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Melt-spun bioactive sutures containing nanohybrids for local delivery of anti-inflammatory drugs

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ABSTRACT

In this work, a novel concept is introduced in drug-eluting fibres to ensure a good control of drug delivery features and wide applicability to different bioactive compounds. Composite bioactive sutures based on fibre grade poly(ε-caprolactone) (PCL) and loaded with the anti-inflammatory drug Diclofenac (Dic) or a Dic nanohybrid where the drug is intercalated in a synthetic hydrotalcite (Mg/Al hydroxycarbonate) (HT–Dic) were developed. Fibres were prepared by melt-spinning at different PCL/HT–Dic/Dic ratios and analysed in terms of morphology, mechanical properties and drug release features. Results emphasized that tensile properties of fibres are clearly affected by Dic or HT–Dic addition, while the presence of knots has limited influence on the mechanical behaviour of the sutures. Release of Dic strongly depends on how Dic is loaded in the fibre (as free or nanohybrid) whereas the combination of free Dic and HT–Dic can allow a further tuning of release profile. In vivo experiments show a reduction of inflammatory responses associated with Dic-loaded fibers. Thus, a proof of principle is provided for a novel class of bioactive sutures integrating advanced controlled-release technologies.

1. Introduction

Sutures are biomedical devices of natural or synthetic origin used to held together tissues that have been separated due to surgery or traumatic injury. Despite the presence of different devices for wound closure (staples, tapes and glues) available on the market, sutures are the most widely diffused in the medical practice and have a market of around 1.3 billion dollars a year [1]. Since a suture should fulfil a number of requirements, unfortunately, no ideal product is available and the surgeon generally operates a selection on the basis of availability and familiarity [2,3]. Nevertheless, an appropriate suture should take into account aspects such as mechanical properties, resorption rate, risk of infection, and inflammatory reactions that may occur during wound healing process. Over the years, new suture materials have been developed to better respond to particular surgical needs. Recently, the research has switched toward a novel concept of medicated suture that includes a bioactive compound which can be released in a defined time frame and help tissue repair.

Research in this area, although being very attractive, has led to very few products successfully entering the market [4–6]. The first commercial antimicrobial suture, a Polyglactin 910 suture loaded with triclosan, a broad-spectrum antibacterial agent (Vicryl Plus®), was approved for clinical uses by the US Food and Drug Administration (US FDA) since 2002 [7]. The basic concept in these sutures consists in coating a preformed polymeric filament with a second biodegradable polymer layer embedding triclosan with the aim to create a zone of inhibition to the spread of bacteria and to exert a preventive action against the possible infection of the surgical site [8]. Nowadays antimicrobial sutures are successfully used in a number of surgical procedures [7, 9–13] with a reduction of wound site infection and consequent cost saving [14]. For all these reasons this treatment strategy was found to be very promising and sutures coated with other drugs such as antithrombotic, analgesics, antineoplastic and antiproliferative agents are under investigation [15].

Recently Lee et al. [16] have proposed a new method to obtain medicated suture where a commercial suture is covered with a polymeric sheet loaded with a pain relief drug. These sutures have been proven

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to have suitable mechanical properties and a drug release only for 6 days. Nevertheless, control of drug release rate is a critical factor to design a bioactive suture in view of an optimized biological effect. For this reason, more suitable strategies are needed to attain both efficient control over drug release rate and adequate mechanical properties. As an alternative to coated fibres, electrospun aligned fibres have been developed where different active agents are dispersed in a polymer to give a matrix-like structure [17–20]. Unfortunately, fibres show weak mechanical properties and electrospinning is difficult to scale-up, making these systems difficult to be applied.

A promising alternative is represented by melt-spinning technology. In this case, a polymer melt is forced through a spinneret capillary to obtain fibres with properties strongly related to the applied drawing extent. The application of this process, even if scalable up to industrial level, is limited in the biomedical field where the usual thermolability of bioactive molecules as drugs and/or the relatively poor elongational properties of biocompatible polymer melts, further worsened by incorporation of additives, may prevent a satisfactory drawing of fibres compromising their ultimate mechanical properties.

Among the strategies useful to control drug release from a polymer matrix, the inclusion of lamellar structures opens new opportunities to develop smart systems. Recently, magnesium and aluminium hydroxycarbonates referred to as hydrotalcite-like compounds (HT) intercalating bioactive molecules have been proposed [21–24]. These systems consist of a layer of inorganic clays which, under specific conditions, self-organize to form a bilayer. In particular Mg/Al LDHs, where some Mg(II) cations are isomorphously replaced by Al(III) cations, generate positive charges balanced by the presence of counteranions located in the interlamellar region. The possibility of replacing these anions by simple ion-exchange procedures makes LDHs a unique class of layered solids to be used as hosts of drugs bearing a negative charge. HTs have already been proven to be biocompatible and some of them are already used in clinical practice as antiacids because of their antipepsin activity [24]. In specific conditions, HT can intercalate different anions or biologically active molecules such as anionic non-steroidal anti-inflammatory drugs (NSAID) [25], antibiotics [26], up to around 50% by weight and form organic–inorganic nanohybrids. Depending on drug features (solubility, molecular weight, affinity to HT), fast dissolution or sustained release of the drug can be accomplished as a consequence of a de-intercalation process [27,28]. Furthermore, a body of interest is growing on the development of novel composites based on inorganic layered materials and organic polymers. Recent studies report on the possibility to introduce organically-modified HT in different polymers as fillers opening a new way to integration of bioactive HT in polymeric films, membranes or fibres with different potential applications in industrial and biomedical field [29–31]. In this context, on the basis of an European patent owned by some participants to this research [32], Sammartino et al. [31] incorporated nanohybrids containing the NSAID Diclofenac (Dic) into poly(ε-caprolactone) (PCL) films and demonstrated effective control of drug release as compared to free drug directly dispersed into the polymer.

Prompted by these considerations, in this paper we offer a proof of principle on the possibility to obtain an anti-inflammatory sustained-release biodegradable suture through the incorporation of a free drug and/or a drug-HT nanohybrid in a thermoplastic polymer. To this purpose a Dic-HT nanohybrid was incorporated in a fibre of poly(ε-caprolactone) (Fig. 1). The fibre was produced by melt spinning and characterized in terms of morphology, size, mechanical properties, drug release and in vivo performance.

