Semi-interpenetrated hydrogels-microfibers electroactive assemblies for release and real-time monitoring of drugs

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Abstract: Simultaneous drug release and monitoring using a single polymeric platform represents a significant advance in the utilization of biomaterials for therapeutic use. Tracking drug release by real-time electrochemical detection using the same platform is a simple way to guide the dosage of the drug, improve the desired therapeutic effect, and reduce the adverse side effects. The platform developed in this work takes advantage of the flexibility and loading capacity of hydrogels, the mechanical strength of microfibers, and the capacity of conducting polymers to detect the redox properties of drugs. The engineered platform is prepared by assembling two spin-coated layers of poly-γ-glutamic acid hydrogel, loaded with poly(3,4-ethylenedioxythiophene) (PEDOT) microparticles, and separated by a electrospun layer of poly-ε-caprolactone microfibers. Loaded PEDOT microparticles are used as reaction nuclei for the polymerization of poly(hydroxymethyl-3,4-ethylenedioxythiophene) (PHMeDOT), that semi-interpenetrate the whole three layered system while forming a dense network of electrical conduction paths. After demonstrating its properties, the platform has been loaded with levofloxacin and its release monitored externally by UV-Vis spectroscopy and in situ by using the PHMeDOT network. In situ real-time electrochemical monitoring of
the drug release from the engineered platform holds great promise for the development of multi-functional devices for advanced biomedical applications.

1. Introduction
Drug delivery systems, as engineered technologies for the targeted delivery and/or the controlled release of therapeutic agents, have long been used to improve health. Biomedical engineers have contributed substantially to our understanding of the physiological barriers to efficient drug delivery, while biomaterial engineers have developed platforms able to act as vehicles and/or cargos for therapeutic drug delivery, helping cope with drawbacks of classical pharmaceuticals (e.g. increasing the solubility of the drugs, reducing side effects, and improving biodistribution). Although many materials have been employed as therapeutic platforms for drug delivery, polymers have received special attention because of their properties and versatility.\(^1\)

Over the last years, drug delivery using polymeric platforms based on (nano)particles,\(^2\) (nano)fibers,\(^3,4\) (nano)films,\(^4,5\) and hydrogels\(^6\) have been extensively investigated. Among polyesters, poly-\(\varepsilon\)-caprolactone (PCL) has attracted great interest for drug release due to its biodegradable and biocompatible nature,\(^7\) as well as by the approval of PCL-based devices by the U.S. Food and Drug Administration (FDA) for many medical applications, including drug delivery devices, in the human body. The release mechanism from PCL formulations depends on the degradation of the polymer and on the polarity of the drug. Lipophilic drugs are usually encapsulated inside the PCL formulations (e.g. microsphere, nanoparticles, scaffolds, films and fibers, micelles) and the release occurs slowly upon surface erosion by enzymatic degradation.\(^7,8\) Instead, hydrophilic drugs tend to accumulate at the surface of polymeric platform and a fast burst release occurs by desorption at the initial period or dosage intake.\(^7,9\) Besides, the utilization of polypeptides in drug release applications is receiving increasing attention not only because of their versatility in macromolecular design and
synthesis, but also due to the incorporation of functionalities present in natural proteins, which facilitates their interaction with tissues and cells.[10] Compared to conventional synthetic polymers, polypeptides exhibit some advantages, as for example, non-immunogenicity, enhanced biodegradability, remarkable biocompatibility properties, and multiple functional groups to enhance the loading efficiency.[11]

On the other hand, dual-functional platforms for real-time drug release monitoring have become of major significance in the last few years. Two main approaches have been developed for this purpose. The simplest one is based on the construction of assembled devices in which drug release systems are chemically or physically coupled to modified electrodes for detecting the drug remaining in the platform.[12] The second approach, which is more recent, is based on connecting elements that release the desired drug with elements that visualize such drug via imaging, like specific fluorescent or luminescent reporters.[13]

Although the latter approach represents an improvement with respect to the former in terms of integration, aspects related with the chemical design and experimentation are much more tedious and complex.

Because of the simplicity and fast response of the electrochemical sensors used in the assembled systems, as well as the associative advantages offered by the connected elements in the integrative systems, the design of hybrid platforms by combining the benefits of each approach is very attractive. In this work, we report a new strategy for the fabrication of dual-functional platforms by merging the best of each of the two approaches. More specifically, we propose the preparation of assemblies involving two spin-coated layers of polypeptide hydrogel separated by a mat of electrospun PCL microfibers (MFs). Then, the elements of such three-layered assembly are connected through a semi-interpenetrated (sINP) three-dimensional network. Polypeptide hydrogels, which exhibit poor mechanical properties, act as drug loading systems, while PCL MFs provide strength to the assembly,[14] which is robust and manageable. The semi-interpenetration of the assembly is performed using an
intrinsically conducting polymer (ICP), poly(3,4-ethylenedioxythiophene) (PEDOT), which is incorporated by in situ anodic polymerization. This ICP is recognized as the most stable and electroactive heterocyclic ICP and, in addition, is biocompatible.\textsuperscript{[15]} After complete characterization, the engineered platform, hereafter named sINP/PCL/sINP, was loaded with levofloxacin (LVX), which is one of the outstanding representatives of the third generation of quinolone antibiotics that have been a useful class of broad-spectrum antimicrobials.\textsuperscript{[16]} The release of LVX has been monitored in real time by detecting the oxidation of the antibiotic that remains inside the matrix. Finally, the electrochemical detection through the dual-functional platform has been correlated with spectroscopic measurements to evaluate the sensitivity of the former.

