesh weighing 35 mg was supplemented with a first coating of 12 mg (3 mg of drug mixed with 9 mg of polymer) followed by a second 10 mg of drug-free polymer coating. The drug release profile was studied by incubating ofloxacin-loaded polymer meshes in 10 ml of phosphate-buffered saline, pH 7.4 (PBS) at 37 °C with stirring at 130 r.p.m. (HeidolphUnimax 1010, Schwabach, Germany). The release medium was replaced by fresh PBS at scheduled time points for 7 days and drug concentrations were measured by HPLC with UV detection.

Ten microliters of the medium was injected onto a Waters chromatographic system equipped with a Kinetex C18 column (2.6 µm particle size, 100 × 4.6 mm, Phenomenex, Le Pecq, France) and ofloxacin was detected at 294 nm by a photodiode array detector (Waters PDA 2998). The mobile phase consisted of water/acetonitrile (80/20 vol.%) at a flow rate of 1 ml min⁻¹.

The total amount of drug released over this period was determined by assaying the drug that remained entrapped in the polymer matrix after 7 days. To do this the modified meshes were immersed in 10 ml of CH₂Cl₂ for 15 min to dissolve both polymer and remaining drug. Water (40 ml) was then added while stirring vigorously and the drug, which is more readily soluble in water than in apolar solvents, was quantified in the aqueous phase after 15 min.

2.4. *In vitro* antibacterial activity

Three antibacterial tests were performed with increasing amounts of ofloxacin loaded onto modified meshes to estimate the *in vitro* anti-infectious potential of these active meshes and to determine the required drug dosage.

An initial ofloxacin solution was sprayed onto the surface of the mesh to load different quantities of drug (from 1 to 10 mg mesh⁻¹), and this was followed by a second spray of drug-free polymer solution (20 mg mesh⁻¹). The negative controls corresponded to a coating of drug-free polymer on the meshes.

Genetically engineered green fluorescent protein (*gfp*)⁺ *Escherichia coli* NECS19923 [24] were used to study bacterial adhesion, biofilm formation and changes in inhibition diameter with ofloxacin-eluting coated meshes.

Before each *in vitro* antibacterial test the bacterial strain was first grown aerobically overnight on Muller–Hinton medium (MH) at 37 °C with stirring.

2.4.1. Inhibition diameter

In accordance with the standardized Kirby–Bauer disk diffusion method, 1 cm² sample meshes were tested for each group in duplicate. After overnight culture at 37 °C bacterial suspensions (OD₆₀₀ 0.5, corresponding to 5 × 10⁸ c.f.u. ml⁻¹) were diluted 1/10 and inoculated onto an MH agar plate. Samples were then placed in contact with an agar plate inoculated with bacteria and inhibition diameters, corresponding to the bacteria-free zone, were measured (in mm) after 18 h incubation at 37 °C.

2.4.2. Bacterial adhesion

Bacterial adhesion to the modified meshes was assessed in accordance with the Balazs method [25], by sample immersion in a bacterial suspension (OD₆₀₀ 0.05) for 1 h. Unattached bacteria

were then removed from the sample by several washes with sterilized water, and the mesh was incubated in neutral medium (AP). After 24, 48 and 72 h incubation at 37 °C adherent bacteria were removed from the prosthesis surface by vortexing and sonication in PBS. The number of detached viable bacteria was determined by a colony count method, whereas undetached viable bacteria were quantified by pressing the samples directly onto the surface of an MH agar plate about 15 times. Total bacterial adhesion was calculated by counting colony forming units (c.f.u.) after overnight incubation of the agar plates at 37 °C.

2.4.3. Biofilm formation and quantification

Modified mesh susceptibility to biofilm formation was evaluated by creating a liquid–air interface between the sample and bacterial suspension (OD₆₀₀ 0.05). After 72 h incubation at 37 °C in a 100% humidity, CO₂ enriched environment unattached bacteria were removed from the samples by repeated PBS washes. Biofilm forming bacteria were dissolved by DMSO immersion and 200 µl of this DMSO-containing bacterial solution was analyzed in a luminometer (Mitras, Berthold). Biofilm formation is expressed in relative luminescence units (RLU).