2. Materials and methods

2.1. Materials

A nanohybrid containing synthetic hydrotalcite and Diclofenac (HT–Dic, $[Mg_2Al(OH)_6](C_{14}H_{10}Cl_2NO_2) * 2H_2O$, Mg/Al ratio 1.8, distance between layers = 23.6 Å, moisture 7.0%, Diclofenac loading = 59.6%) was obtained from Prolabin & Tefarm (Italy). Diclofenac sodium (Dic) was purchased from Farmalabor (Italy). Poly(ε-caprolactone), PCL, used for this study (CAPA® 6800) was from Perstorp Corporation (UK). HPLC-grade tetrahydrofuran (THF), acetonitrile and methanol, analysis-grade acetone and dichloromethane were from Carlo Erba (Italy). Synthetic hydrotalcite (HT, $Mg_6Al_2(CO_3)(OH_{16}) * 4H_2O$), sodium chloride, potassium chloride, HPLC-grade trifluoroacetic acid (TFA), sodium phosphate dibasic and potassium phosphate monobasic (HPLC grade) were obtained from Sigma-Aldrich (USA). Distilled filtered (0.22 μm) water was employed.

2.2. Fibre production

Fibres were prepared through extrusion, drawing and subsequent cold drawing to the final diameter of approximately 300 μm. Prior to the extrusion process, the components were separately sieved to obtain a fine powder (97% of the powder passed through a #140 sieve with a mesh size of 106 μm according to Ph. Eur. 7th edition). The mean diameter and size distribution of powders were determined by laser light scattering (Coulter LS 100Q, USA). Particle size is expressed as volume mean diameter (μm) ± SD of values collected from three different batches. For Zeta potential measurements, HT–Dic was dispersed in water and analysed on a Zetasizer Nano Z (Malvern Instruments, UK).

The base materials were mixed in a HAAKE twin screw extruder using a screw speed of 20 rpm and applying a temperature profile going from 60 °C, at feed zone, to 100 °C at the die. The filament was cooled in stagnant air (at 23 °C) and collected with a take-up speed of 4 m/min. The as-spun fibres (with a diameter of about 900 μm) were drawn at 50 °C using a Conditioning Unit (DSM Xplore, The Netherland) to the final diameter of about 300 μm (corresponding to a draw ratio of 9).

Different compositions of the fibres, reported in Table 1, were selected in order to investigate i) the effect of Dic intercalated in HT (Dic–HT) as compared to free Dic (PCL/HT–Dic vs. PCL/Dic); ii) the influence of HT in fibres containing free Dic (PCL/HT/Dic vs. PCL/Dic) and iii) the

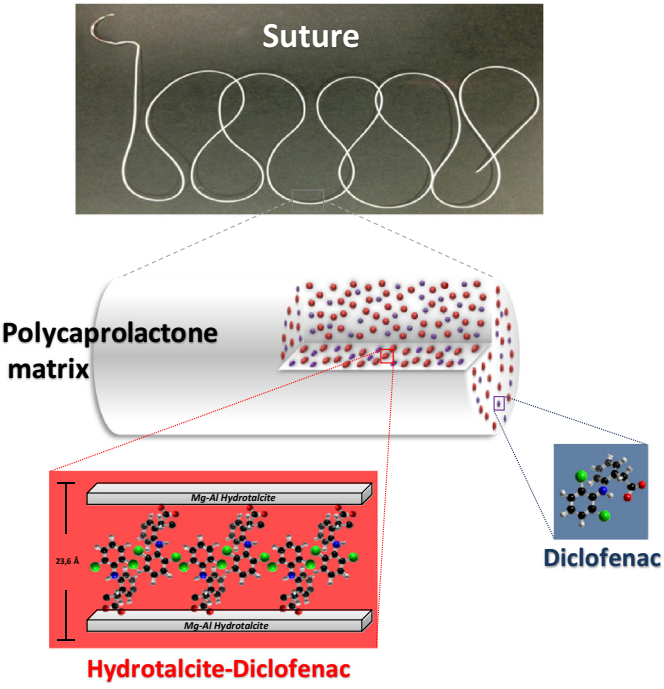


Fig. 1. The concept of anti-inflammatory fibres developed in the study.

Table 1
Composition of PCL fibres.

Fibre	PCL (%)	HT–Dic (%)	HT (%)	Dic (%)	Dic (mg/cm)	Diameter (μm)
PCL/HT/Dic	75	–	8	17	0.095	264 ± 44
PCL/HT–Dic	82	18	–	–	0.105	315 ± 30
PCL/HT–Dic/Dic	75	17		8	0.080	328 ± 67
PCL/Dic	82	–	–	18	0.149	324 ± 21

possible effect of free Dic in fibres involving intercalated Dic (PCL/HT–Dic vs. PCL/HT–Dic/Dic). We fixed Dic loadings in the fibre at 9 and 18% by wt in order to test the system at high loading where control of drug release is generally poor.

2.3. Mechanical properties

Tensile properties of straight and simple knotted (according to Ph. Eur. 7th edition) fibres were measured using a universal testing machine (Alpha Technologies mod. 2020) equipped with a 10 N load cell. All tensile experiments were carried out at a strain rate of 300 mm/min, using a gage length of 150 mm, at 23 °C. For each sample, at least five specimens were analysed (if the fibre broke in a clamp or within 1 cm, the result was discarded and the test was repeated) and average results are reported with their standard deviation. The mean diameter was obtained after three measurements at points evenly spaced along the suture using a 0.001 mm accuracy digital micrometer. Mechanical properties of fibres are expressed in terms of elastic modulus, breaking load, breaking stress and percent elongation at break.

2.4. Scanning electron microscopy/Energy Dispersive X-ray microanalysis

Suture shape and morphology were analysed by scanning electron microscopy (SEM) (Quanta 200 FEG; FEI, USA). To analyse internal structure, fibre wire sections were prepared. The sample was included into Tissue Tek® OCT (Sakura, Japan) and then cut into 40 μm slice using a Tissue-Tek® Cryo3® Microtome/Cryostat (Sakura, Japan). The samples were stuck on a metal stub and coated with gold under vacuum evaporator for 90–120 s. Surface composition of the samples was investigated by Energy Dispersive X-ray spectroscopy (EDS) microanalysis through a X-EDS detector (Oxford Inca Energy System 250 equipped with INCAx-act LN2-free detector).

