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Antibiotic-Loaded Smart Platelet: A Highly Effective Invisible Mode of Killing Both Antibiotic-Sensitive and -Resistant Bacteria

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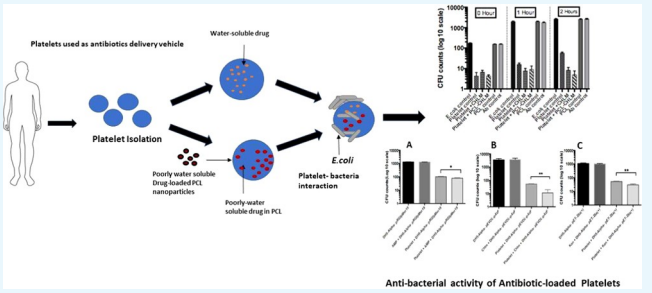
ABSTRACT: Microbial pathogenesis is considered one of the most critical health challenges worldwide. Although several antibiotics have been procured and used, the microbes often manage to escape and become resistant to antibiotics. Thus, the discovery of new antibiotics and designing smart approaches toward their delivery are of great importance. In many cases, the delivery agents using foreign chemicals like lipids or polymers induce immunogenic responses of varying degrees and are limited to a shorter circulatory time and burst release. In the current work, we have designed a novel antibiotic delivery system where the antibiotic is encapsulated into a blood component—platelet. Platelets have been previously reported as efficient drug delivery vehicles for targeting cancer cells. On the other hand, during platelet−bacterial interaction, platelets can act as covercytes. Keeping this in mind, smart antibiotic-loaded platelets have been used for killing bacterial cells. The loading of the antibiotic was done using its typical nature of engulfing surrounding small molecules. The water-soluble antibiotics were loaded directly into the platelet, whereas the hydrophobic antibiotics were preloaded in polycaprolactone (FDA-approved polymer)-based nanovesicles to make them solubilized prior to loading inside the platelets. The antibiotic-loaded platelets (containing hydrophilic antibiotics or hydrophobic antibiotic -encapsulated polymer nanoparticles) were found to be stable when studied through platelet aggregometry. The carrier showed bactericidal effects at a significantly lower concentration at which the free antibiotic has negligible efficacy. This could be attributed to the molecular confinement of the antibiotics inside the platelets, therefore causing localization of the drug and leading to efficient activity against bacteria. Interestingly, the smart antibiotic-loaded platelets were capable of killing the resistant strains too at the same lower concentration regime. Therefore, the antibiotic-loaded platelet could emerge as a potential strategy for efficient delivery of antibiotics with a significant reduction of the dose required to achieve the intended antibacterial efficacy. Moreover, this antibiotic delivery method can be very useful to minimize immunogenic responses due to antibiotic administration and to avoid the development of drug resistance due to the invisible mode of delivery.



INTRODUCTION

Despite major advancements in the medical field, microbial pathogenesis is still considered one of the most critical health challenges to occur worldwide.[1] To combat this microbial explosion on public health, several ranges of antibiotics have been used on a regular basis. Still, the microbes manage to bypass the effect of antibiotics and become resistant to drug molecules. In some cases, the bacteria display intrinsic resistance properties, while in other cases, the drug molecules encounter difficulties in permeating through the bacterial cell wall because of the high molecular weight and the large spatial structure.[2,3] This in turn has compelled scientists to discover more efficient antibiotics and design new delivery vehicles for the same. Due to the decade-long duration and huge financial investments needed in the discovery of new drug molecules, strategic advancements and improvement of the existing delivery systems can be an efficient alternative approach. Over the last few decades, innovative technological research has been done to change the pharmacokinetic profile of known antibiotics to reduce the clinical significance of acquired bacterial resistance. Additionally, several drug carriers have been developed for treating pathogens, including antibiotics loaded into liposomes and other lipid formulations, microspheres, polymeric carriers, dendrimers, and nanoplexes.[4−7] With significant advancements in novel drug delivery systems, nanomedicine has emerged as a promising strategy to achieve enhanced bioavailability and improved therapeutic efficacy

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while minimizing adverse effects associated with higher doses of potent drugs.[8] Of all of the nanocarriers, biocompatible polymer and lipid-based nanoparticles, in particular, have been extensively researched for delivery of hydrophobic as well water-soluble compounds.[9,10] Polymer nanoparticles are synthesized using FDA-approved biodegradable polymers such as poly(ε-caprolactone) (PCL), poly(ethylene glycol) (PEG), poly(lactic-*co*-glycolic acid) (PLGA), chitosan, and albumin.[11,12] Poly(ε-caprolactone) (PCL)-based nanoparticles have been used to enhance bioavailability and encapsulate poorly water-soluble drugs against cancer,[13−16] infectious diseases such as malaria,[17−19] leishmaniasis,[20−22] etc., as well as chronic conditions like hypertension[23,24] and diabetes.[25,26] Poly(ε-caprolactone)-based nanoparticles have also been used for the delivery of antibiotics via different routes of administration targeting a range of bacterial infections.[27−30]