2. Results and discussion

2.1. Preparation of sINP hydrogel and the sINP/PCL/sINP platform

The hydrogel used in this work for the dual-functional device was based on poly-$\gamma$-glutamic acid ($\gamma$PGA), which is a water soluble, anionic, biodegradable and edible biopolymer.\textsuperscript{[17]} In this polypeptide, which is produced by Bacillus subtilis,\textsuperscript{[18]} the peptide bonds are formed between the amino group of $\alpha$-glutamic acid (Glu) and the carboxyl group at the end of the Glu side chain. $\gamma$PGA hydrogels, which are produced by the chemical crosslink of the polypeptide in its free form, have been tested in multifarious potential applications in healthcare,\textsuperscript{[19]} water treatment\textsuperscript{[20]} and energy storage.\textsuperscript{[21,22]} In this work, the $\gamma$PGA hydrogel was prepared using a condensation reaction with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (CDI) and cystamine (Cys) as condensation agent and cross-linker, respectively.\textsuperscript{[21]} The $\gamma$PGA:CDI:Cys molar ratio was 5:5:4. Details about the synthetic procedure are given in the Electronic Supporting Information.

sINP $\gamma$PGA hydrogels have been prepared using a recently reported strategy.\textsuperscript{[22]} For this purpose, poly(3,4-ethylenedioxythiophene) (PEDOT) microparticles (MPs) were obtained by
sonicating films previously synthesized by anodic polymerization. The resulting MPs were loaded *in situ* during the formation of γPGA hydrogels. Thus, PEDOT-γPGA hydrogels were prepared using the condensation reaction of γPGA with CDI and Cys but incorporating 20% w/w of PEDOT MPs to the initial γPGA solution. This solution was stirred for 12 hours at 1000 rpm prior to the condensation reaction. Details about the preparation of PEDOT films, their sonication to obtain PEDOT MPs, and the synthesis of PEDOT-γPGA hydrogels are provided in the Electronic Supporting Information.

PEDOT MPs embedded inside the γPGA were used as reaction nuclei for the *in situ* polymerization of hydroxymethyl-3,4-ethylenedioxythiophene (HMeDOT) and, thus, produce the sINP [PEDOT-γPGA]PHMeDOT hydrogel, where PHMeDOT refers to poly(hydroxymethyl-3,4-ethylenedioxythiophene). For this purpose, PEDOT-γPGA hydrogel samples supported onto steel tweezers were kept immersed in an aqueous solution containing 10 mM HMeEDOT and 0.1 M LiClO₄ for 12 hours under stirring. This step previous to the polymerization guaranteed the penetration into the PEDOT-γPGA matrix of the HMeDOT monomers, which are more soluble in water than EDOT due to the exocyclic hydroxymethyl group. After that, the anodic polymerization was performed applying a constant potential of 1.10 V for 7 h. This process, which is detailed in the Electronic Supporting Information, allowed the formation of conductive PHMeDOT networks extending inside the hydrogel matrix ([Figure 1a]).[22]

The procedure used to prepare the three-layered dual-functional platform, sINP/PCL/sINP, is depicted in [Figure 1b]. Firstly, 600 µL of a 20% w/w PEDOT MPs-containing γPGA solution with CDI and Cys, formulated as described for the preparation of the PEDOT-γPGA hydrogels (5:5:4 molar ratio), was spin-coated onto a square glass substrate (area: 2 × 2 cm²) at 500 rpm for 1 min. Then, a mat of PCL MFs was electrospun on the top of the PEDOT-γPGA:CDI:Cys layers. After this, the top layer of PEDOT-γPGA:CDI:Cys was spin coated onto the PCL mat using the same experimental conditions that for the first layer. The
operational parameters used for both the spin-coating and electrospinning processes are described in the Electronic Supporting Information. The final three-layered system was let to gel at room temperature for 1 h. To remove any compound in excess the resulting system was washed with distilled water three times.

After gelation, the whole three-layered system, which was separated from the glass substrate using steel tweezers of 0.5 cm width, was used as working electrode for the anodic polymerization of HMeDOT using the experimental conditions described above for the preparation of sINP [PEDOT-γPGA]PHMeDOT hydrogels. After the anodic polymerization step, the assembled elements of the resulting three-layered system, sINP/PCL/sINP, were expected to be completely crossed and, therefore, interconnected by PHMeDOT conductive networks. sINP/PCL/sINP behaved as free-standing platforms once detached from the steel tweezers. Figure 1b includes photographs of sINP/PCL/sINP before and after being detached from the steel tweezers.

On the other hand, bi-layered γPGA/γPGA, PEDOT-γPGA/PEDOT-γPGA and sINP/sINP platforms, which were prepared using the same process but omitting the incorporation of PEDOT MPs, the anodic polymerization step or the intermediate PCL layer, respectively, were used as control systems for a better understanding of the role played by the different elements in the platform.

2.2. Morphology

As described in the previous section, the resulting platforms are composed of several elements. Hence, special attention was placed to characterize their morphological features in an effort to understand the effect of each one of the constitutive elements on the overall performance of the device. The surface structure of lyophilized γPGA hydrogels consists in a cellular architecture with macropores, which are relatively uniform in both size and shape (Figure 2a). Thus, although the pores are irregularly shaped, differences among them are small enough to
allow their organization into a pseudo-honeycomb pattern. Besides, the effective pore size was measured to be 7 ± 2 μm.

The process employed to transform PEDOT films into PEDOT MPs is of vital importance to control the final size of the MPs (Figure 2b). More specifically, sonication of PEDOT films in acetonitrile and subsequent centrifugation resulted in dense agglomerates of PEDOT MPs, which exhibited an effective diameter of 1.7 ± 0.3 μm (Figure 2b, left). In order to reduce the size and increase the dispersibility of the MPs, the sonication and centrifugation processes were successively repeated in acetonitrile, acetone and milli-Q water. After this treatment, well-dispersed PEDOT MPs with an effective diameter of 0.9 ± 0.5 μm were obtained (Figure 2b, right).

The morphological characterization of the PEDOT-γPGA hydrogel was carried out before and after the semi-interpenetration with PHMeDOT (Figures 2c and 2d, respectively). The incorporation of PEDOT MPs to the γPGA hydrogel does not affect the morphology of the latter (Figure 2c). Furthermore, the dispersion of these MPs, which are not in contact, indicates that their electrochemical response could be improved creating conduction paths to connect them. Instead, the sINP [PEDOT-γPGA]PHMeDOT hydrogel exhibits a drastic morphological change (Figure 2d). More specifically, PHMeDOT completely coats the surface of the hydrogel, the largest pores being the only ones that remain uncovered by the ICP after the anodic polymerization process.

The shape and the diameter distribution of electrospun PCL MFs were also determined (Figure 3a). MFs present a cylindrical morphology with an average diameter ($D$) of 742 ± 44 nm. An important observation is that electrospun MFs are collected forming a very porous mat, which is consequence of their random alignment. Because of this porosity, the mat of PCL MFs is expected to be penetrated by the PHMeDOT chains in three-layered sINP/PCL/sINP platforms, facilitating the formation of conduction paths connecting the two
external hydrogel layers. Finally, the two hydrogel layers and the intermediate PCL fibrous mat are well-assembled in the sINP/PCL/sINP platform (Figure 3b), which is also demonstrated below by the peel test. Furthermore, micrographs reveal that the electropolymerized PHMeDOT network is not restricted to the two hydrogel layers but successfully extends over the PCL fibrous mat, thus favoring the electrochemical response of the platform as a whole (see below).