2.5. HPLC analysis of Diclofenac sodium

Dic analysis was carried out by RP-HPLC on a system consisting of a FCV-10ALvp mixer, a LC-10ADvp pump equipped with a SIL-10ADvp autoinjector, a SPD-10Avp UV–vis detector and a C-R6 integrator from Shimadzu (Japan). The analysis was performed on a Luna 5μ C18 (250 × 4.6 mm) (Phenomenex, USA) at a flow rate of 1.0 mL/min. The injection volume was 20 μL in all the experiments and the detection wavelength fixed at 238 nm.

For Dic quantitative analysis the mobile phase was acetonitrile/acid water both modified with 0.1% TFA mixture in the ratio 70:30 v/v. A

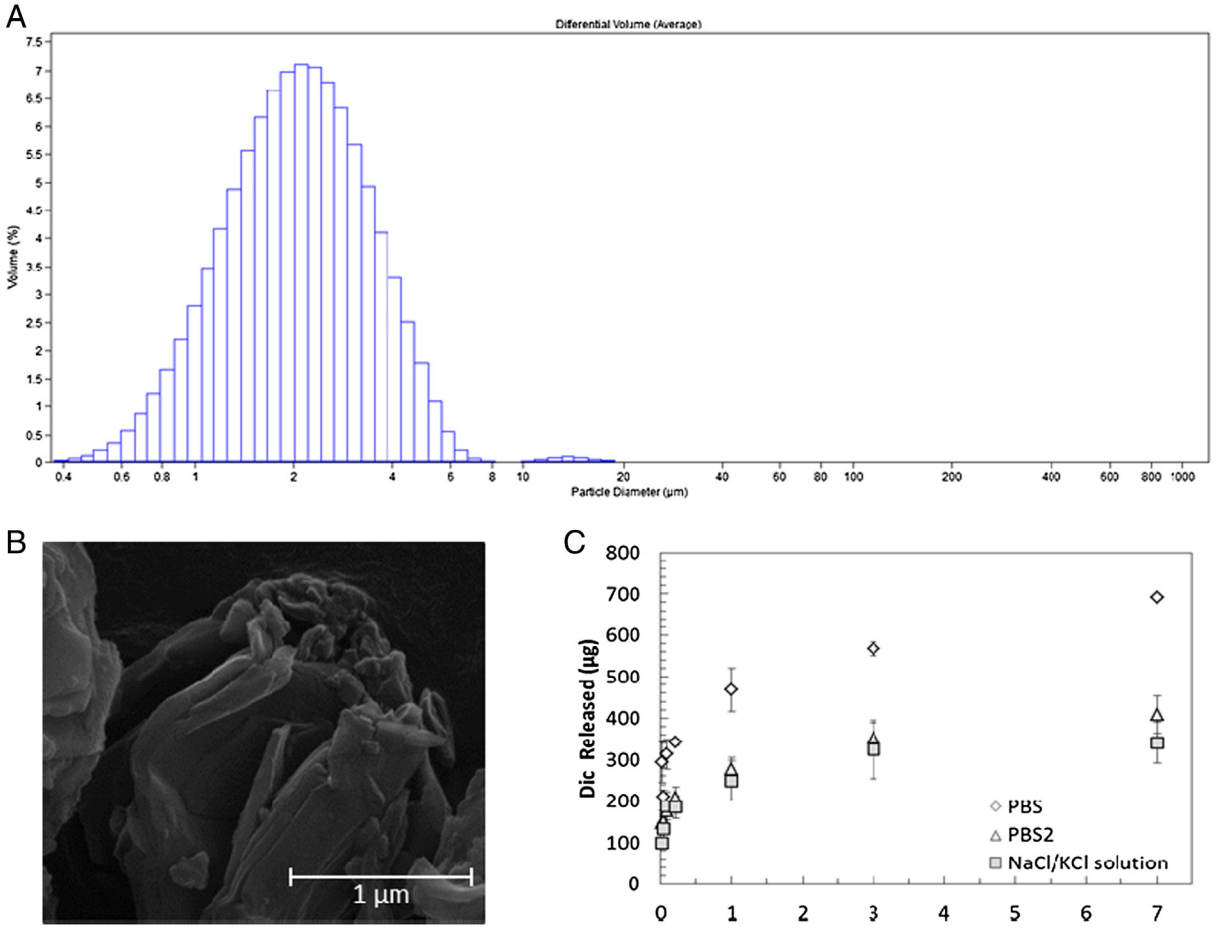


Fig. 2. Properties of HT–Dic. A) Size distribution; B) SEM micrograph; and C) release profile of Dic in PBS (NaCl 120 mM, KCl 2.7 mM, Na_2HPO_4 10 mM), PBS2 (NaCl 240 mM, KCl 5.4 mM, Na_2HPO_4 10 mM) and NaCl/KCl (NaCl 120 mM, KCl 2.7 mM) solution at pH 7.4 and 37 °C. Results are reported as mean ± SD of three measurements.

Dic standard solution in water was prepared and stored at 4 °C until use. Calibration curve was constructed by injecting solutions with concentrations in the range 0.3–30 μg/mL LOD was 0.08 μg/mL whereas QOD was 0.26 μg/mL.

2.6. In vitro release studies

In vitro release of Dic from the HT–Dic nanohybrid was evaluated by dispersing 2 mg of HT–Dic in 5 mL of release medium at 37 °C. Three different release media were prepared: NaCl/KCl solution (NaCl 120 mM, KCl 2.7 mM), Phosphate Buffer Saline (PBS, NaCl 120 mM, KCl 2.7 mM, Na_2HPO_4 10 mM) and Phosphate Buffer Saline at double chloride concentrations (PBS2, NaCl 240 mM, KCl 5.4 mM, Na_2HPO_4 10 mM) both at pH 7,4. At predetermined time intervals 1 mL of supernatant was collected after centrifugation (5000 rpm 15 min 4 °C) and analysed by the analytical method previously described for Dic quantification.