However, the clinical success of nanomedicine has been limited due to major challenges associated with existing delivery systems such as rapid clearance, lack of site-specificity, phagocytic clearance, inability to cross the blood−brain barrier, in vivo degradation, etc.[31] Consequently, despite advancements in the field of nanotechnology, there still exists a requirement for a smart drug delivery system to bridge laboratory-scale nanotherapeutics with clinical success. Moreover, widely varying physiochemical properties of drugs demand versatile carrier systems for effective drug delivery and targeting. Therefore, keeping in mind the challenges in the design and development of nanomedicines, we aim to develop an alternative drug delivery system that combines nanotechnology with a circulatory cell-based carrier system, which inherently possesses outstanding qualities of site-specific biological payload delivery.[32] With a better understanding of their physiological roles, circulatory cells have received significant interest as a promising strategy to address drawbacks associated with nanocarriers. Circulatory cells possess a unique structure, surface functionality, and distinctive properties such as a long circulation time, in vivo stability, inherent biocompatibility, ability to cross the blood−brain barrier, and high payload capacity due to the large internal volume, rendering them as potential carrier systems to achieve anticipated clinical success.[33,34] Cell-based drug delivery systems include erythrocytes, leukocytes, platelets, stem cells, monocytes, macrophages, lymphocytes, dendritic cells, and other extracellular vesicles such as microvesicles and exosomes.[30,34] However, in this report, we have focused on platelets and introduced a platelet-based delivery system for antibiotics. Platelet is the smallest anucleated component of blood. They are cytoplasmic fragments derived from the megakaryocytes of the bone marrow.[35,36] Thus, the system should not have any biocompatibility issues. In normal physiological conditions, platelets play a vital role in homeostasis. Besides, this platelet has been recently shown to actively participate in immunity. Clinical investigation suggests that thrombocytopenia occurs during infection and platelets can bind infectious agents or engulf them.[37] Previously, we have used platelets as drug delivery vehicles for cancer treatment and management.[38] Now, we have targeted "platelet−pathogen interaction" and used platelets as antibiotic carriers. This well-known interaction of platelets with pathogens, especially bacteria, inspired us to adopt the present concept. The hypothesis has been verified using *Escherichia coli* as model bacteria and chloramphenicol as model antibiotic. We also believe that the present study will open a new era and provide a safer approach toward antibiotic delivery. We have also, for the very first time, assessed the capability of drug-loaded platelet carriers against drug-resistant bacterial strains.

Additionally, we aim to deliver poorly water-soluble antibiotic molecules encapsulated into polymer nanoparticles, which will further be attempted to be loaded into the platelets. As mentioned above, the drug-loaded PCL−platelet complex will also be investigated for its antibacterial effect in this study.

MATERIALS AND METHODS

Materials. Chloramphenicol, ampicillin, kanamycin, poly(ε-caprolactone) (PCL) (MW 14,000), and Pluronic F-127 were purchased from Sigma Aldrich Pvt. Ltd, India. The organic solvents used were of analytical grade.

Platelet Isolation and Antibiotic Loading. Blood was collected from healthy donors by venipuncture into plastic tubes containing sodium citrate buffer. The collected blood was then centrifuged at 200*g* for 12 min to isolate the platelet-rich plasma (PRP). Platelet-poor plasma (PPP) was obtained by centrifugation at 1200*g* for 10 min. The PPP served as a blank for the aggregometry study. All in vitro experiments were done within 4 h of blood draw. Gram-negative bacteria *E. coli* ATCC 25922 were used to observe the bacteria−platelet interaction and platelet-mediated antibiotic delivery. Confocal microscopy was used to see the interaction between the platelet and bacteria. Bactericidal activity was investigated by exposing bacteria to various treatment groups, including antibiotic solutions, control platelets, and platelets loaded with antibiotics. At different time intervals, the treated samples were placed on an agar plate and surviving bacterial cells where colonies were counted after overnight incubation.

Platelet Aggregometry. Platelet aggregation study was done to detect the effect of applied chloramphenicol on platelets. For this purpose, adenosine diphosphate (ADP) (final concentration 10 μM), collagen (final concentration 4 μg/mL), and arachidonic acid (AA) (final concentration 500 μg/mL) were used as agonists. All of the reagents were purchased from Chrono-log, and a Chrono-log aggregometer (model no 700) Chrono-log, Havertown, was used for the platelet functioning test. Briefly, PRP was isolated by the same procedure stated earlier. Then, chloramphenicol (final concentration 6.25 μg/mL) was added to the PRP and incubated at 37 °C. Another portion of PRP was kept as the control. After different time intervals (in our case, 0, 2, and 4 h), PRP was taken from both the control and test, and the functionality of the platelet was measured by ADP, collagen, and AA. The aggregation experiment was run for 4 min with a predetermined stirring rate of 1000 rpm at 37 °C. This experiment was repeated three times.

Confocal Microscopy to Study Platelet−Bacteria Interaction. Interaction of platelets with bacteria was studied using a confocal microscope. Briefly, the LB media were inoculated with *E. coli*. ATCC 25922 was incubated to allow the bacterial cells to attain the mid-logarithm growth phase. The primary culture was further grown to obtain optical density equivalent to 10^6 CFU/mL. On the other hand, platelets were prepared following the same procedure as mentioned in the platelet isolation section. After preparation of platelets, *E. coli* cells were stained by DAPI (concentrate 0.5 μg/mL) following PBS washing to remove excess stain and was mixed with platelets (*E. coli* and platelet ratio is 2:1) and incubated at 37 °C with a rocking speed of 200 rpm for 1 h. Next, the platelet−*E. coli* mixture was fixed with 4%

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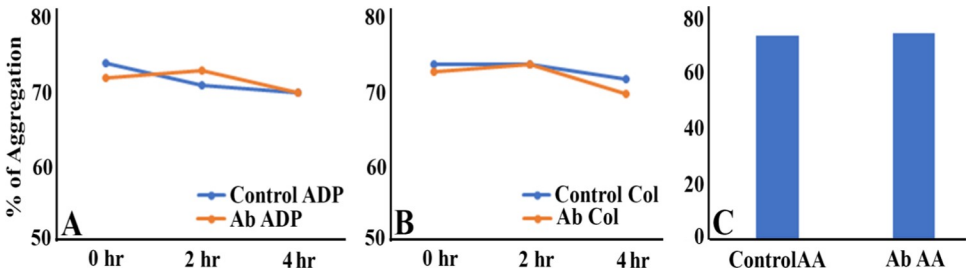

Figure 1. Aggregation profile of the platelets in the presence of ADP and collagen as agonists at different time intervals (0, 2, and 4 h). The percentages of aggregation of platelets in the absence (control ADP) and presence (Ab ADP) of chloramphenicol were 74, 71, 70% and 72, 73, 70%, respectively, at 0, 2, and 4 h when ADP was used (A). For collagen-induced aggregation, the percentages of aggregation were 74, 74, 72% and 73, 74, 70%, respectively, in the absence (control Col) and presence (Ab Col) of the antibiotic for the same time intervals (B). (C) Arachidonic acid-induced aggregation of platelets at 4 h where the percentages of aggregation were 74 and 75% for control platelets and antibiotic-loaded platelets, respectively.

paraformaldehyde. In a grease-free glass slide, 10 μl of the suspension was taken and a coverslip was placed on the slide, with the edges sealed. The slides were then viewed under a laser confocal microscope of Olympus model no. IX 81 FV 1,000 with 60× objective lenses.