3. Results

3.1. Morphological characterization of the coated meshes

Existing meshes were modified using an airbrush spraying technique that coats the prostheses with biocompatible and degradable polymers. This airbrush coating technique has only been described previously for drug-eluting stent coatings, where it is able to form a homogeneous coating without having an impact on the morphological characteristics of the initial device [26,27].

We therefore decided to study the effect of a polymer coating on mesh morphology. Different amounts of polymer were used to obtain a coating that did not cover the mesh knots. Ultimately, 20 mg of polymer, corresponding to the most suitable amount with regard to the morphological appearance of the mesh, were loaded onto 3 × 3 cm meshes initially weighing 35 mg and observed by ESEM and optical microscopy. Fig. 1 shows a homogeneous and regular coating on the filament that does not affect the specific morphology of the mesh. Unfortunately, meshes coated with PCL showed microporous structures with interstices (Fig. 1C). Similar images were obtained for meshes coated with PLA.

Rapid heating was applied to the surfaces of the polymer-coated meshes above the glass transition temperature of PLA (*T*_g = 50 °C) or above the melting point of PCL (*T*_m = 60 °C), respectively. ESEM observations of PCL-coated meshes before (Fig. 1C) and after heating (Fig. 1D) indicated that heated meshes present a smoother surface than unheated meshes. The homogeneity of the polymer coating was also studied to precisely characterize the morphology of the coated meshes. A fluorescent probe was dissolved in the polymer solution before coating and filament sections were observed by ESEM (Fig. 1E) and fluorescence optical microscopy (Fig. 1F). The biodegradable polymer shell showed a homogeneous thickness of 40–60 µm. Its microstructure appeared to be uniform, devoid of pores and asperities on the external surface or at the interface between the mesh and the polymer coating.

3.2. Biomechanical studies

Even though the mechanical properties of PP type I mesh are not similar to those of native tissue, such meshes are routinely used in clinical practice for dysfunctional pelvic floor or abdominal wall repair surgery. Several mechanical tests, described by authors such as Cobb and Elsner, have been performed on native and

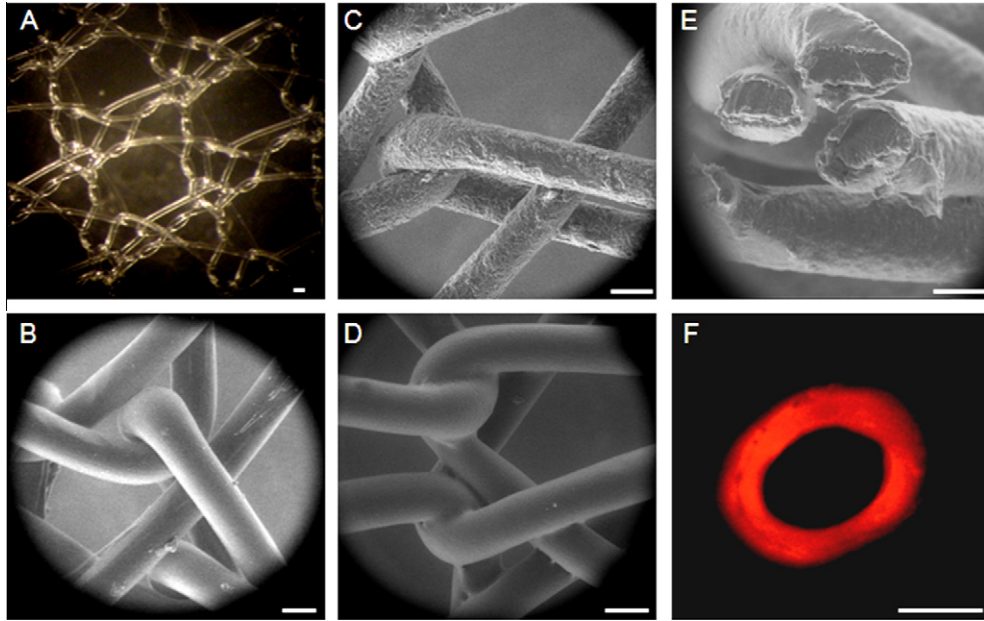


Fig. 1. (A) Optical microscopy and (B) ESEM images of native meshes. ESEM images of respectively (C) PCL Cold and (D) PCL Hot coated meshes. (E) ESEM and (F) fluorescence optical microscopy images of sections of PCL Hot coated meshes containing a fluorescent probe. Scale bars represent 100 μm for each image.

