

Supporting Information for

**Lipid-Polymer Hybrid Nanoparticles Enhance the Potency of Ampicillin against *Enterococcus faecalis* in a Protozoa Infection Model**

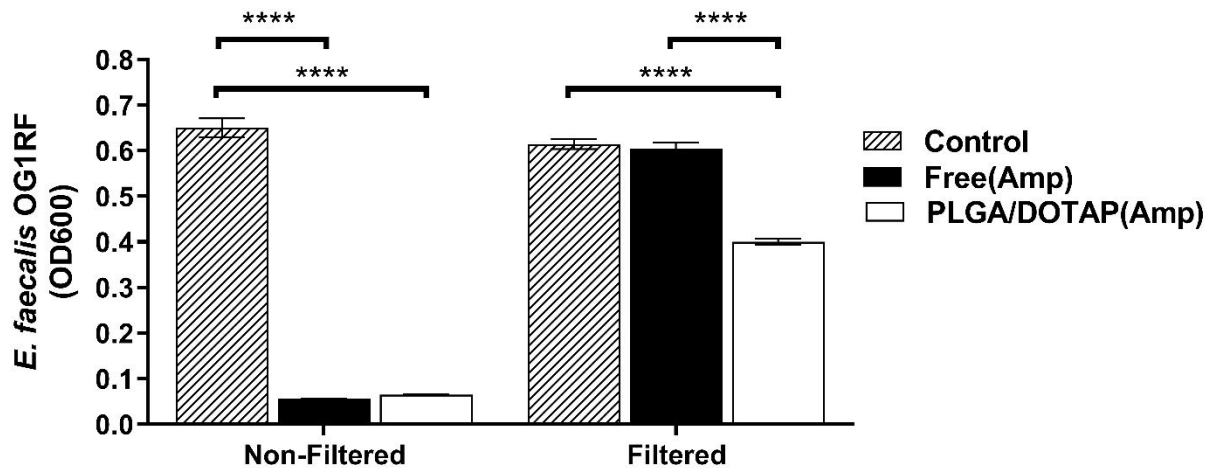
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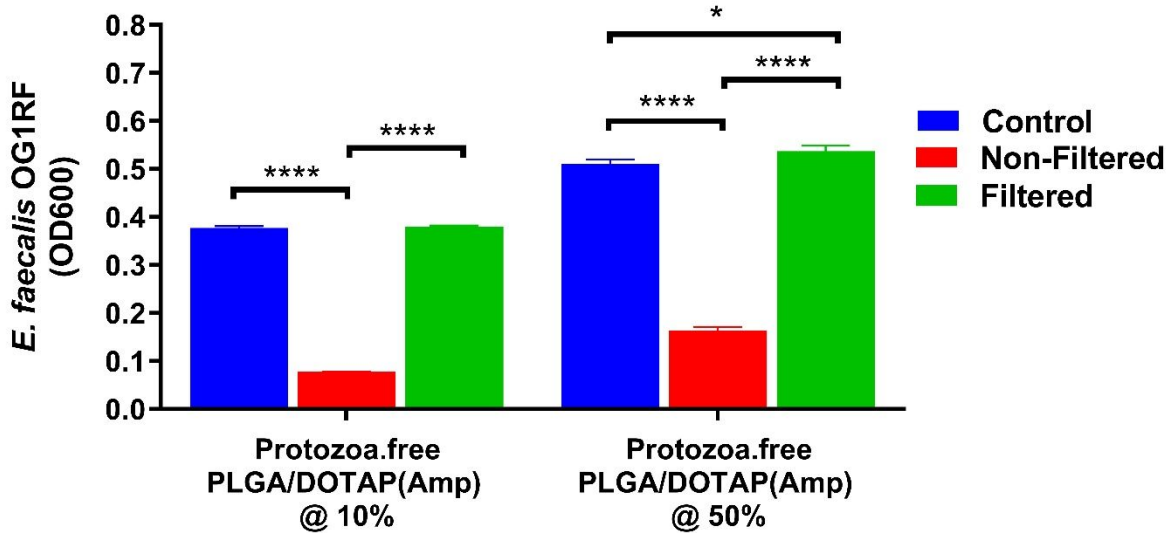
**This PDF file includes:**

6 Pages; Figure S1 to S6



**Figure S1. Residual Amp activity in the filter well, after filtration and washing, was assessed using the *E. faecalis* culture.**

To assess whether the extracellular Amp in the filter wells was diffused out and diluted successfully, the residual Amp activity in the filter well, after medium exchange for 5 times, was determined using the *E. faecalis* culture. Nearly no bacteria grew in the non-filtered media containing either free Amp or Amp-LPNs, confirming their effectiveness against extracellular *E. faecalis*. No bactericidal effect was exhibited in the medium from the free Amp group after filtration and washing, indicating complete removal of extracellular free Amp. In the group pre-treated with Amp-LPNs, the medium after filtration and washing caused a reduction in the number of *E. faecalis* (indicated by the reduction in the OD reading), suggesting there was still Amp present in the 5extracellular environment. One-way ANOVA test with Tukey’s multiple comparisons was performed. The statistical differences are indicated as follows: \*\*\*\* P < 0.0001.