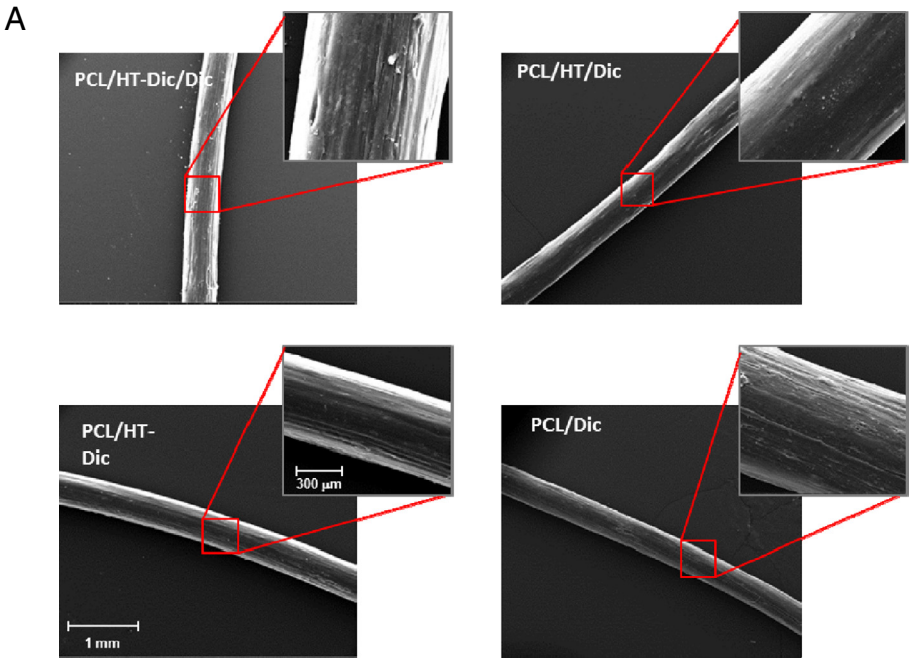
The in vitro release of Dic was evaluated by immersing three fibre portions (3 cm) taken from different parts of extruded suture in 5 mL of Phosphate Buffer Saline (PBS, NaCl 120 mM, KCl 2.7 mM e Na_2HPO_4 10 mM) at pH 7.4 and 37 °C under gentle shaking. Periodically 1 mL of release medium was collected and replaced with the same volume of fresh medium. The samples were analysed by the analytical methods previously described and results are reported in terms of released Dic percent.

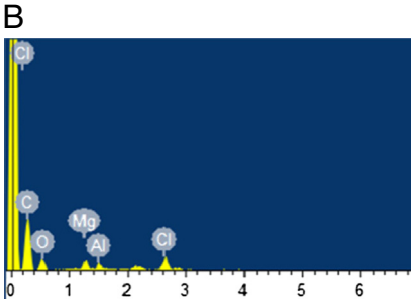
Fibre morphology was evaluated also at the end of the release experiment. To this purpose, fibre was washed with water three times, freeze-dried and then analysed by SEM.

2.7. In vivo experiments

Male CD1 mice (30–35 g) (Harlan, Italy) were purchased from Harlan Italy (San Pietro al Natisone, UD, Italy) and housed in stainless steel cages in a room kept at 22 ± 1 °C with a 12:12 h light/dark cycle. The animals were acclimated to their environment for 1 week and had ad libitum access to standard rodent chow pellets. All procedures met the guidelines of the Italian Ministry of Health (D.L. no. 116 of January 27, 1992) and guidelines in the European Communities Council (Directive of November 24, 1986, 86/609/ECC).

Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), the back was shaved and scrubbed with betadine, and an incision (4 cm in length, and 0.2 cm in deep) was made in the middle using a number 12 blade. Five different sutures were used: 1) PCL suture; 2) PCL/HT/Dic, 3) PCL/Dic; 4) PCL/HT–Dic/Dic; and 5) PCL/HT–Dic. The wounds were closed with three sutures for subcutaneous and four sutures for cutaneous tissues. After 3 days from surgery, mice were killed and cutaneous and sub-cutaneous tissues were removed. The samples were fixed in 10% neutral buffered formalin, then processed and embedded in paraffin, according to standardized protocol. Sections of 4 μm were stained with haematoxylin and eosin.





	Mean (%)	Standard Deviation
C	66.74	0.20
O	26.16	0.18
Mg	2.06	0.21
Al	1.21	0.07
Cl	3.83	0.10

Fig. 3. SEM images of fibre surface (A) and EDS analysis of PCL/HT–Dic fibre surface (B). The sample is representative of outer fibre surface.

Two histological parameters were evaluated in each sample: inflammatory infiltrate and granulation tissue. They were graded according to semi quantitative score as:

+ = weak inflammatory infiltrate/low amount of granulation tissue

++ = moderate inflammatory infiltrate/moderate amount of granulation tissue

+++ = intense inflammatory infiltrate/intense amount of granulation tissue.

3. Results and discussion

3.1. Fibre extrusion and morphology

The aim of this work was to develop an anti-inflammatory drug-eluting synthetic suture and to test its effects in vivo. Modulation of drug delivery rate was considered as a key parameter to achieve regional prolonged release and to promote healing process. We focused on melt-spinning technique to produce polymeric fibres with regular circular cross section since it couples good versatility, fast industrial scale-up and does not require organic solvents. Nevertheless, melt-spinning perfectly fits loading of very hydrophilic drugs in water-insoluble polymers such as PCL ensuring high entrapment efficiency. After characterization, the HT–Dic nanohybrid was included in PCL fibres, which were then drawn to obtain suitable diameter and fully investigated.

Properties of HT–Dic nanohybrid are reported in Fig. 2. The size distribution curve showed a monomodal trend and a HT–Dic mean particle diameter of 2.4 ± 0.2 μm. Surface charge of the nanohybrid was slightly negative. SEM analysis confirmed the size and the HT lamellar structure.

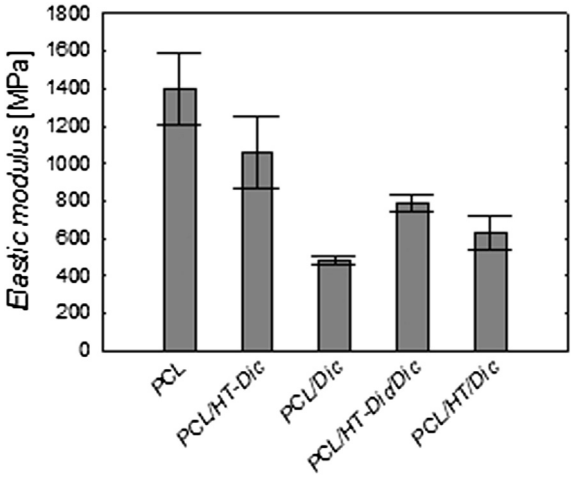


Fig. 5. Elastic modulus of fibres with different compositions. Results are the mean of five measurements, error bars are standard deviations.

