# Supplementary Information

## A Bacteria-resistant and Self-healing Spray Dressing Based on Lyotropic Liquid Crystalline to Treat Infected Post-Operative Wounds

Xiao **Yue**[a, b, #], Xuejuan **Zhang**[a, #], Chen **Wang**[b], Ying **Huang**[a, *], Ping **Hu**[a], Guanlin **Wang**[b], Yingtong **Cui**[b], Xiao **Xia**[a], Ziqiang **Zhou**[a], Xin Pan[b, **], Chuanbin **Wu**[a]

[a] *College of Pharmacy, Jinan University, Guangzhou 511443, Guangdong, P. R. China*

[b] *School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510006, Guangdong, P. R. China*

# These authors contributed equally to this paper.

* and ** are corresponding authors.

Xin Pan, Ph.D.

School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510006, Guangdong, P. R. China

Tel: +86 020 39943427; Fax: +86 020 39943115

E-mail: panxin2@mail.sysu.edu.cn

Ying Huang, Ph.D.

College of Pharmacy, Jinan University, Guangzhou 511443, Guangdong, P. R. China

Tel: +86 020 39943427; Fax: +86 020 39943115

E-mail: huangy2007@jnu.edu.cn

E-mail addresses of the authors are listed below.

Xiao Yue (X. Yue, X. Y.): yuex7@mail2.sysu.edu.cn

Xuejuan Zhang (X. Zhang, X. Z.): zhanghongdou0223@126.com

Chen Wang (C. Wang, C. W.): wangch296@mail2.sysu.edu.cn

Ying Huang (Y. Huang, Y. H.): huangy2007@jnu.edu.cn

Ping Hu (P. Hu, P. H.): pinghu@jnu.edu.cn

Guanlin Wang (G. Wang, G. W.): wangglin3@mail2.sysu.edu.cn

Yingtong Cui (Y. Cui, Y. C.): cuiyt3@mail2.sysu.edu.cn

Xiao Xia (X. Xia, X. X.): 2067134115xiao@stu2020.jnu.edu.cn

Ziqiang Zhou (Z. Zhou, Z. Z.): zhouziqiang@stu2020.jnu.edu.cn

Xin Pan (X. Pan, X. P.): panxin2@mail.sysu.edu.cn

Chuanbin Wu (C. Wu, C. W.): chuanbinwu@jnu.edu.cn

## 1. The design and preparation of PLL-LLCPs

In the formulation of lyotropic liquid crystalline (LLC) system, GMO and EtOH were chosen as matrix and solvent, respectively. And PEG400 was added as a release regulator. PLL was selected as a model AMP to realize the effective clearance against drug-resistance bacteria. Molten GMO was dissolved in pre-heated EtOH and PEG400 at different weight ratios, and PLL was dissolved in the water at a concentration of 133.33 mg/g (w/w). Then the aqueous solution of PLL was added into the lipid matrix, followed by vortex mixing until the uniform precursor solution formed. The detailed composition of formulations was showed in Table S1.

**Table S1** The composition of formulations

| Formulation | GMO (%) | EtOH (%) | PEG400 (%) | PLL aqueous solution（%） |
|---|---|---|---|---|
| PLL-LLCP$_1$ | 74 | 18.5 | - | 7.5 |
| PLL-LLCP$_2$ | 54 | 13.5 | 25 | 7.5 |
| PLL-LLCP$_3$ | 64.75 | 27.75 | - | 7.5 |
| PLL-LLCP$_4$ | 47.25 | 20.25 | 25 | 7.5 |
| PLL-LLCP$_5$ | 55.5 | 37 | - | 7.5 |
| PLL-LLCP$_6$ | 40.5 | 27 | 25 | 7.5 |

## 2. The optimization of PLL-LLCPs

The PLL-LLCPs was optimized with the apparent viscosity, gelation time and *in vitro* release to achieve the convenient spraying, fast gelation and sustained release.

### 2.1 Apparent viscosity and gelation time

The apparent viscosity of PLL-LLCPs (approximate 1 mL of sample) was measured by a rotational rheometer (Kinexus Lab+, Malvern Instruments Ltd, Worcestershire, UK) with a cone plate geometry (CP 1° / 60 mm). To mimic the spraying process during administration, the shear rate was set at 10 $s^{-1}$ and the

temperature was kept at 25 ℃.

The gelation time was the time from PLL-LLCPs contacting with the water to the gel formed in situ, which was recorded by visual. Briefly, 15 mL of PBS was added into a glass container, and placed in a water bath at 37 ± 0.5 ℃. Then PLL-LLCPs were added to the saline using a pipette at a constant rate.