**Figure S2. Residual Amp activity in a *T. pyriformis*-free control well, after filtration and washing, was assessed using the *E. faecalis* culture.**

To determine whether Amp-LPNs presented in the extracellular environment in Fig. S1 was due to insufficient filtration/washing, the residual Amp activity after filtration and washing was assessed in a protozoa free control well. Filtered media even at 50% showed no bactericidal activity towards *E. faecalis*, confirming the effectiveness of our procedure in removing Amp in both free and encapsulated forms in the extracellular environment. One-way ANOVA test with Tukey's multiple comparisons was performed. The statistical differences are indicated as follows:

\*  $P < 0.05$ , \*\*\*\*  $P < 0.0001$ .

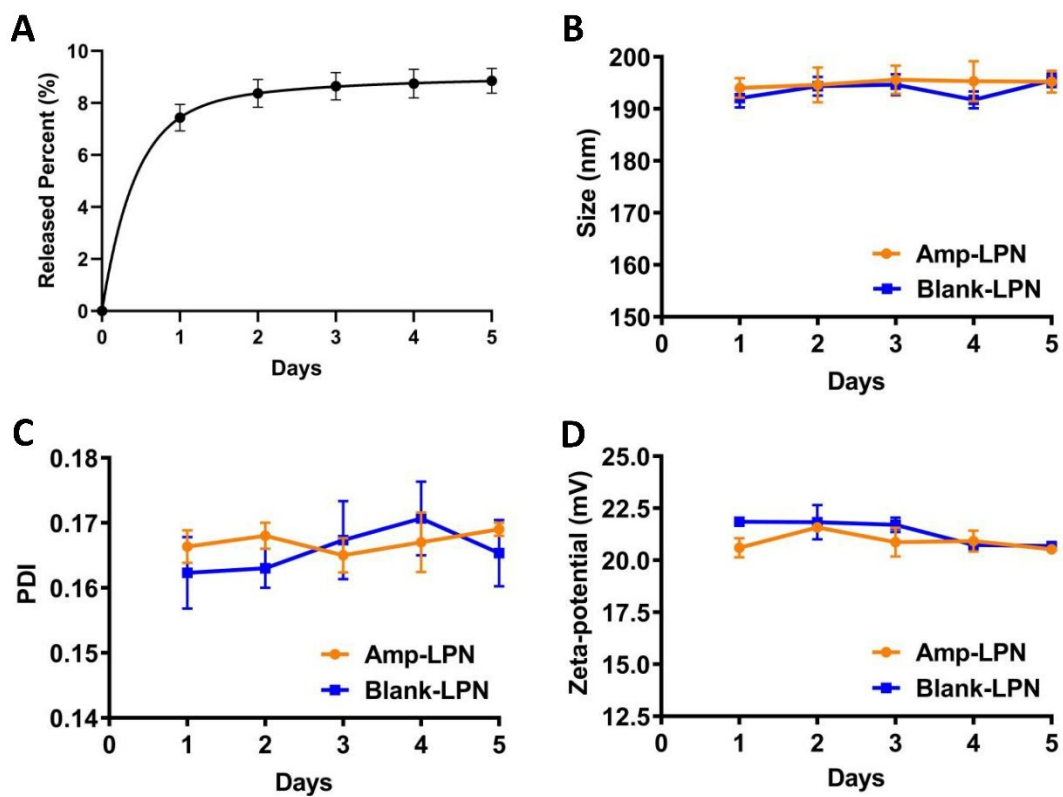
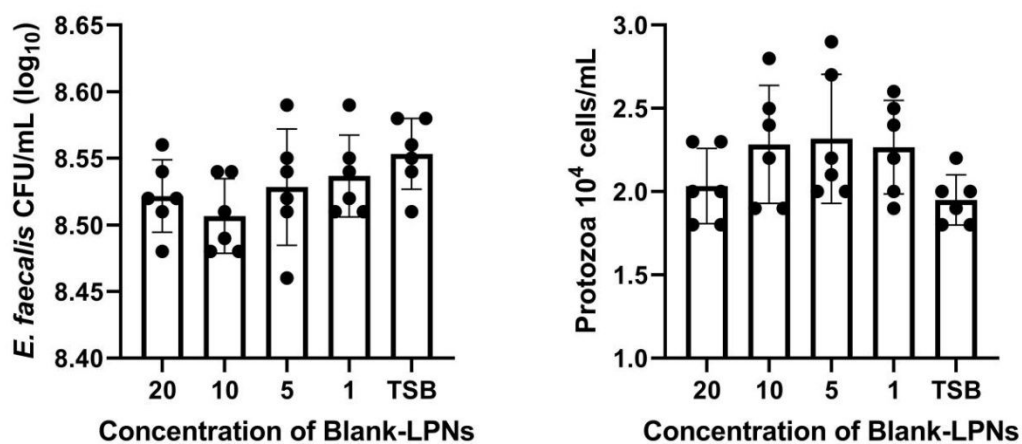