***E. coli* Culture and Killing Assay.** Gram-negative bacteria *E. coli* ATCC 25922 were used for the study. The bacterial cells were grown overnight in LB media until the mid-logarithm phase was achieved. The optical density of the culture was measured and adjusted to 10^6 CFU/mL. The bacterial culture was then exposed to various treatment groups including antibiotic solution, control platelets, and platelets loaded with antibiotics and further incubated at 37 °C. The aliquots were withdrawn immediately and at time intervals of 30, 60, and 120 min and placed on agar plates. After overnight incubation, the colonies were counted manually, and the bactericidal activity was calculated.

Formulation of Chloramphenicol-Loaded Poly(ε-caprolactone) (CHLM-PC) Nanoparticles. Sparingly soluble chloramphenicol was encapsulated into poly(ε-caprolactone) nanoparticles by nanoprecipitation as reported by Kalita and team,[39] with modifications. Briefly, water-insoluble polymer and drug were solubilized in acetone. The resultant organic phase was added dropwise to 1% Pluronic F-127 containing aqueous solution with continuous stirring. The resultant colloidal suspension was left for overnight stirring to allow complete elimination of the organic solvent. The particle size of drug-loaded nanoparticles (CHLM-PCL) was determined by dynamic laser scattering.

Antibiotic Loading and Encapsulation Efficiency. Antibiotics such as chloramphenicol, ampicillin, and kanamycin were loaded into the platelets using the diffusion method. In short, antibiotics (final concentration 6.25 μg/mL) were incubated with PRP at 37 °C for 1 h. To discard the excess antibiotic remaining in the PRP solution, apyrase (final concentration 0.2 U/mL) was added and centrifuged at 2,000 rpm in a swing-out centrifugation machine. The excess drug-containing supernatant was discarded carefully, and the pellet was dissolved in PBS. The antibiotic-loaded platelet was washed another two times following the same procedure. After final washing, the pellet was resuspended in LB broth keeping the platelet count 1,50,000/mL. The above-mentioned protocol was followed for loading the chloramphenicol (1 mg/mL)-encapsulated nanoparticles (PCL-CHLM) into the platelets.

For antibiotic (Ab) loading purposes, 6.25 μg/mL antibiotics (chloramphenicol, kanamycin, and ampicillin) were individually incubated with a platelet suspension (150,000/mL). The platelet count was kept constant using a hemocytometer and a conventional counting technique and kept constant for all of the experiments unless mentioned otherwise. To quantify the entrapment efficiency, a freshly prepared drug-loaded platelet suspension was centrifuged at 2,000 rpm for 10 min. The supernatant containing the free drug was collected to quantify the drug content, which was measured using a UV−vis spectrophotometer at wavelengths of 273 nm for chloramphenicol and 222 nm for ampicillin.[38,39]

In Vitro Drug Release. The dynamic dialysis method was used to study the release kinetic of chloramphenicol from platelets.[40] Briefly, freshly prepared chloramphenicol-loaded platelets were dispersed in PBS (pH 7.4) with a final concentration of 5 mg/mL. Then, 1 mL of a drug-loaded platelet suspension was transferred into a dialysis bag, which acted as a donor compartment and was further submerged in 50 mL of PBS (7.4). The experiment was carried out at 37 °C under gentle stirring at 100 rpm. A total of 75 ul of the sample aliquot was taken at predetermined time intervals, which was replaced by fresh PBS solution keeping the total dissolution volume unchanged. The chloramphenicol concentration present in the samples withdrawn at predesignated time intervals was calculated using a UV spectrophotometer at 273 nm.[38]

Antibiotic-Resistant *E. coli* Culture and Killing Assay. To study the effect of antibiotic-loaded platelet carriers on the viability of drug-resistant Gram-negative bacteria *E. coli* ATCC 25922, three different drug-resistant strains were chosen. The mutant strains used in this study were DH5-*α-pRGfpMer19*, DH5-*α-pEVOL-pAzF*, and DH5- *α-pET-28a*(+), which were resistant to ampicillin, chloramphenicol, and kanamycin, respectively. Initially, the mean % viability of the mutant strains at varying drug concentrations was calculated to ensure successful transformation and was compared to the viability observed in the wild type. Later, the bacterial cells were grown, and the killing assay was performed in a similar fashion that has been explained in the previous section of *E. coli* culture and killing assay.