The results of apparent viscosity and gelation time were shown in Table S2. A shear rate of 10 $s^{-1}$ was used to simulate the spraying process in the detection of apparent viscosity. The apparent viscosities of all the formulations were below 50 $mPa \cdot s^{-1}$, suggesting a favorable spraying performance[1]. And the apparent viscosity reduced with the decreasing content of GMO. Nonetheless, the lower apparent viscosity meant a longer gelation time, which was longer than 1 s observed in PLL-$LLCP_5$ and PLL-$LLCP_6$. Furthermore, the gels formed of PLL-$LLCP_5$ and PLL-$LLCP_6$ showed loose floc structure with extremely poor mechanical properties, which were destroyed rapidly by gentle shaking. Based on the results, four formulations (PLL-$LLCP_1$ ~ PLL-$LLCP_4$) with acceptable apparent viscosities and short gelation time were selected for further studies.

**Table S2** Apparent viscosity and gelation time of formulations ($n$ = 3)

| Formulation | Apparent viscosity ($mPa \cdot s^{-1}$) | Gelation time (s) |
|---|---|---|
| PLL-$LLCP_1$ | 28.08 ± 0.09 | ＜1 |
| PLL-$LLCP_2$ | 45.51 ± 1.92 | ＜1 |
| PLL-$LLCP_3$ | 14.73 ± 0.10 | 3 |
| PLL-$LLCP_4$ | 27.11 ± 1.44 | 3 |
| PLL-$LLCP_5$ | 10.33± 0.07 | ＞5 |
| PLL-$LLCP_6$ | 17.03 ± 0.08 | ＞5 |

### 2.2 *In vitro* release profiles of PLL-LLCPs

The membraneless dissolution model was adopted to evaluate the *in vitro* release behavior of PLL-LLCPs as reported in our previous studies[2, 3]. Briefly, 5 mL of PBS (pH 7.4) was placed in an Eppendorf tube as the release medium. Subsequently, about

0.15 g of PLL-LLCPs was injected into the medium. At predetermined time intervals (1, 3, 6, 12, 24 and 48 h), 5 mL of the release medium was withdrawn and replaced with an equal volume of fresh PBS. The system was under rotation at 100 rpm and 37 ± 0.5 ℃ throughout the test. The collected samples were assayed by a commercially bicinchoninic acid (BCA) protein quantification kit, strictly following the manufacturer's instructions. The determined PLL concentration at each time interval was converted into the cumulative release by *Eq.* S1.

$$\text{Cumulative release}\ (\%) = \frac{M_t}{M_0} = \frac{\sum_{i=1}^{t} C_t V}{M_0} \times 100\% \qquad (Eq.\ \text{S1})$$

where $M_t$, $M_0$, $C_t$, and V represent the cumulative release of PLL at a certain time, initial total amount of PLL, determined PLL concentration at a certain time and the withdrawn volume of release medium, respectively.

In the experiment, PLL-LLCPs rapidly transformed into a gel state upon contacting aqueous release medium. Formed gels floated on the release medium to simulate the drug release on the surface of skin. Release profiles (Figure S1) illustrated the cumulative release of PLL-LLCP$_2$ ~ PLL-LLCP$_4$ in 48 h were 97.87 ± 3.95%, 89.89 ± 3.76% and 100.0 ± 4.19%, respectively, which were significantly higher than that of PLL-LLCP$_1$ (62.527 ± 3.75%). All samples showed a rapid release in the first 1 h, which was potentially beneficial to kill bacteria promptly once applied. In addition, PLL-LLCP$_2$ exhibited a rapid release behavior in the early stage and a sustained release behavior up to 48 h, which was theoretically favorable to kill the bacteria in the wound rapidly and prevent the invasion of external bacteria in a long term. Overall, PLL-LLCP$_2$ was considered as the optimal formulation based on its release profile.

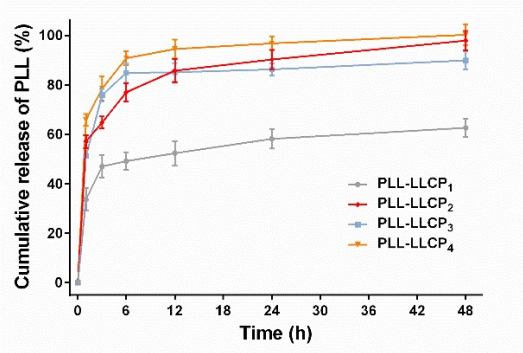


**Figure S1** *In vitro* release profiles of PLL from different delivery systems ($n$ = 3).

## 3. *In vitro* cytotoxicity evaluation

Skin and tissues around the wound were vulnerable, especially cells of the fragile new tissues [4]. Thus, the safety of preparations was crucial to the wounds. In this study, CCK-8 assay and Live/Dead staining test were used to investigate *in vitro* cytocompatibility of the optimized PLL-LLCP.