The release profile of Dic from the HT–Dic nanohybrid was evaluated in physiologically-simulated conditions. HT consists of layers of magnesium hydroxide, with aluminium isomorphically substituted to give a net positive charge inside the layers that is balanced by interlayer hydrated Dic anions. To achieve drug release, the presence of

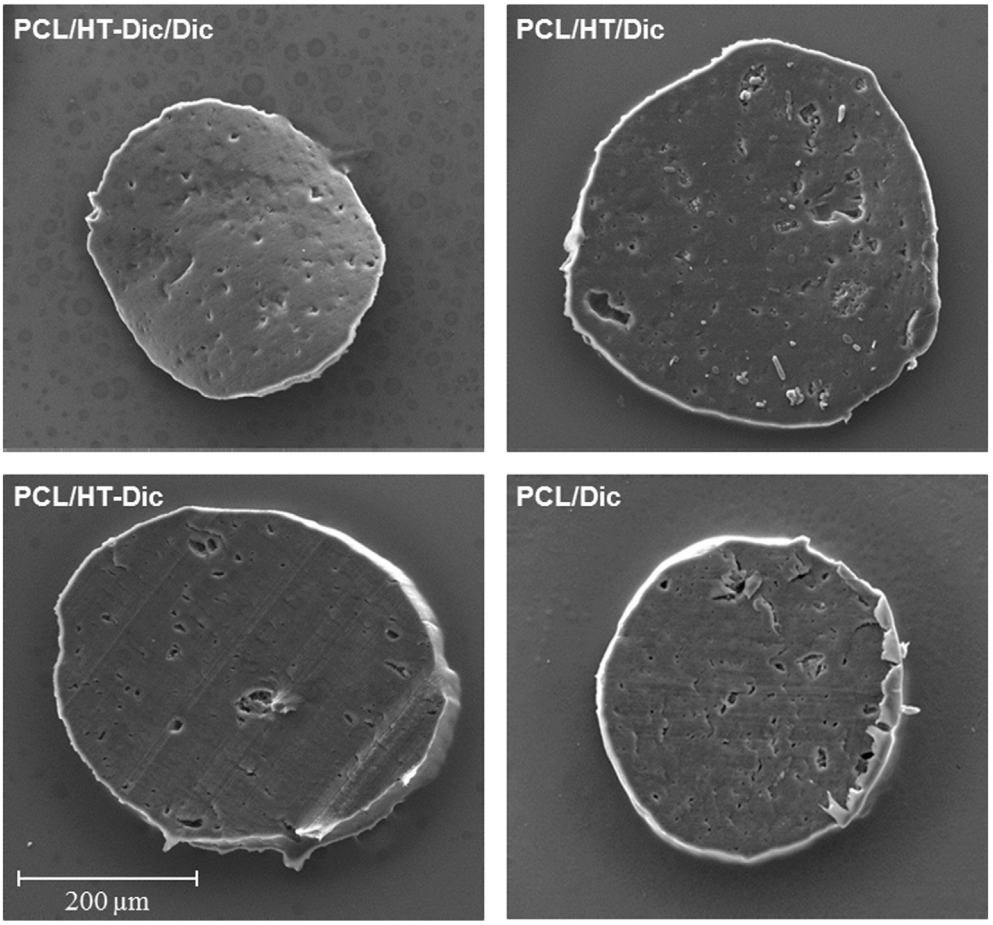


Fig. 4. SEM images of fibre section. The sample is representative of other inner sections.

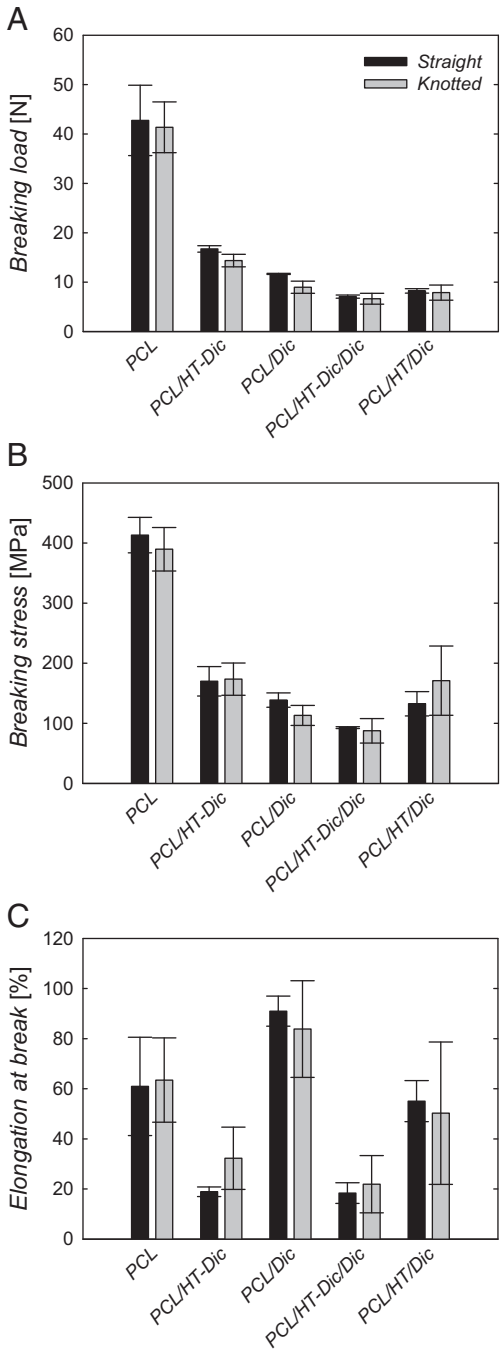


Fig. 6. Mechanical properties of straight and knotted fibres: breaking load (A); breaking stress (B); and elongation at break (C). Results are the mean of five measurements, error bars are standard deviations.