modified meshes (Fig. 2) [19,28,29]. Fig. 2A gives a typical tensile stress–strain curve showing two distinctive regions. The first, from 0% to 15% elongation, is used to determine the elastic modulus E_I , while the second corresponds to the plastic properties of the material (E_{II} modulus). The elastic modulus is generally lower than the plastic modulus, indicating that the mesh can be easily deformed, even by a weak force. Our native meshes were characterized by increased elasticity and a substantial displacement capability before failure (Fig. 2). Fig. 2B shows that addition of a polymer

coating increased the mesh elasticity modulus E_I from 4 MPa for the native mesh to a maximum average value of 34 MPa for the heated PCL-coated mesh (this analysis was performed in the soft knitting and perpendicular directions of the prosthesis). The rigidity of the coated prostheses seemed to be related to the polymer coating, but also to the heating process: E_I average values for PLA- and PCL-coated meshes corresponded to 16.0 and 10.3 MPa, respectively, for non-heated meshes, and to 20.4 and 34.1 MPa, respectively, for heated meshes.

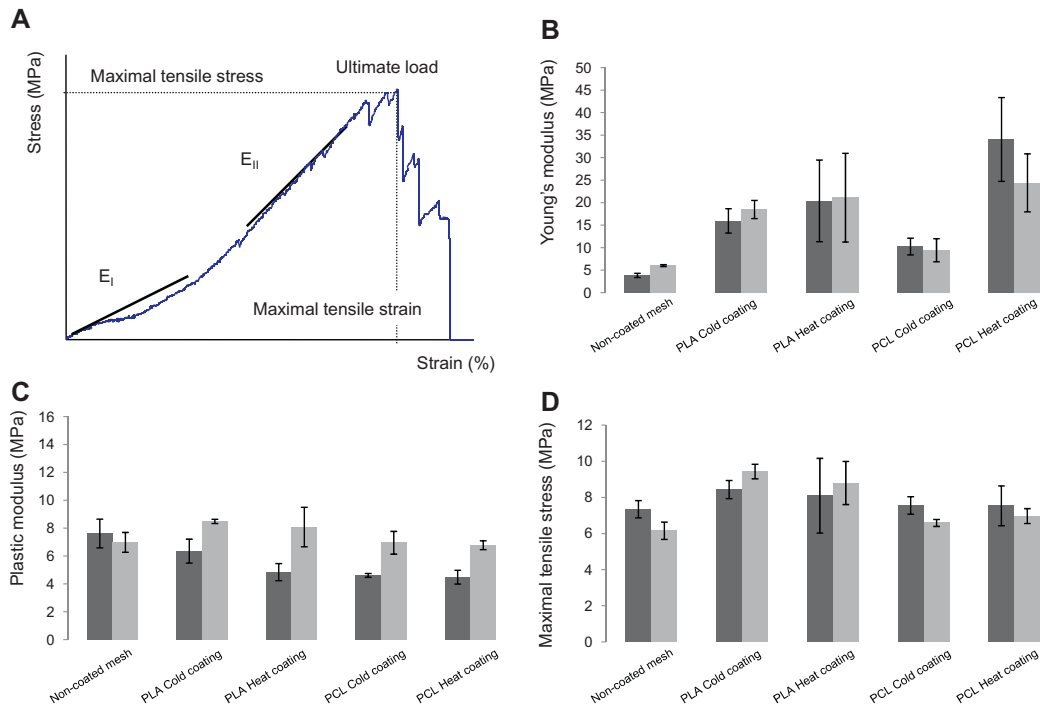


Fig. 2. (A) A typical tensile stress–strain curve obtained for a coated mesh. (B) Young's modulus E_I and (C) plastic modulus E_{II} and (D) maximal tensile stress obtained for non-coated compared with coated meshes in the soft knitting (dark gray square) and perpendicular directions (light gray square) of the prostheses.