Antibacterial Activity of Antibiotic-Loaded PCL Nanoparticles and CHLM-PCL-Platelet. The antibiotic-loaded biodegradable polymer nanoparticles (CHLM-PCL) were assessed for their antibacterial activity after their uptake by platelets. A time-dependent bacterial cell killing assay was performed as mentioned in the earlier section at different time intervals. Treatment groups included antibiotic control (Ab ctrl), vehicle control (PCL ctrl), platelet control, CHLM-PCL-

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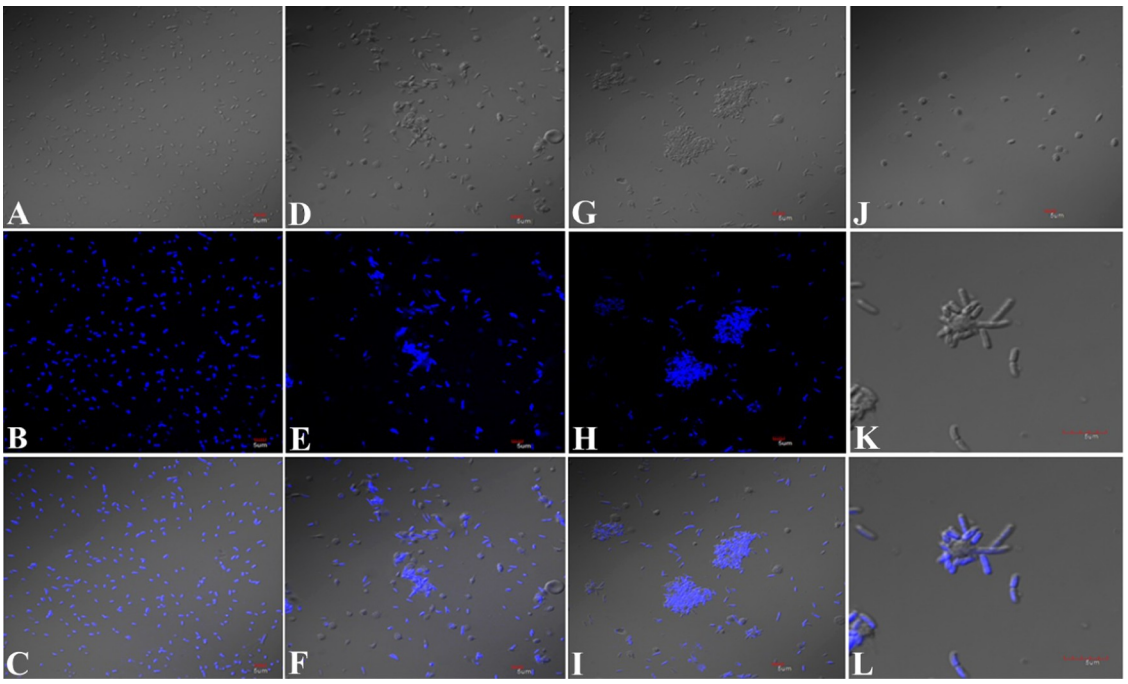

Figure 2. Confocal microscopic images of *E. coli* and platelets. (A−C) Bright-field, fluorescent, and corresponding overlapping images of *E. coli* cells, respectively. (D−F) Respective bright-field, fluorescent, and corresponding overlapping images of *E. coli* in the presence of platelets after 1 h of incubation. (G−I) Bright-field, fluorescent, and corresponding overlapping images of *E. coli* in the presence of platelets after 2 h of incubation. (J) Bright-field image of platelets. (A)−(J) were captured at 60× magnification. (K)−(L) Higher-magnification (2.4× optical zoom) images of platelets and bacterial cell interaction in bright field and its fluorescent overlapping mode, respectively.

loaded platelets (platelet + PCL), chloramphenicol-loaded platelets (platelets + Ab), and negative control.

RESULTS AND DISCUSSION

Platelet Aggregometry. In this study, we have used the platelet, a blood component itself, as an antibiotic delivery system. Platelets have a normal tendency to form micro/large aggregates in the presence of some chemicals, and in general, the use of these chemicals is prohibited for platelet-based studies. To check whether the used antibiotic chloramphenicol has any adverse effects on platelet functionality, a platelet aggregometry study was performed using different agonists (ADP, collagen, and AA) at different time intervals. The experimental results (Figures 1 and S1) show that the percentage of aggregation of Ab-loaded platelets remains almost the same as that of unloaded control over time for all of the agonist-associated pathways, which in turn suggest that platelets can be used for antibiotic delivery.

The concept of delivering antibiotics using platelets evolved from the fact that platelets express a variety of potential receptors for bacteria binding. The mechanism behind this interaction is primarily of three types: (1) indirect binding of a plasma protein, with bacteria, which serve as a ligand of platelet receptor, (2) binding of secreted bacterial products, particularly toxins, to platelets; and lastly (3) direct binding of bacteria to platelet receptors.[36] Besides these, there is also evidence of platelets engulfing bacteria within it.[40] A confocal microscopic study (Figure 2) suggested a similar kind of interaction between platelets and *E. coli*. The bacterial cells were stained with DAPI (DNA-binding fluorescent dye) prior to the incubation with platelets so that they could be easily detected. Figure 1 clearly indicates aggregate formation in the presence of platelets (Figure 2D−I), whereas the bacterial population remains as a separate entity in the absence of platelets (Figure 2A−C). The size of the aggregates becomes larger in a time-dependent manner (Figure 2D−F and G−I). This interaction is more clearly visible in higher-magnification images (Figure 2K,L). Not only the superficial interaction but also the Z sectioning of platelet−*E. coli* clump revealed that platelets also engulfed *E. coli* cells within it (Figure S2).

Encapsulation Efficiency. The encapsulation efficiencies of chloramphenicol and ampicillin into platelets were calculated. As mentioned above, platelets were incubated with a drug solution and the free drug was separated by centrifugation at 2,000 rpm. The free drug content was quantified in the supernatant by UV−vis spectrophotometry. The encapsulation efficiencies of chloramphenicol and ampicillin were found to be 38.6 ± 2.68 and 59.71 ± 3.49%, respectively, confirming that platelets are capable of encapsulating drugs with varying physiochemical properties.