2.3. Chemical and electrochemical characterization of sINP/PCL/sINP platforms

Figure 4a compares the FTIR spectra recorded for γPGA hydrogel, PEDOT film, sINP hydrogel and PCL electrospun mat with that of the sINP/PCL/sINP platform, detailed discussion of the spectra former four being provided in Supporting Information. The γPGA hydrogel spectrum shows the bands typical ascribed to the amide bond (i.e. amide I, amide II, C–N stretch and N–H stretch), whereas the absence of the free carboxylic acid, asymmetric COO\(^{-}\) and symmetric COO\(^{-}\) reflects the success of the condensation reaction used to form the hydrogel.\(^{[21]}\) On the other hand, PEDOT film displays the characteristic absorption bands of the thiophene and ethylenedioxy groups. The spectrum of the sINP [PEDOT-γPGA]PHMeDOT hydrogel includes the amide peaks of γPGA hydrogel, the characteristic vibrations of the thiophene and ethylenedioxy groups, and a very intense and broad band associated to the vibrations of the hydroxyl groups of PHMeDOT.\(^{[22]}\) The FTIR spectrum of PCL is fully consistent with those reported in the literature.\(^{[23]}\) Regarding the sINP/PCL/sINP spectrum, although clear identification of the absorption bands is not possible for all individual components, the most characteristic trends of sINP hydrogels and PLC mats are observed (marked by semi-transparent boxes in Figure 4a).

The success of the in situ PHMeDOT electropolymerization was also demonstrated by Raman spectrometry comparing the spectra obtained for PEDOT-γPGA and sINP [PEDOT-γPGA]PHMeDOT hydrogels (Figure S1). Indeed, after the incorporation of PHMeDOT, the
peaks at 1430 and 1485 cm\(^{-1}\), which correspond to the C=C symmetrical and asymmetrical stretching, respectively, shift to 1433 and 1496 cm\(^{-1}\), respectively, and become more intense. Moreover, the peaks at 2878 and 2960 cm\(^{-1}\), which have been associated with the exocyclic hydroxyl group, are only detected for the sINP hydrogel.

Cyclic voltammograms recorded for \(\gamma\)-PGA/\(\gamma\)-PGA, PEDOT-\(\gamma\)-PGA/PEDOT-\(\gamma\)-PGA and sINP/sINP bi-layered systems in water with 0.1 M LiClO\(_4\) are compared in Figure 4b. As it is reflected by the very small cathodic and anodic areas, the electrochemical activity of \(\gamma\)-PGA/\(\gamma\)-PGA is practically inexistnet. However, these areas increase considerably when conducting PEDOT MPs are incorporated into the biopolymer matrix. Thus, PEDOT-\(\gamma\)-PGA/ PEDOT-\(\gamma\)-PGA shows an oxidation peak at 0.82 V and a reduction shoulder between -0.1 and 0.1 V, which have been associated with the reversible formation of polaron and bipolaron in PEDOT chains, supporting the successful loading of PEDOT MPs and showing their essential contribution to the redox charge storage capacity. As expected, the electrochemical response is more pronounced for sINP/sINP than for PEDOT-\(\gamma\)-PGA/PEDOT-\(\gamma\)-PGA on account of the formation of PHMeDOT networks semi-interpenetrating the hydrogel matrix. The effect of PHMeDOT conduction paths is also evidenced by the enhancement of the current density at both the initial/final and reversal potentials.

Figure 4b, which includes the voltammogram recorded for the sINP/PCL/sINP platform in the same electrolytic medium, shows that the electrochemical activity increases when the two sINP hydrogels are separated by the fibrous PCL layer. This feature has been attributed to the fact that PHMeDOT conduction networks grow not only inside the sINP hydrogel layers but especially inside the fibrous PCL layer. Thus, the diffusion of HMeDOT monomers across the porous PCL mat is favored, facilitating the anodic polymerization of PHMeDOT and, therefore, promoting the connection between the two sINP hydrogel layers.
The areal specific capacitance (SC, in mF/cm²) values, which were determined by applying Eqn S1, are fully consistent with the discussion of the electrochemical activity. SC increases as follows: γPGA/γPGA < PEDOT-γPGA/PEDOT-γPGA < sINP/sINP < sINP/PCL/sINP (Figure 4c). On the other hand, the loss of electrochemical activity (LEA, in %), which refers to the stability of the platform against consecutive oxidation-reduction cycles, was calculated through the variation of voltammetric charge (Eqn S2). Thus, the electrochemical stability decreases with increasing LEA values. The LEA parameter, which indicates the electrochemical stability of the system, increases as follows: PEDOT-γPGA/PEDOT-γPGA < sINP/sINP < sINP/PCL/sINP (Figure 4d). Hence, not only do the conduction paths provide higher ability to store charge (as it is reflected by the SC value) by semi-interpenetrating the hydrogel layers and connecting them across the PCL layer, but also enhanced longevity against redox processes.

2.4. Thermal stability, swellability and mechanical characterization of sINP/PCL/sINP platforms

Thermal gravimetric analysis (TGA) was conducted for γPGA/γPGA, PEDOT-γPGA/PEDOT-γPGA, sINP/sINP, γPGA/PCL/γPGA and sINP/PCL/sINP to examine their degradation profiles. In all cases, a small weight loss (i.e. ~10 %) is detected at around 100 °C, which has been attributed to the evaporation of absorbed water (Figure 5a). The thermal degradation of γPGA/γPGA, PEDOT-γPGA/PEDOT-γPGA and sINP/sINP starts at a similar temperature, the predominant decomposition step occurring at ~275 °C for such three bi-layered systems. Moreover, the chemical heterogeneity of γPGA hydrogels, which is related to the influence of the long and short molecular tracts (i.e. the polypeptide backbone and the crosslinks, respectively) in the diffusion of the degraded molecules, explains the different degradation steps observed for γPGA/γPGA in the DGTA profile. The incorporation of PEDOT MPs enhances the problems associated with the diffusion of the degradation products,
adding complexity to the degradation process. In contrast, the anodic polymerization of PHMeDOT provides homogeneity to the polymeric matrix, facilitating the diffusion of the degraded molecules (i.e. a single predominant peak is observed in the DGTA profile).