The plasticity modulus of coated meshes (Fig. 2C) was then assessed. No significant differences with respect to non-coated meshes were noted in the perpendicular knitting direction, whereas this modulus was slightly reduced in the soft knitting direction. The polymer coating would therefore appear to mainly have an impact on the elastic properties of the mesh (elastic modulus), while above this reversible region the elongation profile of the modified meshes was similar to that of the native meshes.

We also measured the maximal tensile stress of native and modified meshes (Fig. 2D). Measurement of the tensile strength before failure of the modified meshes indicated that in both orientations PLA coating slightly increased the strength required to break the mesh, whereas PCL coating did not modify this mechanical parameter.

In order to simulate loading conditions similar to those on the human fascia after mesh implantation biomimetic tests based on cyclical displacement were performed on the prosthetic material and the coating integrity was assessed by ESEM (Fig. 3). Globally the tensile strength after 20% or 50% elongation was higher for modified meshes than for native meshes, indicating that the material is stiffer. However, despite a decrease in tensile strength, preservation of the coat integrity was noticed for the PCL-coated meshes compared with the native meshes, even after 20% or 50% displacement in both mesh orientations (Fig. 3E).

To provide an efficient drug delivery system it is essential that the coating integrity is preserved for the required length of time while subject to possible in situ forces. The ESEM pictures taken after the biomimetic test (Fig. 3) show that of all the coated meshes tested the heated PLA- and PCL-coated meshes conserved their surface integrity without breakage, in contrast to the other non-heated meshes.

This mechanical characterization was completed by bending measurements in a Handle-O-Meter to determine the “handle

capability” of the medical device, which is of interest to surgeons during implantation. The results indicated that both native meshes and PCL Hot coated meshes were highly flexible, whereas PLA Hot coated meshes appeared to be stiffer (1.6 ± 0.1 , 7.0 ± 0.7 and 17.9 ± 1.7 g, respectively) (data not shown).

3.3. Antibacterial drug release profile

Antibiotic elution from ofloxacin containing PLA- or PCL-coated meshes was monitored by HPLC assay to assess the impact of polymer hydrophobicity and crystallinity and the effect of the heating process on ofloxacin release. The initial coating, composed of drug dispersed in the polymer matrix, was loaded onto the mesh for sustained drug release, while a second drug-free polymer coating was used to minimize the initial drug burst release.

Ofloxacin release profiles, illustrated in Fig. 4, indicated that these differed not only with the composition of the polymer matrix but also in relation to the heating process.

Ofloxacin release profiles were similar when the drug was incorporated into PLA Hot or Cold coated meshes, with an initial burst of about 30–35% over the first 5 h, followed by a continuous slow release. Drug release from PCL-coated meshes was generally faster than from PLA-coated meshes and, above all, was dependent on the macroscopic surface of the coating layer. Because the PCL coating surface is rough and porous, water penetration and ofloxacin diffusion through the polymer matrix is faster than with the PCL Hot coated mesh surface, which is regular and smooth.

However, the main difference between the PLA and PCL coatings concerned the amount of drug that remained trapped in the polymer matrix at the end of the release phase (7 days). We clearly demonstrated that the PCL matrix no longer contained any drug after 3 or 4 days (100% was released after 3 days), whereas only 80% was released from the PLA matrix after 3 days