In Vitro Drug Release. The drug release kinetics was studied in PBS 7.4 for chloramphenicol-loaded platelets (CHLM-platelets) as well as an aqueous solution of chloramphenicol enclosed inside a dialysis bag. The drug release pattern (Figure 3) of CHLM-platelets showed delayed release during the initial hours; however, slow release of the drug was observed after 6 h. In the first 24 h, almost 37.21 ± 0.728% drug was released from the CHLM-platelets, which further increased to 50.48 ± 1.68% after 72 h. Overall, a sustained in vitro drug release pattern was observed for the duration of 240 h with a cumulative drug release of 67.55 ± 1.84% after 10 days. However, the release pattern from the aqueous solution of chloramphenicol enclosed in the dialysis bag showed that 100% of the drug was released within the first 24 h. Thus, it was inferred that a sustained drug release pattern could be obtained using platelets as drug delivery vehicles for a

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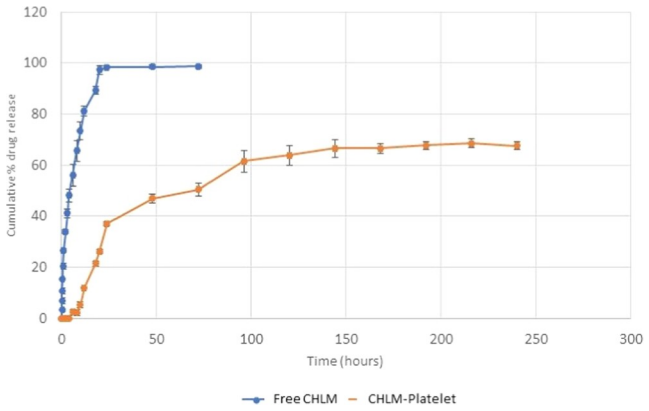

Figure 3. In vitro chloramphenicol release kinetics from an aqueous solution and chloramphenicol-loaded platelets at pH 7.4.

longer period of time. The longer in vivo circulation time of platelets can be further exploited to achieve sustained or controlled drug delivery.

Antibacterial Activity of CHLM-Loaded Platelets (Ab-Platelet) and Antibiotic-Loaded PCL Nanoparticles (CHLM-PCL) Entrapped in Platelets. The main objective of the study was to develop an invisible and highly biocompatible mode of delivery system that can effectively kill bacteria. Choosing platelets for this purpose is not a coincidence; there are several factors. First, being a blood component, they will be nonimmunogenic. Second is a well-defined interaction of platelets with microorganisms. Third, platelets can uptake a substantial amount of drug, and upon aggregation, they can release the same (Figure 4), strongly suggesting that when *E. coli* was treated with platelets loaded with chloramphenicol (final concentration of 2.4 μg/mL), the viable bacterial count (in terms of CFU) at 30 min was 45.8%, which further reduced to almost nil after 2 h of incubation. However, in the case of the free drug (6.25 μg/mL), an initial lagging of growth was observed, which increased exponentially after 1 h and reached 552% after 2 h. Furthermore, the observations show a typical growth pattern of *E. coli* when treated with only platelets, wherein a subsequent decrease in the percentage viable count was observed in the first 60 min of treatment (74 and 50% after 30 and 60 mins, respectively), which increased to 88% after 2 h (Figure 4A,B). The initial growth arrest could be attributed to the formation of platelet−bacterial aggregates due to their natural tendency to interact with microbial cells. On the other hand, untreated *E. coli* cells used as positive controls show a normal growth pattern, as anticipated. The most striking aspect of this method is the substantial reduction of the antibiotic dose to achieve significant antimicrobial activity. The final applied dose (2.45 μg/mL) of 6.25 μg/mL free chloramphenicol showed negligible killing of bacterial cells; however, the applied dose

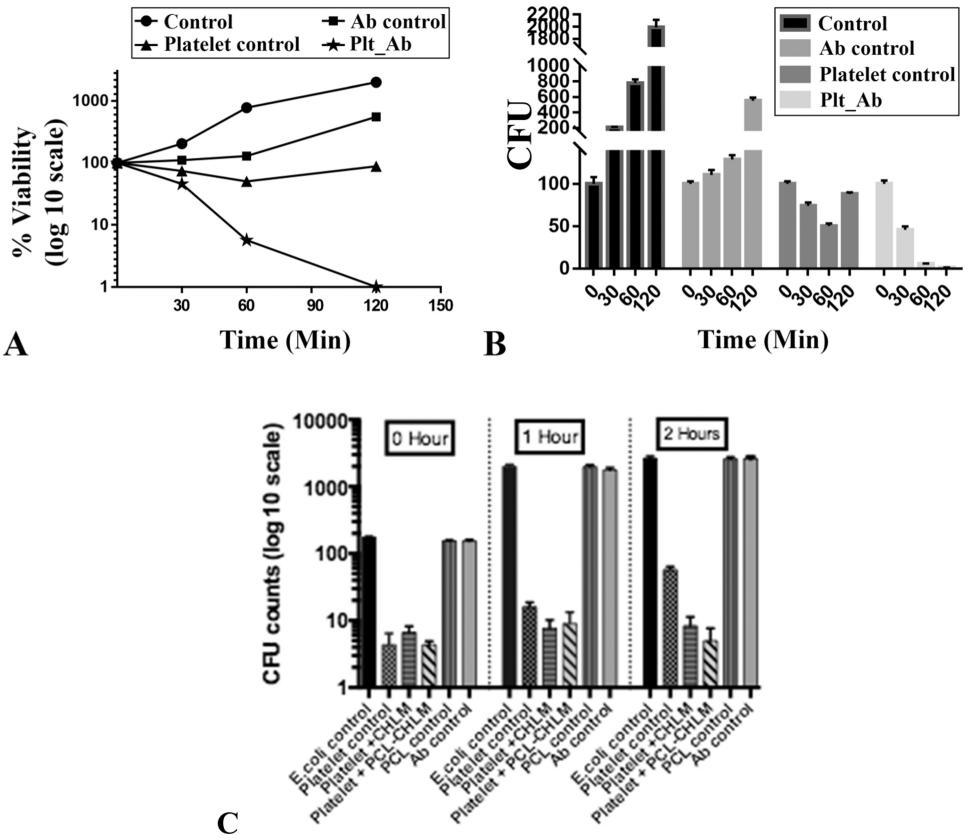

Figure 4. *E. coli* cell viability with different treatments. (A) Percentage viability of *E. coli* cells when treated with free chloramphenicol (Ab control), platelet (platelet control), and chloramphenicol-loaded platelet (Plt-Ab) in a time-dependent manner. *E. coli* cells without any treatment serve as controls. (B) Bar diagram with the viability percentage of *E. coli* cells of individual treatment groups at 0, 30, 60, and 120 min. (C) Comparative time-dependent antibacterial effect of PCL-entrapped chloramphenicol (PCL-CHLM), chloramphenicol solution (Ab control), platelet with chloramphenicol (platelet + CHLM), PCL-CHLM (platelet + PCL-CHLM), and control groups (*E. coli* control and platelet control). The data sets are represented as mean ± standard error of the mean (n = 3).