### 3.1 CCK-8 assay

CCK-8 assay was used to evaluate the cytotoxicity of PLL-LLCP against HUVECs, HacaTs and L929. The samples used in the experiment were extract of PLL-LLCP. PLL-LLCP at the weight of 10 mg was added into 1 mL of DMEM, and incubated at 37℃ for 48 h, followed by being filtering through a sterile filter with 0.22 μm diameter and diluted to different PLL concentrations. Cells were seeded into a 96-well plate at density of 5000 cells per well and incubated for 24 h at 37℃ with 5% carbon dioxide. After incubation, 100 μL of fresh medium with different PLL concentrations at 500, 250, 125, 62.5, 31.25, 15.625 μg/ mL was added into the wells, respectively. The cells were incubated for another 24 h before the medium was replaced by 110 μL of CCK-8 solution (CCK-8: medium=1:10). Followed by additional 1 h of incubation, the optical density at 450 nm ($OD_{450}$) was measured using an ELx800 microplate absorbance reader (BioTek Instruments, Inc, Winooski, VT). Cytotoxicity of the samples, indicated by the relative cell viability, was calculated by the following equation (*Eq*. S2). Data was shown as the mean ± SD ($n$ = 6). PBS without cells was regarded as the group zero.

$$\text{Relative cell viability (\%)} = \frac{OD_{\text{samples}} - OD_{\text{zero}}}{OD_{\text{blank}} - OD_{\text{zero}}} \times 100\% \qquad (Eq.\ \text{S2})$$

As revealed in Figure S2, a high viability over than 95% was exhibited in HacaTs, HUVECs and L929 after incubation with PLL-LLCP extracts at different PLL concentration for 24 h. No obvious inhibition of cell proliferation was observed in the PLL-LLCP extracts even at the highest PLL concentration of 500 μg/mL, a much higher concentration than MBC, suggesting the safety of PLL-LLCP to the skin.

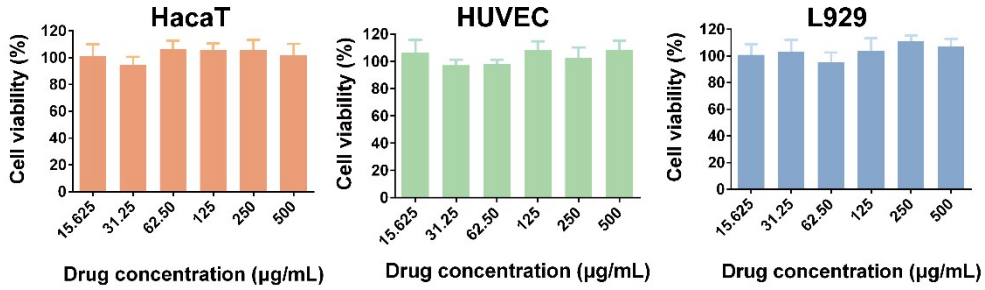


**Figure S2** Cell viabilities of HacaT, HUVEC and L929 treated with PLL-LLCP extracts at various PLL concentrations ($n$ = 6).

### 3.2 Live/Dead staining

In order to visually evaluate the influence of PLL-LLCP on the viability of HUVECs, HacaTs and L929 cells, the Live/Dead staining was adopted in this study. The live and dead cells were stained with green and red fluorescence using Calcein AM and PI, respectively. The cellular imaging system (EVOS FL Auto, Life Technologies, America) was employed to observe the fluorescence of cells. Briefly, the cells were seeded into 24-well plates at density of $5\times10^4$ cells per well and incubated at 37 ℃ under 5% carbon dioxide. After 24 h of incubation, the cells have adhered to the well. The medium was then replaced with 400 μL of freshly extracted fluid of PLL-LLCP to incubate for another 24 h. Cells treated with medium and 0.64% phenol were regarded as the negative and positive controls. After incubation, cells were stained using a double stain kit according to the instructions of manufacture, before observed by the cellular imaging system.

As shown in Figure S3, cells treated with PBS and PLL-LLCP extract appeared

green, stained by Calcein AM fluorochrome during metabolizing. It suggested that few dead cells were observed in PBS and PLL-LLCP groups. In contrast, cells presented red color of 0.64% phenol group due to the damage of cell membranes. It was indicated that cells treated with PLL-LLCP extract remained survival throughout the culture period up to 24 h. Thus, no obvious cytotoxic was caused by PLL-LLCP extract even at a high concentration of 500 μg/mL, which was in accordance with that of CCK-8 assay. Overall, PLL-LLCP had favorable cytocompatibility to HacaTs, HUVECs and L929.