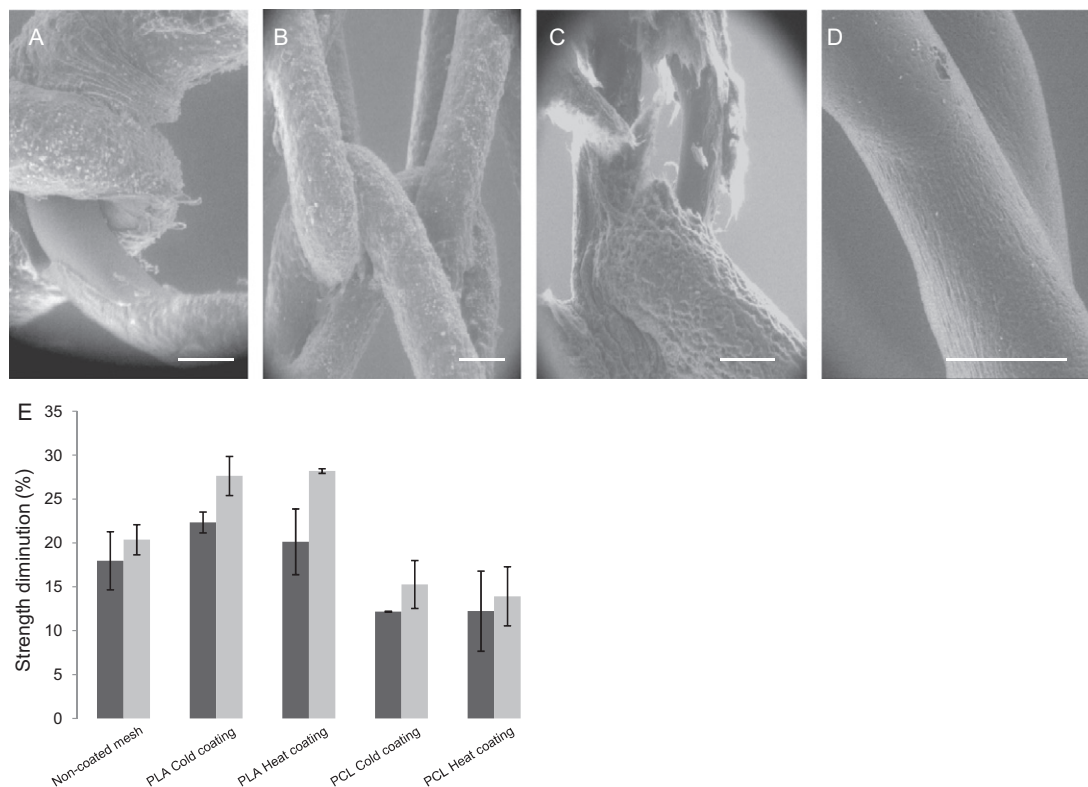


Fig. 3. ESEM images of (A and B) PLA Cold and Hot coated meshes and (C and D) PCL Cold and Hot coated meshes after cyclic elongation testing. Scale bars represent 100 μm. (E) Results for elongation decrease after cyclic displacements of 20% (dark gray square) and 50% (light gray square) of the different meshes in the soft knitting orientation of the meshes.

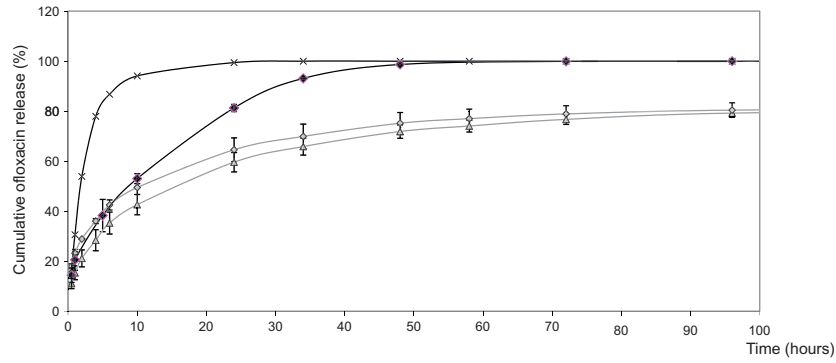


Fig. 4. Cumulative release of ofloxacin from PLA Cold (light gray diamond) and Hot (light gray triangle) coated meshes and from PCL Cold (cross) and Hot (black diamond) coated meshes in PBS at 37 °C.

and low antibiotic doses continued to be released for 7 days. The hydrophobicity and the high degree of crystallinity of PCL compared with the amorphous character of PLA₅₀ restricted drug retention in the polymer matrix and resulted in total release after 3 days.