It is well-known that the thermal degradation of PCL fibrous mats starts at 350 ºC.[24] This value, which is higher than that found for γPGA hydrogel, explains the enhanced thermal stability of γPGA/PCL/γPGA and sINP/PCL/sINP with respect to γPGA/γPGA and sINP/sINP, respectively. The most prominent peak in the DGTA curve of γPGA/PCL/γPGA and sINP/PCL/sINP appears at 305 and 280 ºC, respectively. The difference between such two systems is again caused by the chemical heterogeneity of γPGA in γPGA/PCL/γPGA (i.e. the predominant peak is surrounded by local peaks at 281 and 342 ºC, evidencing restrictions related to degraded products diffusion, as in γPGA/γPGA) and the homogeneity caused by the anodic polymerization in the sINP/PCL/sINP platform (i.e. the predominant peak is much clearly defined, as for sINP/sINP).

The incorporation of PCL MFs between the two hydrogel layers affects no only the thermal stability, but also the swellability of the platform (Figure 5b). The swelling ratio (SR, Eqn S3) of γPGA/γPGA and sINP/sINP decreases upon the incorporation of the intermediate PCL layer by 65% and 49%, respectively. This observation is attributed to the poor wettability of PCL fibrous mats, which behave as hydrophobic systems with water contact angles of ~120º.[25] On the other hand, the incorporation of PEDOT MPs into γPGA/γPGA enhanced the SR by 65%, this effect being slightly more pronounced (i.e. 8%) after the anodic polymerization of PHMeDOT. The increment of the SR for PEDOT-γPGA/PEDOT-γPGA and sINP/sINP with respect to γPGA/γPGA has been attributed to the incorporation of doped PEDOT and PHMeDOT chains, which enhance the hydrophilicity of the γPGA matrix due to the charges (i.e. each ICP chain stores n positive charges balanced with n perchlorate anions).
As the mechanical integrity of anodically polymerized ICPs is null, the influence of the PCL intermediate layer on the strength of the platform was evaluated by conducting stress-strain assays in γPGA/γPGA and γPGA/PCL/γPGA samples. An important factor in mechanical tests is good sample grip, which is particularly difficult when the specimens are formed by sticky and soft hydrogels. The strategy used to overcome this challenge includes the utilization of elastic materials to help grip the samples (i.e. to reduce the pressure of grip), as shown in Figure S2. The measured stress-strain curves which are compared in Figure 5c, reflect the notable influence of the PCL layer. Thus, γPGA/γPGA displays an elastic behavior with a very low Young modulus of 0.2 ± 0.1 kPa. Unfortunately, tensile strength measurements at strain values higher than ~50% were not possible because of the loss of grip. Interestingly, the incorporation of the fibrous layer not only improved the gripping, allowing measures at higher strains, but also reinforced the mechanical resistance of the platform. Thus, the Young modulus estimated for γPGA/PCL/γPGA was 1.1 ± 0.5 kPa, which represents an increase of more than five times with respect to γPGA/γPGA.

A peel test was conducted using a tensile testing equipment to determine the strength of the adhesive bond between the γPGA hydrogel and the PCL fibrous mat (Figure S3). For this purpose, a bi-layered γPGA/PCL system was prepared by applying spin coating and electrospinning successively. After drying at room temperature for 24 h, the resulting stress-strain curve, which is shown in Figure 5d, indicate that the peel strength required to detach the two layers is of around 1.5 MPa. This adhesive strength is very high considering that the interface between hydrophilic γPGA hydrogel and the hydrophobic PCL MFs does not involve covalent bonds. Thus, the adhesive strength of hydrogels is usually around 1 MPa or even lower. For example, hydrophilic adhesive formulations made by blending poly(N-vinylpyrrolidone) and hydroxyl terminated poly(ethylene glycol) exhibited debonding stresses comprised between 0.6 and 1.1 MPa.
2.5. Loading of levofloxacin

sINP/PCL/sINP platforms loaded with levofloxacin (LVX), hereafter named [sINP/PCL/sINP]LVX, were prepared by adapting the last step of the procedure sketched in Figure 1b. More specifically, the antibiotic (2 mM) was added to the aqueous solution with HMeEDOT (10 mM) and LiClO₄ (0.1 M) used in the anodic polymerization step. As mentioned above, the PEDOT-γPGA/PCL/ PEDOT-γPGA was kept immersed in this reaction medium for 12 h under stirring before to initiate the polymerization of PHMeDOT, which favoured not only the penetration of the HMeDOT monomer but also of the antibiotic LVX inside the platform.

The successful incorporation of LVX into the bioplatform was evidenced by UV-Vis spectrometry (Figure S4a). Indeed, the absorption peak centered at 286 nm and the shoulder at ~320 nm indicate the presence of LVX in the [sINP/PCL/sINP]LVX platform. According to the calibration curve obtained with the peak at 286 nm in a 0.1 M LiClO₄ aqueous solution (Figure S4b), the amount of antibiotic loaded in the [sINP/PCL/sINP]LVX platform was of 219.5 ± 40.1 µM.

2.6. Release and detection of levofloxacin

The release of the antibiotic from [sINP/PCL/sINP]LVX was expected to depend on the relative strength of the interactions between the drug and the different polymeric components of the platform or the molecules from the 0.1 M LiClO₄ aqueous solution used as release medium (i.e. water and ions). [sINP/PCL/sINP]LVX square pieces of 0.5×0.5 cm² were immersed in the release medium using Eppendorf tubes. At regular time intervals (i.e. 5 min, 15 min, 30 min, 1 h and 24 h), the release medium (1 mL) was withdrawn from the tube and analyzed by UV-Vis spectroscopy. The amount of released LVX was quantified by UV-vis
spectroscopy, using the band centered at 286 nm and the calibration curve displayed in Figure S4b.

The antibiotic was rapidly released to the medium (Figure 6a). More specifically, ~80% of LVX was released to the medium during the first 30 min, whereas only 7% of the antibiotic content remained inside the platform after 1 h. This fast release has been attributed to the weakness of the interactions between LVX and the polymeric components of the platform, which are rapidly compensated by the strong interactions between the carboxylate of LVX and the ions of the medium. Despite this inconvenience, it should be noted that this work focuses on the conceptualization of a dual platform for real-time detection of the released drug. Hence, investigations on the dependence between the chemical structure of potential drugs and the kinetics of their release are beyond the scope of this study.