Figure S3. The characteristics of Amp-LPN and Blank-LPN. (A) the Release Profile of Amp-LPN in PBS (pH = 7). (B-D) the stability study under the same condition as release study, including (B) size (C) PDI (D) Zeta-potential. Mean  $\pm$  SD, n = 3.

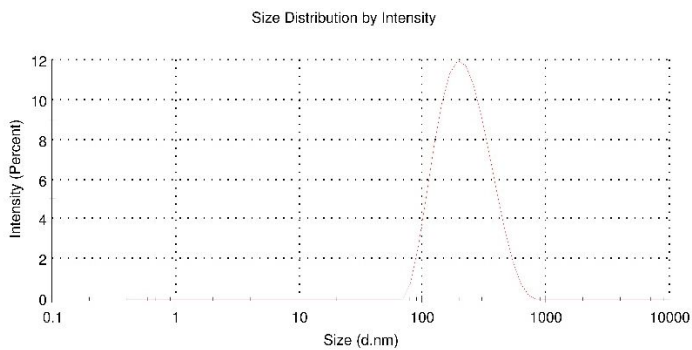


**Figure S4. Toxicity test of blank LPNs (mg/mL) on *E. faecalis* (left) and protozoa (right). Mean  $\pm$  SD, n = 6.** No statistical significance was identified between groups receiving different concentrations of blank LPNs. Based on the drug loading (around 25  $\mu$ g ampicillin/mg particles), the corresponding concentrations of Blank-LPNs of maximum 250  $\mu$ g/mL Amp in the report corresponds to 10 mg/mL Blank LPNs in the toxicity studies.

**Results**

|                                | Size (d.nm...)       | % Intensity: | St Dev (d.n... |
|--------------------------------|----------------------|--------------|----------------|
| <b>Z-Average (d.nm):</b> 194.0 | <b>Peak 1:</b> 231.7 | 100.0        | 108.8          |
| <b>Pdl:</b> 0.167              | <b>Peak 2:</b> 0.000 | 0.0          | 0.000          |
| <b>Intercept:</b> 0.957        | <b>Peak 3:</b> 0.000 | 0.0          | 0.000          |

**Result quality** Good



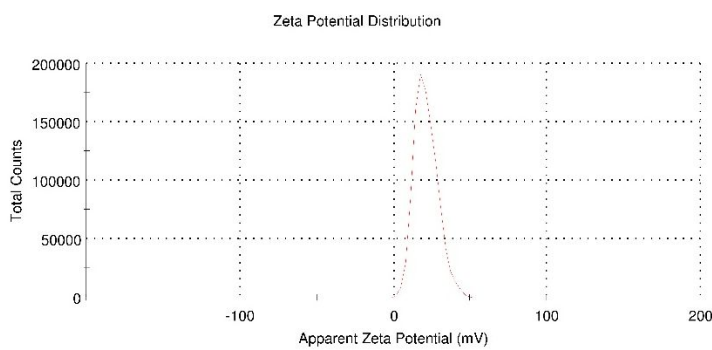
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**Figure S5. Raw data report of size distribution by Zetasizer.**

**Results**

|                                     | Mean (mV)           | Area (%) | St Dev (mV) |
|-------------------------------------|---------------------|----------|-------------|
| <b>Zeta Potential (mV):</b> 20.9    | <b>Peak 1:</b> 20.9 | 100.0    | 7.59        |
| <b>Zeta Deviation (mV):</b> 7.59    | <b>Peak 2:</b> 0.00 | 0.0      | 0.00        |
| <b>Conductivity (mS/cm):</b> 0.0898 | <b>Peak 3:</b> 0.00 | 0.0      | 0.00        |

**Result quality** Good



Record 629: PLGA AMP\_DOTAP FILM 20200306 1

**Figure S6. Raw data report of Zeta-potential distribution by Zetasizer.**