3.4. Antibacterial drug-eluting mesh activity

Several post-operative investigations following surgery for hernia or pelvic organ prolapse reported that mesh-related infections were caused by *Staphylococcus aureus*, Enterobacteriaceae or *Streptococcus* spp. [30,31]. In our study a genetically modified fluorescent *gfp*⁺ *E. coli* strain was selected to evaluate the efficiency of the antimicrobial-coated device. The antimicrobial tests indicated

that the ofloxacin-containing polymer-coated meshes exerted a potent antibacterial activity even at low drug dosages (Fig. 5).

For instance, 1 mg of ofloxacin on a 3 × 3 cm PCL Hot coated prosthesis dramatically reduced bacterial adhesion for 72 h (c.f.u. = 15) compared with the control, for which a value of 7 × 10⁴ c.f.u. was found (Fig. 5D).

Inhibition diameters (Fig. 5A) indicated that quantities of drug exceeding the minimal inhibitory concentration (MIC) were released from this coated mesh, then diffused through the agar plate, eradicating the bacteria. The PCL Hot coating mesh containing 1 mg of ofloxacin gave a 39 mm inhibition diameter (Fig. 5C). This bacteria-free diameter was close to the maximum diameter obtained with higher ofloxacin concentrations and equivalent to 45 mm (Fig. 5B shows the absence of any inhibition with a drug-free mesh).

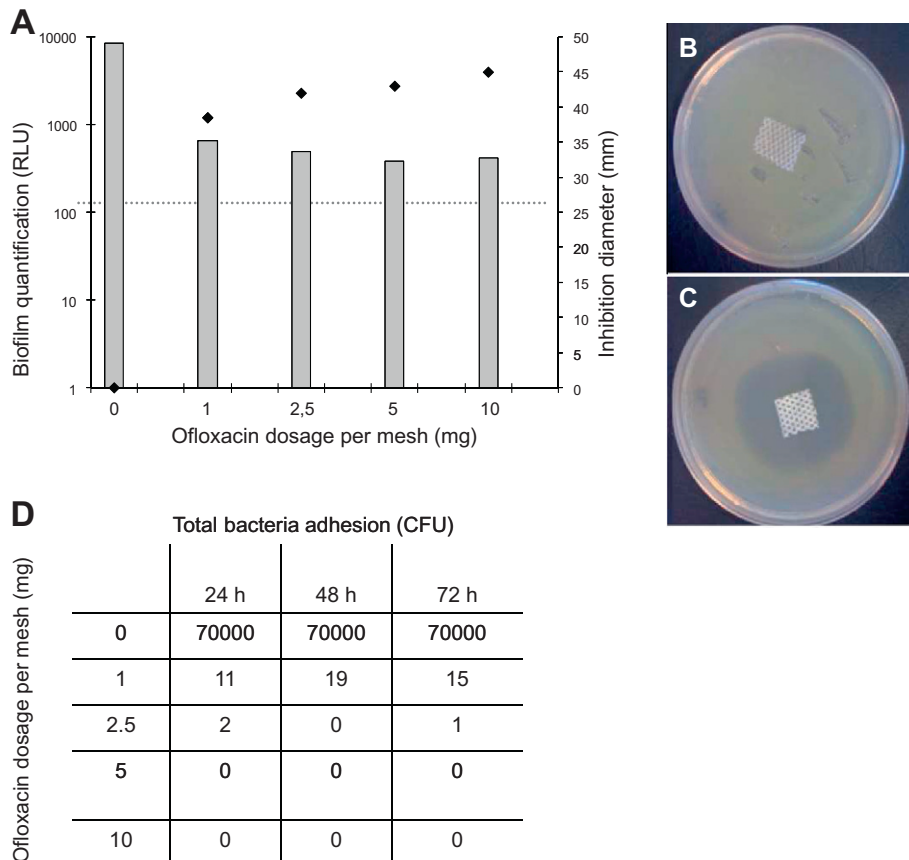


Fig. 5. (A) In vitro anti-biofilm activity (light gray square, background value) and inhibition diameters (black diamond), (B and C) inhibition diameter images and (D) anti-adherence properties of ofloxacin-loaded PCL-coated meshes.