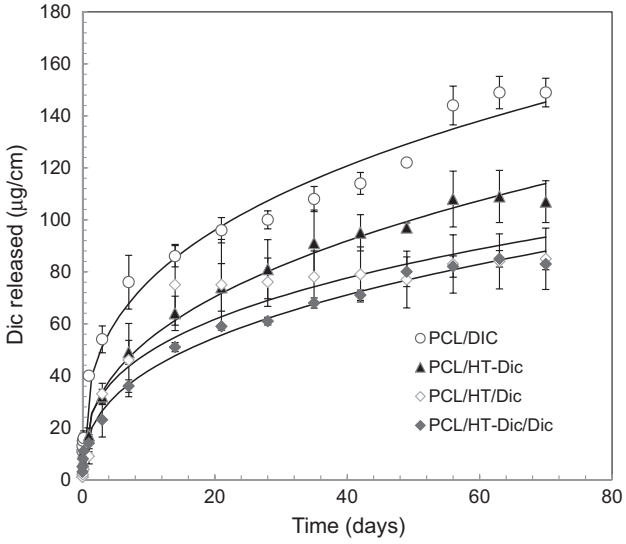


Fig. 7. Dic release from fibres in PBS (10 mM) at pH 7.4 and 37 °C. Results are reported as mean ± standard deviation of three measurements. Lines through data points are to guide the eye.

anions in the medium capable of exchange with those located inside the nanohybrid is needed. Since in a physiological environment the most available anions are chlorines and phosphates, Dic release from HT–Dic was tested in media at different chloride and phosphate concentrations (Fig. 2c). As expected for this type of system, release rate was strongly influenced by the ionic composition of the medium. PBS ensured a complete release of Dic from the intercalation product and was selected for further release experiments on fibres.

Biodegradable PCL fibres with different compositions were processed by a two-step melt-spinning method (melt extrusion and hot draw) (Table 1). Melt spinning has proven to be a robust method for the production of several biomedical devices and it has been found to be useful in the pharmaceutical industry as well [33]. Since many years this technique has been already used for the industrial production of sutures because it is an economical process with reduced production time, few processing steps, and offering the possibilities to work in continuous. Furthermore, the capacity of melt extrusion to disperse different active pharmaceutical ingredients in a matrix at molecular level (forming solid solutions) has been seen as a possible strategy to increase solubility and bioavailability of water-insoluble compounds [34,35]. One disadvantage of this production method is the necessity to operate at high temperature in order to melt the polymer while most drugs may degrade at high temperature. In the present case, RP-HPLC qualitative analysis of Dic extracted from the PCL/HT–Dic after extrusion confirmed that the production method does not affect drug stability (Supplementary material S1). Nevertheless, this strategy of drug loading through HT can be of benefit for those molecules with poor thermal stability as well as drugs undergoing polymorphism in order to maintain their chemical–physical integrity.

After extrusion, fibres were analysed by scanning electron microscopy (SEM) to evaluate size and surface morphology (Fig. 3). In details, as spun fibre of PCL formed at 100 °C die temperature showed extruding lines on the surface (Fig. 3a), likely due to polymer curing in the applied processing conditions. Nevertheless, the presence of some particles on the surface of the fibres containing HT or HT–Dic was observed. To investigate the nature of these particles, fibre surface was analysed by Energy Dispersive X-ray spectroscopy (EDS). Particles were composed mainly by magnesium and aluminium, thereby suggesting that HT–Dic particles also locate on the filament surface during processing (Fig. 3b).

To evaluate the inner morphology of the fibres, SEM images on fibre sections were taken (Fig. 4). All the fibres had round cross-sectional geometry and non-porous morphology, with an average diameter around 0.3 mm comparable with those measured by a calibre (Table 1). Micrographs indicated a rather homogeneous dispersion of included components with the presence of small internal voids due to a decrease of feeding load during extrusion.

3.2. Mechanical properties

Given that the use of sutures strongly depends on mechanical properties, their strength is the most frequently reported parameter

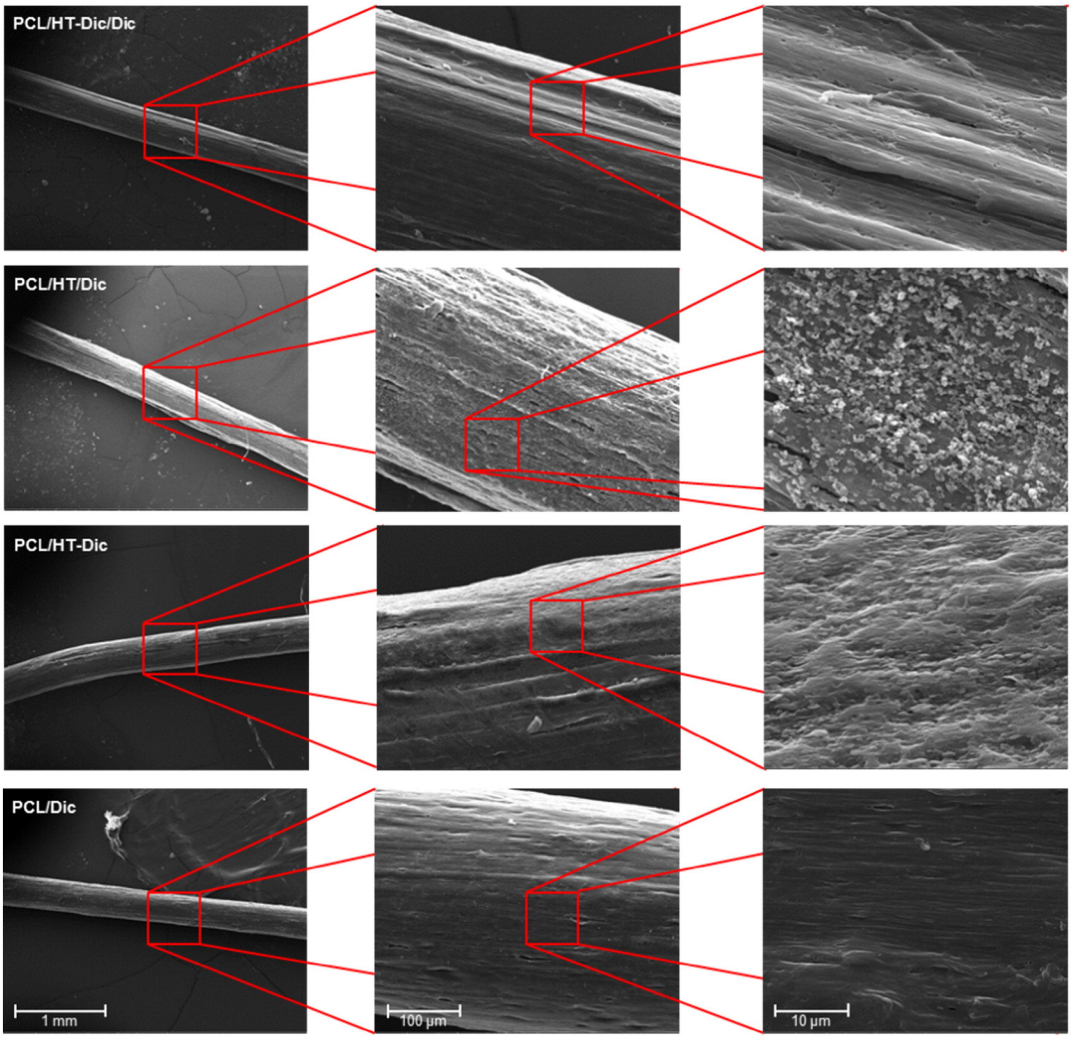


Fig. 8. SEM images of fibres after 70 days of incubation in PBS (10 mM) at pH 7.4 and 37 °C.