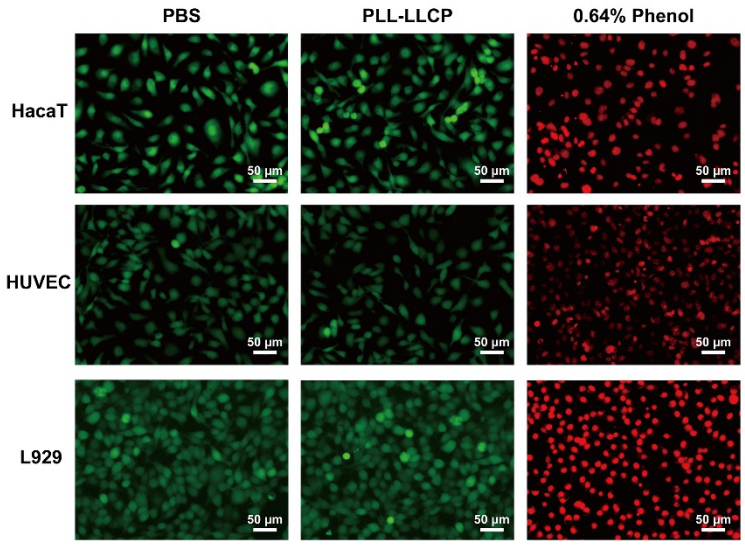


**Figure S3** Live / dead cell images treated with PLL-LLCP extracts. The green fluorescence of Calcein-AM and the red fluorescence of PI reflect live and dead cells, respectively.

## 4. Morphology of gels formed by PLL-LLCP

The morphology of gel formed by PLL-LLCP was evaluated by spraying it onto the glass plane coating with water. Glass plane coating with water was placed on the table, and PLL-LLCP was applied onto the glass plane at an angle of about 45° and a distance about 5 cm. The gelation occurred immediately within several seconds, and the transparent and glossy gel was formed.

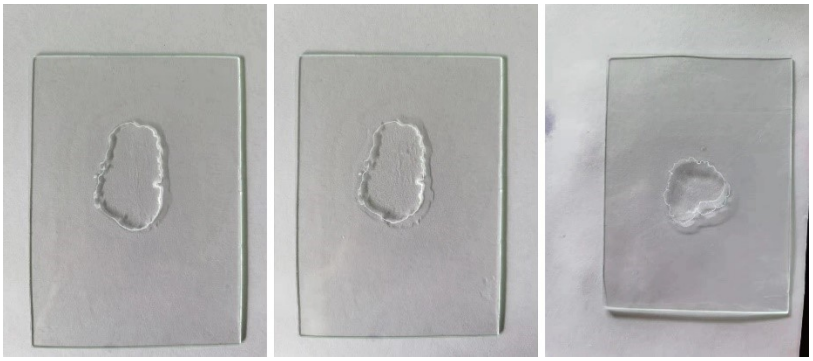

**Figure S4** Morphology of gels formed by PLL-LLCP

## 5. Antimicrobial evaluation of LLCP

The antimicrobial evaluation of LLCP was done via the measurement of the determination of bacteriostatic circle diameter and time-killing curve. As shown in Figure S5, LLCP didn't any antimicrobial effect. After treatment with LLCP, bacterial cells proliferated and cover the entire agar plate, even the area surrounding the gel. Further after incubation with the extract fluid of LLCP, bacterial cells also proliferated rapidly and the time-killing curve was the similar to PBS control group.

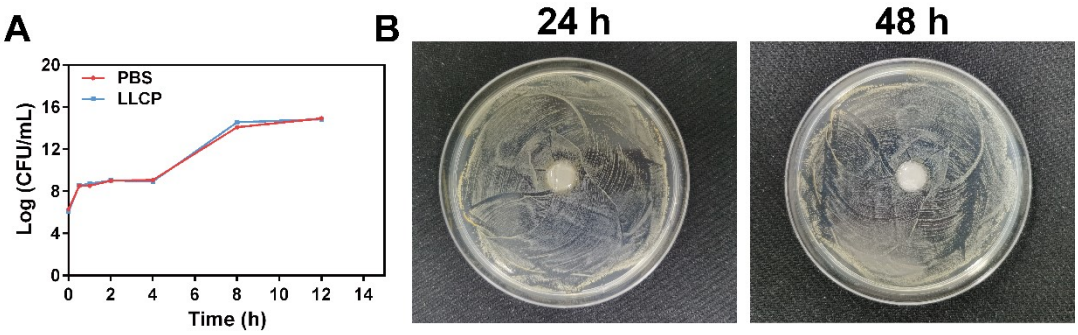


**Figure S5** The antimicrobial activity of LLCP. A) Time-killing curve of LLCP against *MRSA* ($n$ = 3); B) Representative photographs of LLCP against *MRSA* after incubation for 24 and 48 h.

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