On the other hand, Figure S5 shows the LVX release profile from [sINP/PCL/sINP]LVX in 0.1 M phosphate buffer saline (PBS) solution. As expected, the behavior is very similar to that observed in 0.1 M LiClO₄ aqueous solution. Small differences have been attributed to side effects occurring in PBS, as for example the competition of the different anions (perchlorate – used in the synthesis of the ICP -, phosphate and chloride) to act as dopant agent or the competition from the different cations (Li⁺, Na⁺ and K⁺) to interact with LVX, which may affect the release response.

Figure 6b shows the cyclic voltammograms for [sINP/PCL/sINP]LVX samples as prepared and after being immersed in the release medium for 5 min, 15 min, 30 min and 1 h. For comparison, the voltammogram recorded for unloaded sINP/PCL/sINP (control) is included in the graphic. The curve for as prepared [sINP/PCL/sINP]LVX shows shoulders at ~0.3 V (cathodic scan) and 1.10 V (anodic scan), which are associated with the oxidation and reduction of the loaded LVX, respectively, and are not observed for as prepared sINP/PCL/sINP. The peak intensity of the loaded LVX oxidation decreases with increasing immersion time, which evidences that the release of antibiotic to the medium is successfully
detected by the integrated bioplatform. Consistently, the voltammograms of sINP/PCL/sINP (control) did not show such oxidation and reduction shoulders after 60 min of immersion in the release medium (Figure S6).

The detection of loaded LVX is clearly observed in Figure 6c, which represents the current density at a potential of 1.10 V. The current density decreases linearly with the immersion time of the [sINP/PCL/sINP]LVX platform into the release medium, evidencing that the integrated platform is sensible enough to detect very low concentrations of LVX. Apparently, this sensitivity is independent of area of the voltammogram, which decreases with the number of redox cycles.

The sensitivity of the detection process is demonstrated by the graphics displayed in Figures 6d and 6e. The former graphic represents the current density at a potential of 1.10 V measured by cyclic voltammetry for [sINP/PCL/sINP]LVX samples as prepared and after being immersed in the release medium for 5 min, 15 min, 30 min and 1 h (Figure 6b) vs the LVX concentration remaining at the platform at such time intervals, as determined by UV-Vis spectroscopy (Figure 6a). The linear relation, $R^2 = 0.9802$, reflects that the ICP internally distributed across the hydrogels and MFs layer can be successfully applied to detect the antibiotic loaded into the semi-interpenetrated platform.

The calibration curve for the detection of LVX (Figure 6e) was obtained using flat PHMeDOT films prepared by anodic polymerization on steel tweezers by applying a constant potential of 1.10 V for 600 s. As expected, a linear correlation with $R^2 = 0.9371$ was achieved, confirming that this ICP-based method is able to detect LVX. Most importantly, application of the linear equation obtained in Figure 6d to the LVX concentrations used for the latter calibration curve provides very similar current densities (empty symbols in Figure 6e). The small differences between the experimental and theoretical calibration curves should be attributed to the fact that (i) the accessibility of ICP is much easier in the film than in the
semi-interpenetrated platform; and (ii) the amount of ICP, which is different in the compared systems.

2.7. Activity of released levofloxacin

Investigation of the antibacterial mechanism of released LVX is out of the scope of this work, even though the possible mechanisms of action of antibacterial agents released from hydrogels have been exhaustively reviewed.\[^{29}\] In this section we demonstrate that the antibiotic, which was loaded by diffusion before the anodic polymerization of HMeDOT monomers, was not damaged by the applied potential, preserving its activity. For this purpose, the antibacterial activity of the platform loaded with LVX was tested against *Escherichia coli* (*E. coli*) and *Staphylococcus sanguinis* (*S. sanguinis*) as representative of Gram-positive and Gram-negative bacteria, respectively, using the agar diffusion test. Thus, [sINP/PCL/sINP]LVX and control samples were emplaced in an agar plate where bacteria were grown to test the extent to which bacteria are affected by released LVX. Both free LVX and discs of nalidixic acid (NAX), which is a well-known Gram-negative antibacterial agent, were used as positive controls, while unloaded sINP/PCL/sINP was considered as the negative control. After incubating for 24 h, inhibition zones around [sINP/PCL/sINP]LVX, free LVX and NAX samples were clearly identified for the two tested bacteria indicating that compounds with bactericidal activity diffuse outward from the samples (Figure 7). Thus, such clear zones are halos of inhibited bacterial growth, which indicate the inability of the tested organisms to survive in presence of free LVX and antibiotic released from the platform. Moreover, the size of the halos obtained for [sINP/PCL/sINP]LVX and free LVX are very similar, indicating that the susceptibility of the tested bacteria towards the released antibiotic is similar to that towards the free one. This observation is fully consistent with the fast release of the antibiotic from the [sINP/PCL/sINP]LVX platform. Besides, the antimicrobial action is more
pronounced for *E. coli* than for *S. sanguinis*, as it is revealed by the size of the halos, since both LVX and NAX are more effective against Gram-negative bacteria. On the other hand, no inhibition zone (*S. sanguinis*) or a small halo (*E. coli*) was detected for sINP/PLC/sINP samples, thus confirming that the polymeric matrix is harmless to bacteria. The small halo found for sINP/PLC/sINP controls in *E. coli* cultures has been attributed to the bacteriostatic effect of residual solvent or reagent, which resulted innocuous in *S. sanguinis* cultures. However, as both [sINP/PCL/sINP]LVX and sINP/PLC/sINP were prepared using identical procedures and materials, such small bacteriostatic effect does not affect the qualitative conclusions extracted from the experiments.

3. Conclusion

Four different biocompatible polymers (*i.e.* γPGA, PEDOT, PCL and PHMeDOT) have been used to manufacture the sINP/PCL/sINP and sINP/PCL/sINP]LVX platforms. These systems consist on electrospun PCL MFs in the middle of two PEDOT-γPGA hydrogel layers, the whole three-layered system being semi-interpenetrated by PHMeDOT conducting networks. In addition of good mechanical and electrochemical properties, sINP/PCL/sINP exhibits drug-loading capacity, which has been used to prepare sINP/PCL/sINP]LVX. Although LVX, which has been loaded during the semi-interpenetration step, was released very rapidly, the antibiotic was electrochemically monitored in real-time while remaining inside the platform. Moreover, the antibiotic has been demonstrated to preserve its antimicrobial activity. The design of dual-functional polymeric devices with drug-loading capacity and electrochemical response is a very promising approach for the development of advanced biomedical applications. However, much work is still required to use this multi-functionality as a practical loading-monitoring tool. Future studies will focus on the effect of the multi-component nature of the sINP/PCL/sINP platform in the drug loading capacity, establishing
relationships with the chemical structure of the loaded drug, as well as in its (bio)degradability rate.