[1]. There should be a proper match between suture strength and tissue strength [36] and for this reason the selection of proper suture depends also from the tissue involved. Furthermore, tensile properties of sutures are important when making a knot. If the material is too weak and the knotting force is stronger than tensile strength of suture material, suture can easily break while tightening the knot [37,38].

Results of tensile tests performed on melt-spun straight and knotted filaments are shown in Figs. 5 and 6.

The elastic modulus data (Fig. 5) indicated that the inclusion of particulate components decreased the stiffness of the fibre with respect to pure PCL. In particular, the addition of HT–Dic caused a reduction of tensile modulus from 1400 MPa to about 1100 MPa (−25%) while the inclusion of Dic caused a remarkable reduction of the modulus up to about 480 MPa (corresponding to −66%). Systems including both Dic and HT as well as Dic and HT–Dic also showed a significant decrease in modulus.

Tensile properties (breaking stress and elongation at break) of straight and knotted fibres are shown in Fig. 6. Data indicated that within experimental errors:

- the knotted fibres had the same breaking stress and the same elongation at break of straight fibres thus indicating that the knot does not represent a weakness to the fibre;
- composite fibres showed a three-fold decrease with respect to pure PCL fibres in breaking stress;
- fibres containing HT–Dic showed a fragile behaviour with elongations at break around 20% (other systems being around 70%) and a tenacity in the range 10–17 MJ/m [3] (other systems being around 70 MJ/m^3).

The effect of knotting on the strength of various sutures was studied by Kim et al. [38], who concluded that the knotting of a suture reduced its tensile strength. In the present case the knots do only have a marginal influence on the mechanical behaviour of the fibres, and this is an advantage for their use in slow-healing tissues (skin, fascia and tendons).

3.3. In vitro release and degradation

The release profile of Dic from fibres was assessed in PBS at pH 7.4 and 37 °C, simulating physiological conditions (Fig. 7). In all the samples, an initial burst of Dic was observed. The control fibre containing free HT and Dic (PCL/HT/Dic, Dic content 0.095 mg/cm) completely eluted its Dic content in around 14 days, thus showing a poor control over release rate. The fibres containing HT–Dic (PCL/HT–Dic, Dic content 0.105 mg/cm) showed a nicely shaped release profile reaching complete Dic release after 55 days. In the fibre containing both HT–Dic

Table 2
Inflammatory infiltrate and granulation tissue in specimens.

Fibre	Inflammatory infiltrate		Granulation tissue	
	Cutaneous tissue	Sub-cutaneous tissue	Cutaneous tissue	Sub-cutaneous tissue
PCL/Dic	++	++/+++	–	–
PCL/HT/Dic	+/−	+	–	–
PCL/HT–Dic	+	++	–	+
PCL/HT–Dic/Dic	+	++	–	–
PCL	+/++	+/++	–	–

+ = weak inflammatory infiltrate/low amount of granulation tissue.
++ = moderate inflammatory infiltrate/moderate amount of granulation tissue.
+++ = intense inflammatory infiltrate/intense amount of granulation tissue.

and a fraction of free Dic (PCL/HT–Dic/Dic, Dic content 0.080 mg/cm), release rate was again well modulated besides the presence of free Dic. Dic amount released from PCL fibre (PCL/Dic, Dic content 0.0149 mg/cm) was higher than for the other fibres due to a higher Dic loading. After 70 days Dic was still released in native form indicating the protective effect of PCL and HT on its chemical structure (Supplementary material S1). These data suggest that a fine tuning of release profiles can be accomplished playing on reciprocal HT–Dic/Dic ratio and optimal amount of released Dic may be selected according to the therapeutic needs.

In order to evaluate the mode of degradation, fibre morphology was analysed after 70 days of release in PBS at pH 7.4 at 37 °C (Fig. 8). Surface modification of the fibre, likely due to slow polymer progressive degradation, was observed without the occurrence of fractures. As expected, the extent of polymer degradation was very limited and confined to the surface, confirming that this kind of system behaves mainly as a surface eroding system. Nevertheless, surface degradation of PCL determined the outcrop on the surface of HT and HT–Dic as shown by EDS analysis. This phenomenon was particularly evident in the PCL/HT/Dic fibre.

Drug release kinetics from a polymer matrix mainly depends on three distinct steps; (a) liquid penetration into the matrix, (b) dissolution and, (c) diffusion of drug, which may be the rate determining step for drug release. Progressive matrix degradation can affect drug diffusion rate along time. The degradation mode observed for PCL fibres suggests that Dic release is predominantly controlled by