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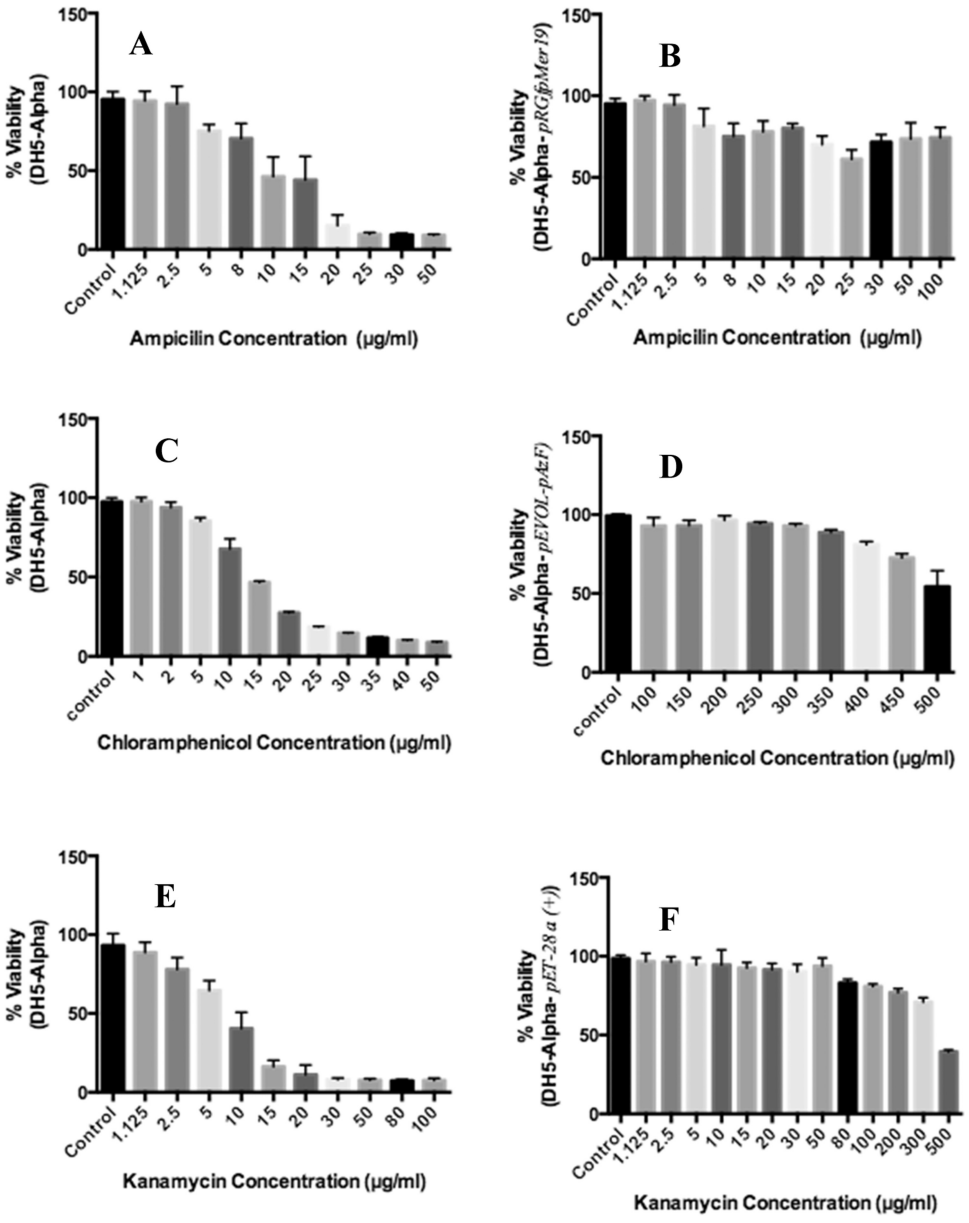

Figure 5. Antibacterial activity of different antibiotics. (A) Viability of *E. coli* (DH5-α) at different concentrations of ampicillin. (B) Survival of ampicillin-resistant DH5-α-*pRGfpMer19* when treated with increasing concentrations of ampicillin. (C) Observed % survival of *E. coli* (DH5-α) at different concentrations of chloramphenicol. (D) Observed % viability of chloramphenicol-resistant DH5-α-*pEVOL-pAzF* at different concentrations of chloramphenicol. (E) Survival % of *E. coli* (DH5-α) at different concentrations of kanamycin. (F) Observed survival % of DH5-α-*pET-28a*(+) at varying concentrations of kanamycin. The data sets are represented as mean ± standard error of the mean (n = 4).

of 2.45 μg/mL ab-loaded platelets showed a significant reduction in the CFU counts (Figure 4A,B). The concentration of free antibody was kept the same as the initial amount of chloramphenicol used for encapsulation into platelets to nullify any ambiguity.