Biofilm quantification (Fig. 5A) indicated that this active mesh inhibited irreversible bacterial attachment to the surface, which corresponds to the first step in implant colonization and formation of a biofilm. The surfaces of the uncoated and drug-free coated meshes offered favorable conditions for biofilm formation. Luminescence decreased from 8500 RLU for the uncoated or drug-free coated mesh to 650 RLU (the value corresponding to background noise) for the PCL-coated mesh containing 1 mg of ofloxacin.

4. Discussion

Of the prostheses available today, lightweight macroporous monofilament PP meshes are those that best match the natural physiological distensibility of the abdominal wall and other soft tissues such as the pelvic floor. Moreover, these meshes have demonstrated that they cause less of an inflammatory reaction, thanks to their excellent tissue integration, which has been attributed to their large pore size ($>75\ \mu\text{m}$). This facilitates the growth of fibroblasts and angiogenesis, and lower long-term infection rates than with other materials, thanks to enhanced macrophage in-growth [2,3,32–34].

Various studies have reported that surgical meshes and other implantable devices may be susceptible to infection and that the number of bacteria inducing implant-related infections is dependent upon the macroscopic and microscopic structure of the device [8].

Consequently, a new antibiotic-eluting system was developed on a pre-existing type I PP mesh used for the treatment of genital prolapse, without having an impact on the intrinsic properties of the material. We used an airbrush system to coat the mesh with two artificial biodegradable polyesters. PLA and PCL may be advantageously used as drug carriers thanks to their FDA approval, associated with their biocompatibility, and their degradation rates, which are suitable for this surgical application [32,35,36].

The two-step coating method (airbrush and rapid heating) offers two main advantages: (1) the shell surface is smooth and it is well known that surface asperities provide encrustation niches for micro-organisms, increase bacterial attachment and biofilm formation, hinder phagocytosis, and promote infection of the new mesh [37]; (2) the shell structure is homogeneous in thickness and as such forms a homogeneous drug reservoir.

In order to evaluate the impact of the polymer coating on the biomechanical properties, the elastic modulus, plastic modulus and maximal tensile stress of native and modified meshes were inspected. The elastic and plastic modulus measurements showed that native meshes are characterized by increased elasticity and that the polymer coating, and especially the heating process, resulted in a slight stiffening of the mesh. The differences between the unheated and heated meshes may be explained by polymer incorporation into the interknitted part of the mesh, caused by the heating process, and the resulting limited filament movement during the tensile tests. The elastic properties of such a mesh are of great importance clinically if it is to be rapidly and efficiently integrated into the surrounding tissue and, consequently, an implanted mesh must be highly flexible. Several groups have studied the elastic properties of commercial meshes used for abdominal or vaginal disorders. Pariente determined the Young's modulus after testing commercial suburethral tapes, and reported values ranged from 5 to 40 MPa, which is comparable with the elasticity of our modified meshes [38].

Maximal tensile stress testing of our native and modified meshes indicated that coating with PLA slightly increased the force required to break the mesh, giving higher values than for the PCL coating and native meshes. These differences are unlikely to be relevant in a clinical setting, as it has been documented that load

before failure of synthetic meshes is generally higher than natural human tissue. Abdominal wall deformability is considered not to exceed 30% for an average maximal stress of 6 MPa, and forces exerted by intra-abdominal pressure do not exceed 10–16 N [39,40].