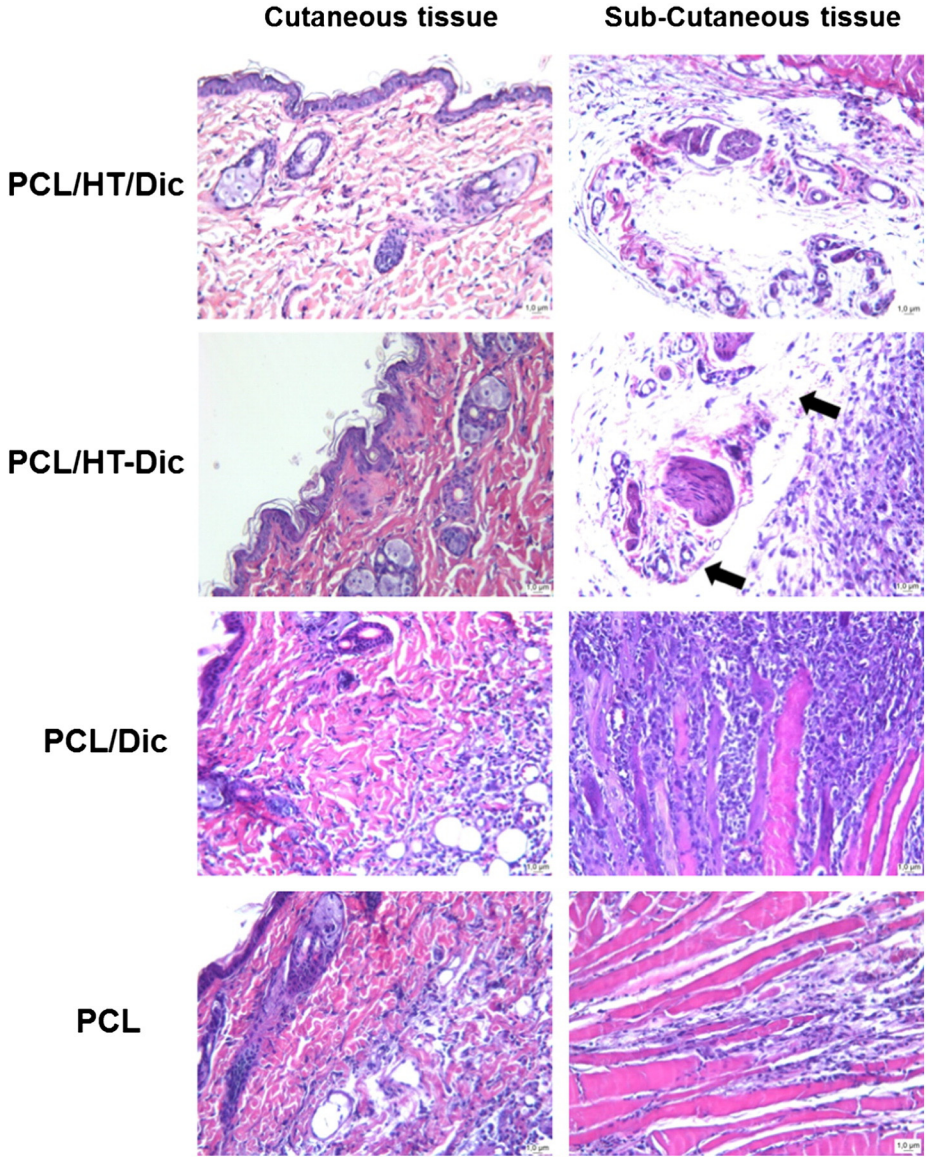


Fig. 9. Haematoxylin and eosin-stained sections of cutaneous and sub-cutaneous tissues from mice at suture site, after 3 days from surgery. Arrows indicate small blood vessels and fibroblasts.

drug dissolution and diffusion in the polymer matrix. Indeed both Dic and HT may affect the PCL structure and, thus, the release mechanisms. In the case of PCL/Dic, drug diffusion in the rubbery PCL phase as well as through polymer mesopores is the prevailing mechanism. In particular, except that the crystallinity of the matrix is not significantly altered by additives, Dic could partially bind to PCL with effects of plasticization confirmed in the case of the PCL/Dic fibre showing stiffness lower than that of pure PCL. This hypothesis also allows to explain the rapid diffusion of drug shown by the PCL/Dic filaments compared to other ones. When the fibre contains HT–Dic nanohybrid, Dic needs to diffuse out of the lamellar structure and then through the polymer matrix, which adds a supplementary release step contributing to the overall release profile. In fact, HT can favour an increase of the tortuosity and, consequently, of the diffusion length with effects that depend on both the content and the distribution of this filler with significant complexity.

Overall, these results suggest that direct incorporation of Dic in PCL fibres controls drug release rate but results in poor suture mechanical properties. On the other side, incorporation of HT–Dic nanohybrids in the fibres allows to control effectively both release profile and mechanical properties.

3.4. In vivo effects

To assess in vivo anti-inflammatory effect, fibres were employed to close a traumatic wound in mice. Two histological parameters were evaluated in each sample: inflammatory infiltrate and granulation tissue (Table 2).

Sections of cutaneous and sub-cutaneous tissue at suture site were stained by haematoxylin and eosin (Fig. 9). In samples containing PCL/Dic an inflammatory infiltrate variable from moderate to intense was observed, especially at the level of the sub-cutaneous tissue, without formation of granulation tissue. In all the other specimens no variations in the inflammatory response was observed, except for PCL/HT/Dic where this reaction was found to be less intense. It is worth of note that the presence of granulation tissue was observed only in the sub-cutaneous tissue of PCL/HT–Dic sample. For PCL fibre, the inflammatory response appeared to be less intense, again without the formation of granulation tissue. Regardless of the surgical site, the inflammatory reaction in the injured tissue reduces the damage, removes damaged tissue components and stimulates the deposition of extracellular matrix, inducing angiogenesis. Therefore the observation that a granulation tissue is present can be related to an expression of a more effective and early reparative process in progress.

Sustained Dic release may represent a clinically relevant therapeutic modality. In fact, besides anti-inflammatory effect, sustained Dic release can have an impact also in the management of postoperative pain, thus minimizing the dose of medications to lessen side effects while still providing adequate analgesia. To date, perioperative administration of a single local dose of nonsteroidal anti-inflammatory drugs has shown inconclusive efficacy [39]. Rather than a single bolus, Lavand'homme et al. [40] found that continuous intrawound infusion of Dic demonstrated a greater opioid-sparing effect and better postoperative analgesia than the same dose administered as an intermittent intravenous bolus. Thus, Dic-eluting sutures can combine delivery concepts to a biomedical device to exert both effective control of inflammatory phase and alleviation of post-operative pain.

4. Conclusions

In summary, we have developed a drug-eluting anti-inflammatory suture that could have the dual function of closing the site of wound excision while providing sustained localized delivery of Diclofenac. The melt spun fibre containing HT–Dic nanohybrid has shown a homogenous distribution of filler, good mechanical properties, tuneable release rate as a function of composition and in vivo activity that suggests its use in surgical practice. Overall, the strategy proposed is of great potential and versatility since melt-spinning is a simple and reproducible process that can be scaled-up at industrial level. Nevertheless, control of fibre properties by nanohybrid should help to extend the applicability of this concept to other bioactive drugs.

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Appendix A. Supplementary data

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