Sparingly soluble chloramphenicol-loaded PCL nanoparticles were prepared by nanoprecipitation. Dynamic laser scattering was used to measure the hydrodynamic radii of drug-loaded nanoparticles, which were found to be 127.1 ± 4.32 nm. The zeta potential of the colloidal suspension was found to be −17.5 mV, which suggested the nanoparticles to be stable. The killing assay of CHLM-PCL was conducted in DH5-α in the presence and absence of platelets to understand the effect of polymers on the antibacterial property of chloramphenicol. The observations were made at time intervals of 0, 1, and 2 h (Figure 3C). The CFU count of CHLM-PCL-platelet was comparable to that of CHLM-platelets, which showed that entrapment of the sparingly soluble antibiotic into the PCL nanoparticles did not hinder the antibacterial activity. Furthermore, the high CFU counts of the PCL control group implied that polymers do not have any antibacterial effect. Hence, it was inferred from the observations that platelets can be used as carrier vehicles for polymer nanoparticles with poorly water-soluble compounds.

Three different mutant strains were used in this study, namely, DH5-α-*pRGfpMer19*, DH5-α-*pEVOL-pAzF*, and DH5-α-*pET-28a*(+), which were resistant to ampicillin, chloramphenicol, and kanamycin, respectively. The mean % viability shown in Figure 5A−F confirms successful transformation as the survival of resistant strains in antibiotic-

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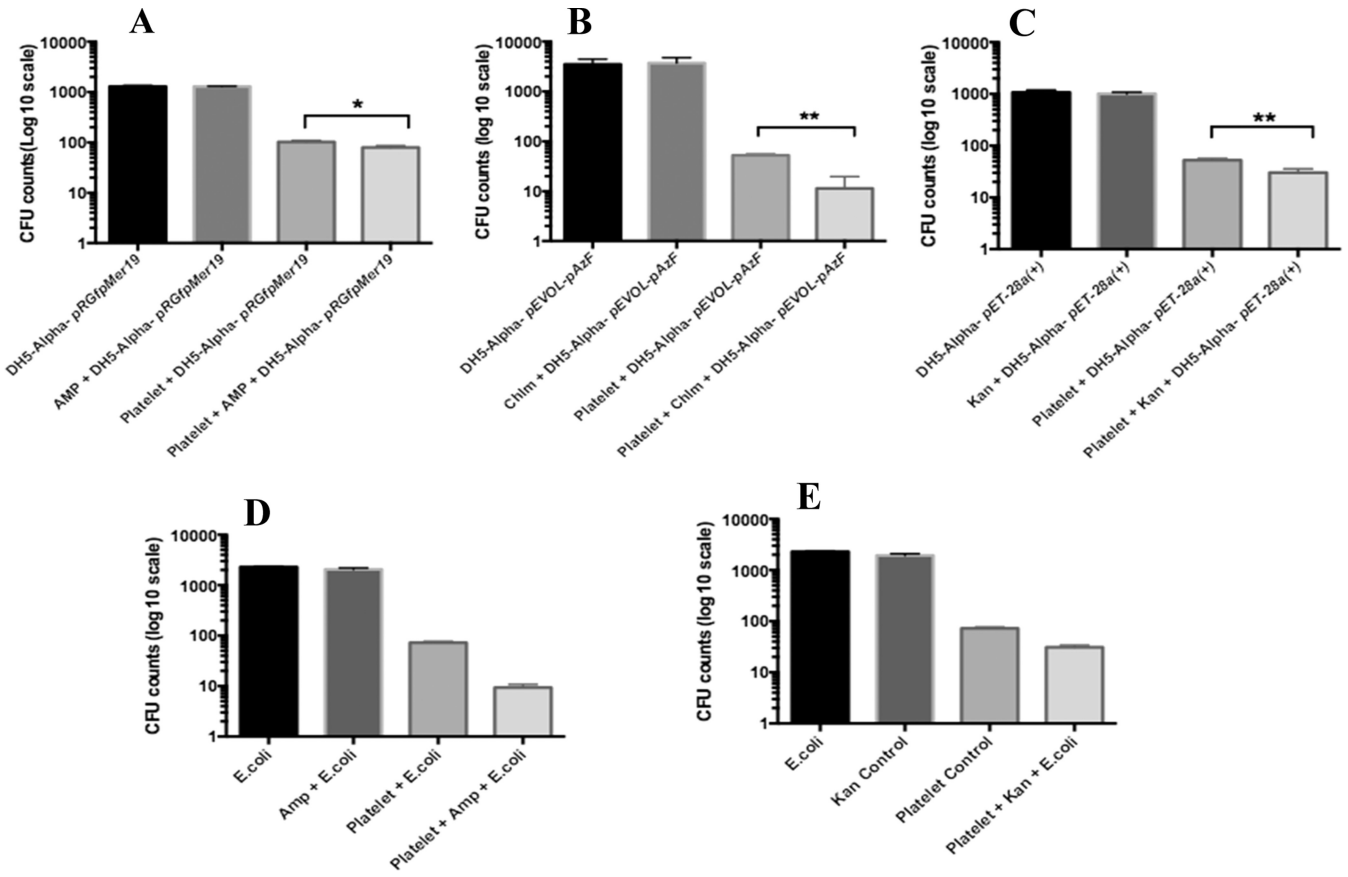

Figure 6. Antibacterial activity of antibiotics loaded in the presence and absence of platelets as carrier systems denotes survival of (A) ampicillin-resistant DH5-*α*-*pRGfpMer19* represented by colony-forming units (CFUs) when treated with ampicillin (AMP + DH5-*α*-*pRGfpMer19*), platelet control (platelet + DH5-*α*-*pRGfpMer19*), and ampicillin-loaded platelets (platelet + AMP + DH5-*α*-*pRGfpMer19*). (B) Survival of chloramphenicol-resistant DH5-*α*-*pEVOL-pAzF* when treated with chloramphenicol, platelet control (platelet + DH5-*α*-*pEVOL-pAzF*), and chloramphenicol-loaded platelets (platelet + CHLM + DH5-*α*-*pEVOL- pAzF*). (C) Observed survival of kanamycin-resistant DH5-*α*-*pET-28a*(+) when treated in the presence of kanamycin (KAN + DH5-*α*-*pET-28a*(+)), platelet control (platelet + DH5-*α*-*pET-28a*(+)), and kanamycin-loaded platelets (platelet + KAN + DH5-*α*-*pET-28a*(+)). Statistical significance was established by the multiple-comparison test (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$). (D) and (E) Enhanced antibacterial activity of ampicillin and kanamycin, respectively, against *E. coli* (DH5-*α*) in the presence and absence of platelets as carrier systems. The data sets are represented as mean ± standard error of the mean ($n = 3$).