Supporting Information
Supporting Information is available from the Wiley Online Library.

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References


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Figure 1. (a) Sketch of the sINP [PEDOT-γPGA]PHMeDOT hydrogel. (b) Scheme of the procedure used to prepare three-layered sINP/PCL/sINP platforms. Photographs of platforms supported onto steel tweezers and free-standing are included.
Figure 2. Representative SEM micrographs of (a) γPGA hydrogel, (b) PEDOT MPs, (c) PEDOT-γPGA hydrogel and (d) sINP [PEDOT-γPGA]PHMeDOT hydrogel. The diameter distribution of the hydrogel pores and the corresponding average value ± standard deviation are displayed in (a). The influence of the sonication and centrifugation process in the dispersibility and size of the MPs is displayed in (b): left side micrograph corresponds to MPs sonicated in acetonitrile and, subsequently, centrifuged, while the right side one refers to MPs sonicated and centrifuged repeatedly in acetonitrile, acetone and milli-Q water.
Figure 3. Representative SEM micrographs of: (a) the PCL fibrous mat; and (b) the sINP/PCL/sINP platform (two different magnifications). The diameter distribution of PCL MFs and the corresponding average value ± standard deviation are displayed in (a).
Figure 4. (a) FTIR spectra, (b) cyclic voltammograms (scan rate: 100 mV/s), (c) areal specific capacitances (SC) and (d) loss of electrochemical activity (LEA) of different bi- and three-layered platforms prepared in this work.
Figure 5. (a) Thermogravimetric (solid lines) and derivative thermogravimetric curves (dashed lines), (b) swelling ratio (SR), (c) strain-stress curves and (d) peel test of different bi- and three-layered bioplatforms prepared in this work.
Figure 6. (a) LVX release profile in 0.1 M LiClO₄ aqueous solution from [sINP/PCL/sINP]LVX bioplatform. (b) Cyclic voltammograms (scan rate: 100 mV/s) obtained using unloaded sINP/PCL/sINP and [sINP/PCL/sINP]LVX. Voltammograms for the latter were recorded for samples as prepared and after 5, 15, 30 and 60 min of immersion into the release medium. (c) Variation of the current density at a potential of 1.10 V for LVX at the integrated [sINP/PCL/sINP]LVX bioplatform against the time immersed into the release medium. (d) Graphic representing the current density at a potential of 1.10 V measured by cyclic voltammetry for [sINP/PCL/sINP]LVX as prepared and after 5 min, 15 min, 30 min and 1 h immersed in the release medium vs the LVX concentration remaining at the platform at such time intervals, as determined by UV-Vis spectroscopy. (e) Calibration curve for the detection of LVX using flat PHMeDOT films (filled symbols) and representation of the current density obtained by applying the linear equation obtained in (d) to the same LVX concentrations (empty symbols).
Figure 7. (a) *S. sanguinis* and (b) *E. coli* bacterial cultures showing the inhibition zones around [sINP/PCL/sINP]LVX, free LVX (positive control) and NAX discs (positive control). Unloaded sINP/PCL/sINP was also tested as negative control.
An electrochemically controlled free-standing platform for drug delivery and real-time monitoring of the released drug is presented. The platform consists of two poly-γ-glutamic acid hydrogel layers separated by poly-ε-caprolactone microfibers, which are all them semi-interpenetrated with a conducting polymer. After investigate properties of the platform, its performance has been examined by monitoring in real-time the release of previously loaded levofloxacin.

**Keyword:** conducting polymer, drug delivery, poly-ε-caprolactone, poly-γ-glutamic acid, biosensors


**Semi-interpenetrated hydrogels-microfibers electroactive assemblies for release and real-time monitoring of drugs**

ToC figure
Supporting Information

Semi-interpenetrated hydrogels-microfibers electroactive assemblies for release and real-time monitoring of drugs

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S.1 Methods

S.1.1. Materials

Free-acid poly-γ-glutamic acid (γPGA, from Bacillus subtilis), with average molecular weight $M_w = 350000$, was purchased from Wako Chemicals GmbH (Neuss, Germany). Cystamine dihydrochloride (Cys; ≥98.0%), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (CDI), 3,4-ethylenedioxythiophene (EDOT; 95%) and hydroxymethyl-3,4-ethylenedioxythiophene (HMeDOT; 95%) were purchased from Sigma-Aldrich. Acetonitrile (Reagent European Pharmacopoeia for analysis, ACS) and NaHCO$_3$ were obtained from Panreac. Anhydrous lithium perchlorate (LiClO$_4$), analytical reagent grade from Aldrich, was stored in an oven at 70 °C before use in electrochemical experiments. Milli-Q water grade (0.055 S/cm) was used in all synthetic processes. Poly-ε-caprolactone (PCL; Aldrich, UK; $M_n$:80,000), chloroform (Scharlau, Spain; stabilized with amylene, 99.8% pure) and acetone (Sigma, South Korea; 99.9% pure) were used as received. Levofloxacin (LVX, ≥98%) and nalidixic acid (NAX, ≥98%) were purchased from Sigma-Aldrich.

S1.2. Synthesis of γPGA hydrogel

The polypeptide hydrogel was prepared by dissolving γPGA and CDI in 0.75 mL of 0.5 M NaHCO$_3$ at 4 °C under magnetic stirring. Then, Cys, previously dissolved in 0.25 mL of 0.5 M NaHCO$_3$, was added to the solution and mixed during 2–3 min. The γPGA:CDI:Cys molar
The solution was let to gel at room temperature for 1 h. To remove any compound in excess, the resulting hydrogel was washed with distilled water three times.

**S.1.3. Synthesis and sonication of poly(3,4-ethylenedioxythiophene) (PEDOT) films**

PEDOT films were prepared by applying a constant potential of 1.40 V during 600 s to the reaction medium, which consisted on an acetonitrile solution with 10 mM EDOT and 0.1 M LiClO₄ (supporting electrolyte). This process was performed in a potentiostat-galvanostat Autolab PGSTAT101 equipped with the ECD module (Ecochimie, The Netherlands) using a three electrode compartment cell under nitrogen atmosphere (99.995% pure) at room temperature. Steel AISI 316 sheets of 6 cm² in area were used as working and counter electrodes. The reference electrode was an Ag|AgCl electrode containing a KCl saturated aqueous solution (offset potential versus the standard hydrogen electrode, E⁰ = 0.222 V at 25 °C).