Biomimetic tests based on cyclical displacement were also performed with the intention of assessing the integrity of the polymer coating. Heating PLA and PCL causes softening or melting of the polymer and macromolecular reorganization of the chains along mesh filaments. This new reorganization of the chains ensures better shell integrity after repeated elongations than with unheated polymer. This fragility of the unheated polymer coating may be explained by shell heterogeneity and porosity, which impede force transmission along the material during tensile test elongation, and predispose to cracking.

Ideally the mesh should release antibiotic into its surroundings immediately after implantation in order to avoid bacterial adhesion and biofilm formation. It should then provide a continuous release of bactericidal doses for up to 3 days. In order to avoid bacterial resistance all the incorporated drug must be released over a period of 3–4 days. Indeed, the release of sub-inhibitory concentrations of antibiotic associated with prolonged therapy are known to be factors that predispose to bacterial resistance to antimicrobial agents [41]. Consequently, we assessed the effect of the polymer coating process (type of polymer and heating process) on the ofloxacin release profile and observed that only PCL Hot coated meshes met the requirements defined above: the release profile of PCL Hot coated meshes was characterized by a steady release of ofloxacin for 3 days without any drastic burst effect and all the drug was released after 4 days, with none remaining trapped in the PCL matrix.

Based on the biomechanical properties and drug release profile of the different coated meshes, PCL Hot was selected as a better coating for a drug-eluting mesh than PLA, and antibacterial tests were performed only on PCL Hot coated meshes. Bacterial adhesion, biofilm formation and inhibition diameter were evaluated in order to determine the in vitro anti-adherence and anti-biofilm effectiveness at the lowest drug dosage. An *E. coli* bacterial model was selected as this microorganism is a common pathogen in abdominal, vaginal and urinary tract post-operative infections. We concluded that 1 mg of ofloxacin on a $3 \times 3\ \text{cm}$ PCL Hot coated prosthesis was sufficient to rapidly decrease bacterial adhesion, prevent biofilm formation and eradicate planktonic bacteria for an appropriate period across a satisfactory area.

However, over the past decade high level fluoroquinolone resistance has been emerging among bacterial strains such as methicillin-resistant *S. aureus* and *E. coli*. This has arisen from multiple chromosomal gene mutations in the antibiotic target enzymes (topoisomerases II and IV) associated with overexpression of antibiotic efflux pumps [42,43]. To avoid such a development combined antibiotherapies could be a possible improvement to this anti-infective mesh. Adding penicillin, which inhibits bacterial cell wall synthesis, could increase the effects of fluoroquinolones that hamper bacterial division. This in vitro testing will be supplemented by implanting the ofloxacin-eluting PCL-coated mesh in an infected incisional abdominal hernia model in the rat.

5. Conclusions

Although hernia and vaginal prolapse repair is considered clean surgery, post-operative mesh-related infection is a common side-effect. Re-operation rates for prolapse surgery may exceed 30% and the incidence of mesh-related infections after hernia repair may be as high as 8% [6,7,44].

Hitherto, infection has been prevented by the intravenous administration of appropriate antibiotics. However, the idea that

the local bioactive system could deliver an effective antibacterial drug (e.g. ofloxacin) is clinically very appealing.

The aim of our study was to create a degradable antibacterial delivery system that could be easily loaded onto the surface of existing prostheses in order to decrease the infectivity of such implantable devices. The spray coating technology described here proved to be suitable for homogeneously loading a drug and polymer coating onto a PP mesh. We created an antibacterial drug-eluting mesh by modifying a commonly used type I PP mesh without dramatically altering the functionality of the prosthetic material.

Antimicrobial tests conducted in a bacterial model showed that local delivery of the antibiotic from the polymer mesh coating may potentially be used to decrease post-operative short-term infection. The advantage of this modified mesh is that the antibiotic delivery vehicle, i.e. the degradable PCL polymer, does not need to be removed at a later stage, thanks to its degradability.

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Appendix A. Figures with essential color discrimination

Certain figures in this article, particularly Figs. 1, 2, 4 and 5, are difficult to interpret in black and white. The full color images can be found in the on-line version, at [doi:10.1016/j.actbio.2011.05.008](https://doi.org/10.1016/j.actbio.2011.05.008).

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