containing media was significantly higher as compared to the wild type. Figure 5A,B shows that the survival of DH5-*α* reduced to less than 50% at ampicillin doses greater than 15 *μ*g/mL, whereas most cells of the resistant strain DH5-*α*-*pRGfpMer19* survived even at a dose of 100 *μ*g/mL. Similarly, less than 50% survival was observed when DH5-*α* was treated with 15 and 10 *μ*g/mL concentrations of chloramphenicol and kanamycin, respectively (Figure 5C,E). However, most populations of *α*-*pEVOL-pAzF* and DH5-*α*-*pET-28a*(+) survived even at a concentration as high as 300 *μ*g/mL for both chloramphenicol and kanamycin (Figure 5D,F). Thus, the observed results affirm successful transformation.

The bacterial killing assay was also carried out to investigate the role of platelets as drug carriers targeted against the drug-resistant mutant strains. Figure 6 shows the mean CFU counts of the wild type as well as drug-resistant bacterial cells on being treated with antibiotics in the presence and absence of platelet carriers. It was observed that in the absence of platelets when DH5-*α* was treated with the antibiotic, the CFU counts were substantially high. However, in the presence of platelet drug carriers, the CFU counts reduced significantly (Figure 6D,E). The reduced CFU counts of groups treated with the platelet control ascertain the inherent antibacterial property of platelets, which is further enhanced in the presence of antibiotics. On the other hand, the killing assay results of the mutant stains also show the successful antibacterial property of antibiotic-loaded platelets against drug-resistant bacteria (Figure 6A−C). The antibiotic control groups show negligible killing with CFU as high as the negative control group. However, the antibiotic-loaded platelets showed significantly reduced CFU, implying the antibacterial activity of antibiotics loaded into platelets against resistant strains. Interestingly, the platelet control group also showed antibacterial property better than the antibiotics. Therefore, on comparing the killing efficacy of platelet controls with antibiotic-loaded platelet carriers, the enhancement of the antibacterial activity in the presence of antibiotics was found to be statistically significant (Figure 6A−C). Thus, the results show the use of the platelet drug carrier as a potential strategy against drug-resistant bacterial strains. The results of the drug-resistant bacterial killing assay showed the usage of platelets as a promising approach to target antibiotic-resistant bacterial strains. The significant enhancement of the antibacterial property of drug-loaded platelets against its respective antibiotic-resistant strains was confirmed using three different antibiotics. The enhanced antimicrobial activity might have been due to the platelet−

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bacterial interaction leading to thrombocytopenia. Furthermore, the localized concentration in the small volume of platelets might have increased in such a way that both the wild-type and resistant strains are killed efficiently at a remarkably low dose. Hence, as a first of its kind, we infer that antibiotic-loaded platelets hold high potential for targeting drug-resistant bacterial infections. Moreover, a large number of existing antibiotics also include compounds with poor aqueous solubility being one of the major factors limiting the therapeutic efficacy. Therefore, as one of the strategies to enhance solubility, we have encapsulated the drug into the polymer nanoparticles and further studied the influence of polymer nanoshells on the activity of chloramphenicol. Comparing the results of chloramphenicol−platelet and CHLM-PCL−platelet complex, it was found that the activity of the antibiotic was not affected by polymer nanoshells. Therefore, platelets can be used as drug carriers for poorly water-soluble molecules too with no significant change in the therapeutic activity.

CONCLUSIONS

Platelets carry biomolecules in their cytoplasmic granules, which are transported and delivered with high specificity upon activation for tissue repair as well as homeostasis.[32] The inherent properties of platelets make them good candidates for a cell-based drug delivery system. In this study, we assessed the antibiotic-loaded platelets for their drug delivery capabilities in *E. coli* as well as drug-resistant mutant strains. Interestingly, it was observed that the Ab-platelet showed an enhanced antibacterial property with significantly lower doses in both wild-type and drug-resistant strains. The antibiotics did not show any effect on the respective drug-resistant mutant strains, whereas substantial killing was observed in antibiotic-loaded platelets as well as platelet control treatment groups. On comparing the killing efficacy of platelets as well as antibiotic-loaded platelets, the enhancement in the antibacterial effect of drug-loaded platelets was found to be significant. Hence, to the best of our knowledge, for the very first time, we report platelets as potential carrier systems for the delivery of antibiotics targeting Gram-negative bacteria as well as drug-resistant microbial pathogenesis. Furthermore, we also investigated the delivery of sparingly water-soluble chloramphenicol-loaded polymer nanoparticles using platelets and concluded that loading of the drug into polymer nanoparticles does not affect the antibacterial properties of the drug. Our observations show that drug-loaded platelets can be a potential strategy to target a wide range of bacterial infections. Extended research is required to elucidate further aspects of platelets for microbial killing. Consequently, our results strongly suggest that platelets can be used as antibiotic delivery vehicles. This method can be used to combat bacterial infection where direct interaction between platelet and bacteria takes place and can be further extended to treat viral infections as a well-documented interaction was observed between platelets and the virus.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c07249.

Aggregation profile of platelets at different time intervals in the presence of ADP, collagen, and ascorbic acid (S1); and confocal microscopy images (Z sections) of platelet−bacteria aggregates (S2) (PDF)

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Notes

The authors declare no competing financial interest.

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