PEDOT films were processed into microparticles (MPs) by sonication (Ultrasons H-D sonicator) in different solvents during 15 min and, subsequent, centrifugation (Sorvall RC5B Plus centrifuge) during 45 min at 11000 rpm. This process was repeated successively in acetonitrile, acetone and milli-Q water. The diameter of the resulting PEDOT particles was determined at room temperature by dynamic light scattering (DLS) in milli-Q water dispersions (0.3% v/v) using a NanoBrook Omni zeta potential analyser from Brookheaven Instruments Corporation.

**S.1.4. Synthesis of PEDOT-γPGA hydrogel**

The procedure previously described for the synthesis of the γPGA hydrogels was also used to prepare γPGA hydrogels loaded with PEDOT MPs, hereafter denoted PEDOT-γPGA. The only difference with respect to the preparation of pure γPGA hydrogels is that the 0.5 M
NaHCO$_3$ solution used to dissolve the polypeptide already contained PEDOT particles (20% w/w with respect to the weight of γPGA).

**S.1.5. Synthesis of the sINP [PEDOT/γPGA]PHMeDOT hydrogel**

PEDOT-γPGA hydrogel samples supported on steel tweezers, which were kept in to the reaction medium overnight, were used as working electrodes for the anodic polymerization of poly(hydroxymethyl-3,4-ethylenedioxythiophene) (PHMeDOT) by chronoamperometry. The reaction medium was a 10 mM HMeDOT aqueous solution with 0.1 M LiClO$_4$ as supporting electrolyte. The anodic polymerization was conducted under a constant potential of 1.10 V using a polymerization time of 7 hours. The experimental setup used for the *in situ* modification of the PEDOT-γPGA hydrogel was identical to that described above for the synthesis of PEDOT films.

**S.1.6. Spin coating**

γPGA:CDI:Cys and PEDOT-γPGA:CDI:Cys solutions, prepared as described for the synthesis of γPGA and PEDOT/γPGA hydrogels (*i.e.* 5:5:4 molar ratio and 20% w/w of PEDOT MPs), were spin-coated using a WS-400BZ6NPP/A1/AR1 spin-coater (Laurell Technologies Corporation). In all cases, glass square plates (area: 2 × 2 cm$^2$), previously cleaned by sonication in milliQ water, acetone and ethanol (5 min each), were used as substrates. This process was conducted at 500 rpm for 1 min.

**S.1.7. Electrospinning of PCL**

Electrospun mats of PCL microfibers (MFs) were obtained at room temperature. The feeding solution was obtained by dissolving 1.3 g of PCL in 3 mL of a 2:1 v/v chloroform:acetone mixture using an stirrer for 12 h. Electrospinning was carried out in a non-conductor chamber. The feeding solution was loaded in a 5 mL BD Discardit (Becton Dickson Co., Franklin.
Lakes, NJ, USA) plastic syringe for delivery through a blunt-tipped (i.e. without bevel) 18 G needle (inner diameter 0.84 mm). The flow rate and the needle tip-collector distance were 5 mL/h and 20 cm, respectively. A voltage of 20 kV h was applied through a highvoltage Gamma High Voltage Research (ES30-5W) power supply.

S.1.8. Characterization

Scanning electron microscopy (SEM) studies were performed using a Focus Ion Beam Zeiss Neon 40 instrument (Carl Zeiss, Germany). Samples of area 1 × 1 cm² were mounted on a double-sided adhesive carbon disc and sputter coated with a thin layer of carbon to prevent sample charging problems. All micrographs were recorded at an accelerating voltage of 5 kV. The size of the pores in the hydrogel and diameter of the PEDOT MPs and PCL MFs were measured with ImageJ software.

FTIR spectra were recorded with a Fourier Transform FTIR 4100 Jasco spectrometer (Jasco Analytical Instruments, Easton, USA) in the 4000–500 cm⁻¹ range. An attenuated total reflection (ATR) system with a heated Diamond ATR Top-Plate (model MKII Golden Gate, Specac, Ltd., Orpington, UK) was used.

Hydrogel samples were characterized by micro-Raman spectroscopy using a commercial Renishaw inVia Qontor confocal Raman microscope. The Raman setup consisted of a laser (at 785 nm with a nominal 300 mW output power) directed through a microscope (specially adapted Leica DM2700 M microscope) to the sample after which the scattered light is collected and directed to a spectrometer with a 1200 lines·mm⁻¹ grating. The exposure time was 10 s, the laser power was adjusted to 1% of its nominal output power and each spectrum was collected with 3 accumulations.

All electrochemical experiments were run in triplicate using water with 0.1 M LiClO₄ as supporting electrolyte. Cyclic voltammetry (CV) was carried out to evaluate the electroactivity, the areal specific capacitance (SC), and the electrochemical stability of the
different systems. The initial and final potentials were −0.50 V in all cases, while the reversal potential was 1.10 V and 1.50 V for unloaded and LVX-loaded samples, respectively. A scan rate of 100 mV/s was used in all cases. For electrochemical measurements using unloaded and LVX-loaded samples, the counter electrode was steel tweezers of 1 cm² and platinum (Pt) sheets of 0.5 cm², respectively. Ag|AgCl 3 M KCl was used as reference electrode in all cases. The SC (in mF/cm²) was determined using the following expression:

\[ SC = \frac{Q}{\Delta V \cdot A} \]  

(S1)

where Q is voltammetric charge determined by integrating the oxidative or the reductive regions of the cyclic voltammograms, \( \Delta V \) is the potential window (in V), and A is the area of the electrode (in cm²).

The electrochemical stability was examined by evaluating the loss of electroactivity (LEA, in %) against the number of oxidation–reduction cycles:

\[ LEA = \frac{\Delta Q}{Q_2} = \frac{Q_1 - Q_2}{Q_2} \]  

(S2)

where \( \Delta Q \) is the difference between the oxidation charge (in C) in the second \( Q_2 \) and the evaluated oxidation–reduction cycle \( Q_i \).

The thermal stability of the prepared system was studied by thermal gravimetric analysis (TGA) at a heating rate of 20 °C/min (sample weight ca. 5 mg) with a Q50 thermogravimetric analyser of TA Instruments and under a flow of dry nitrogen. Test temperatures ranged from 30 to 590 °C.

The swelling ratio (SR, in %) of the studied systems was determined according to:

\[ SR(\%) = \frac{w_w - w_D}{w_D} \times 100 \]  

(S3)

where \( w_w \) is the weight after 30 min plunged in distilled water and \( w_D \) is the weight of the system after freeze-drying (dried system). All swelling experiments were conducted at room temperature.
Mechanical properties and peel tests were evaluated with a Zwick Z2.5/TN1S testing machine with integrated testing software (testXpert, Zwick). The deformation rate for stress-strain assays was 1 mm/min. Samples with a surface area of 1×3 cm² and a thickness of 1.1 ± 0.2 mm were cut for experiments. Peel tests were conducted on a bi-layered γPGA/PCL system to examine the adhesion between the hydrogel and the fibrous mat. After preparation, platforms were allowed to dry at room temperature for 24 h before conducting the assays, which were performed in triplicate.

S.1.9. Release and electrochemical detection of levofloxacin (LVX)
Unloaded and LVX-loaded platforms supported on steel tweezers were put inside an electrochemical cell filled with a 0.1 M LiClO₄ aqueous solution, which was used as the release medium. For the electrochemical detection of the LVX remaining at the platform, CV assays were done at well-defined time intervals (i.e. 5 min, 15 min, 30 min, 60 min and 24 h) using the methodology described above. After doing each CV, the release medium was replaced by a fresh one, the removed medium being used to quantify the released LVX by UV-vis spectroscopy. UV–Vis spectra were recorded using a UV-Vis Cary 100 Bio spectrophotometer (Agilent, Santa Clara, CA, USA). Additional release assays were performed in 0.1 M PBS solution.

S.1.10. Antimicrobial test: Inhibition of bacterial growth
Escherichia coli (E. coli) and Staphylococcus sanguinis (S. sanguinis) were selected to evaluate the activity of loaded LVX against bacteria. The bacteria were previously grown aerobically by exponential phase in Luria-Bertani (LB) broth (at 37 ºC and agitated at 80 rpm). In addition to the LVX-loaded platform, the unloaded platform (negative control), NAX discs (positive control) and free LVX (positive control) were also considered for antimicrobial assays.
The agar diffusion test was performed in Petri dishes of 90 mm. A standardized 0.5 McFarland of the test strain culture was inoculated homogeneously on the surface of LB agar using a sterile nylon swab. Then, samples were pasted onto the agar plate. Bacteria were incubated at 37 °C for 24 h and the inhibition zone for each sample on the plate was observed.

S.2. Results and discussion

S2.1. FTIR spectroscopy

The FTIR spectra recorded for γPGA hydrogel, PEDOT film, sINP hydrogel and PCL electrospun mat are shown in Figure 4a. Detailed discussion of each is provided below:

**γPGA hydrogel.** The spectrum of γPGA shows sharp and intense absorption peaks at 1636, 1542 and 1257 cm\(^{-1}\), which correspond to stretching vibration of the amide carbonyl group (amide I), the –CONH– bond vibration (amide II) and the C–N vibration, respectively, and a broad peak at 3291 cm\(^{-1}\) attributable to the N–H stretching. Such four peaks confirm the presence of amide groups in γPGA, including cross-link bonds, whereas the absence of peaks at approximately 1728 (free carboxylic acid), 1580 (asymmetric COO\(^-\)) and 1400 cm\(^{-1}\) (symmetric COO\(^-\)) reflects the success of the condensation reaction used to form the hydrogel.[S1]

**PEDOT film.** The spectrum displays characteristic absorption bands at 1513 (asymmetric C=C stretching) and 1291 cm\(^{-1}\) (inter-ring C–C stretching). Besides, the bands appearing at 1166, 1120, 1064 and 1031 cm\(^{-1}\) are attributed to the C–O–C vibrations in the ethylenedioxy group, while the C–S–C vibrations in the thiophene ring occur at 954, 902 and 869 cm\(^{-1}\).

**sINP hydrogel.** The spectrum of the [PEDOT-γPGA]PHMeDOT hydrogel includes: the amide I, amide II and the C–N vibrations of γPGA hydrogel (1628, 1536 and 1255 cm\(^{-1}\), respectively); the C=C and C–C stretching modes, which overlaps the amide I and amide II, as well as the C–O–C (1169, 1120, 1065 and 1033 cm\(^{-1}\)) and the C–S (954 cm\(^{-1}\)) vibrations of PEDOT; and a very intense and broad peak centered at 3248 cm\(^{-1}\) associated to the vibrations
of the hydroxyl groups of PHMeDOT. The small peak at 1729 cm$^{-1}$ was associated with enhanced oxidation processes due to the large polymerization time (7 h).\textsuperscript{[S2]}

**PCL electrospun mat.** The spectrum of PCL is well-known.\textsuperscript{[S3]} It shows a strong band associated to the carbonyl stretching mode at 1721 cm$^{-1}$. Other important bands that can be easily identified in fibrous PCL mats are the asymmetric and symmetric CH$_2$ stretching (2939 and 2866 cm$^{-1}$, respectively), the C–O and C–C stretching in the crystalline phase (1293 cm$^{-1}$), asymmetric and symmetric COC stretching (1239 and 1176 cm$^{-1}$, respectively), and the C–O and C–C stretching in the amorphous phase (1160 cm$^{-1}$).\textsuperscript{[S3]}


Figure S1. Raman spectra of PEDOT-γPGA and sINP [PEDOT-γPGA]PHMeDOT hydrogels.

Figure S2. γPGA/γPGA sample preparation for strain-stress tests. The position of the sample is indicated by the red arrow,
Figure S3. Peel test for the bi-layered γPGA/PCL sample.

Figure S4. (a) Comparison between the UV-Vis spectra of sINP/PCL/sINP and [sINP/PCL/sINP]LVX bioplatforms. (b) LVX calibration curve in 0.1 M LiClO₄ aqueous solution using the absorbance at 286 nm.
Figure S5. LVX release profile in 0.1 M PBS solution from [sINP/PCL/sINP]LVX bioplatform.

Figure S6. Cyclic voltammograms (scan rate: 100 mV/s) obtained using unloaded sINP/PCL/sINP Voltammograms were recorded for samples as prepared and after 5, 15, 30 and 60 min of immersion into the release medium.