Biofilm-Inspired Encapsulation of Probiotics for the Treatment of Complex Infections

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Biofilm-inspired encapsulation of probiotics for the treatment of complex infections

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Abstract:

The emergence of antimicrobial resistance poses a major challenge to healthcare. Probiotics offer a potential alternative treatment method but are often incompatible with antibiotics themselves, diminishing their overall therapeutic utility. This work uses biofilm-inspired encapsulation of probiotics to confer temporary antibiotic protection and to enable the co-administration of

probiotics and antibiotics. Probiotics are encapsulated within alginate, a crucial component of pseudomonas biofilms, based on a simple two-step alginate cross-linking procedure. Following exposure to the antibiotic tobramycin, the growth and metabolic activity of encapsulated probiotics are unaffected by tobramycin, and they show a four-log survival advantage over free probiotics. This results from tobramycin sequestration on the periphery of alginate beads which prevents its diffusion into the core but yet allows probiotic byproducts to diffuse outward. We demonstrate that this approach using tobramycin combined with encapsulated probiotic has the ability to completely eradicate methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* in co-culture, the two most widely implicated bacteria in chronic wounds.

Overuse of antibiotics has led to the widespread development of antimicrobial resistance (AMR). AMR poses a growing challenge to healthcare and is projected to cause 10 million deaths per year by 2050 at an estimated cost of 100 trillion USD.[1] There is an ever-dwindling number of effective antimicrobial agents and little resource allocation into the development of novel ones.[2], [3] Probiotics, which are live organisms that confer health benefits when administered in adequate amounts, offer a potentially powerful solution to AMR. Certain probiotics are able to combat pathogens through the secretion of antimicrobial substances and organic acids or through simply competing for resources and space.[4], [5] Importantly, probiotics have demonstrated the ability to be effective in the treatment of some chronic infections, against which most antibiotics are ineffective.[6]

The co-administration of probiotics and traditional antibiotics therefore has the potential to overcome AMR and combat complex infections. However, technically this is very challenging since most probiotics, being bacteria themselves, are susceptible to antibiotics and cannot survive co-

administration. The transformation of bacteria using genetically designed plasmids is a common method to confer antibiotic resistance for probiotics. However, this method is inefficient and results in a permanently resistant strain that has not only the potential to cause pathology but also the propensity to transfer the resistance to other bacteria.[7], [8] Probiotics that possess intrinsic antibiotic resistance have the same risk.[9] Even though antibiotics at subinhibitory concentration may not be bacteriostatic or bactericidal to them, it is well known that it can drastically affect their gene expression and hence their functionality.[10] As an alternative strategy, we suggest that probiotics placed inside a biofilm-inspired capsule would benefit from protection against antibiotics without the need to genetically modify the organism. This temporal antibiotic resistance would allow for the probiotic to exert antimicrobial effects in the presence of antibiotics. While some antibiotic-resistant pathogens may persist after conventional antibiotic treatment, we hypothesize that the co-administration of antibiotics and encapsulated probiotics can complement each other's spectrum of treatment and improve pathogen clearance **(Figure 1a)**. This work aims to combine the delivery of antibiotics and antibiotic-susceptible probiotics to enhance therapeutic efficacy.

Our encapsulation strategy is based on alginate, a crucial extracellular polymeric substance of *Pseudomonas aeruginosa* (PA) biofilms that has been shown to confer AMR.[11], [12] Alginate is an anionic polysaccharide consisting of mannuronic and guluronic acid units, which can be ionically cross-linked with divalent cations (e.g., Ca^{2+}) to form hydrogels.[13] Commercial alginate can be extracted from seaweed and its use as food additives is approved by the U.S. Food and Drug Administration (FDA). Alginate is also commonly used for mammalian cell encapsulatiocn due to its ease of use, low cost, and biocompatibility.[13], [14], [15] Alginate encapsulation of probiotics has been applied in the food industry to protect probiotics from harsh storage conditions and gastrointestinal

environments.[16] However, to our knowledge, it has not been used to facilitate the co-administration of probiotics and antibiotics.

As a model, we selected the commercial probiotic product Bio-K+®. It contains three Lactobacillus strains: *Lactobacillus acidophilus CL1285®*, *Lactobacillus casei LBC80R®* and *Lactobacillus rhamnosus CLR2®*. Lactobacilli belong to the group of lactic acid bacteria (LAB) and make up a large component of the skin, gastrointestinal tract, and urogenital tract microbiota in humans.[17] They are gram-positive, rod-shaped bacteria (Figure 1b) that display an exponential growth with a lag phase of 4 hours (Figure 1c). Bio-K+® was successfully encapsulated in alginate using a simple two-step fabrication method with standard techniques.[13] Briefly, a mixture of sodium alginate solution and Bio-K+® was introduced into a bath containing $CaCl_2$ using electrostatic spraying. Alginate beads quickly cross-linked, encapsulating Bio-K+® (Figure 1d). This first encapsulation step resulted in small beads with a diameter of 700 μm (Figure 1e) containing Bio-K+®. The small beads were mixed with a second alginate solution and underwent cross-linking after being dispensed through a 1-ml pipette. This created large beads 3.3 mm in diameter that contained 7-10 small beads each (Figure 1e) and a barrier of alginate between Bio-K® and the surface. This two-step fabrication procedure resulted in only minor loss of Bio-K+®, with an encapsulation efficiency of 84.0 ± 4.0%.

In order to assess the efficacy of this approach, free and encapsulated Bio-K+® were subjected to tobramycin, a polycationic aminoglycoside antibiotic that inhibits bacterial synthesis by binding to their 30S ribosomal subunit.[18] Alginate's effectiveness at limiting

the penetration of tobramycin and other aminoglycosides (gentamicin, amikacin, neomycin) in the context of PA has been previously demonstrated for both exopolysaccharides extracted from PA biofilms and commercial alginate hydrogels.[19], [20], [21], [22], [23] Furthermore, a mutant PA strain that overproduced alginate had higher resistance levels.[12]

The impact of alginate was evaluated by comparing the antibiotic efficacy of tobramycin on free and encapsulated Bio-K+®. Free and encapsulated Bio-K+® were both incubated in the lactobacilli-specific De Man-Rogosa-Sharpe (MRS) broth with or without tobramycin at the minimal inhibitory concentration (MIC) (Supplemental Table 1) for 24 hours at 37°C. Bio-K+® was inoculated at 10^7 CFU/ml. Free Bio-K+® in MRS broth without tobramycin grew to 10^9 CFU/ml, whereas those exposed to tobramycin decreased to 10^5 CFU/ml, showing a 4-log killing effect of tobramycin. Encapsulated Bio-K+® in both the absence and the presence of tobramycin grew to 10^9 CFU/ml, showing no killing effect of tobramycin. Alginate encapsulation did not have any significant effect on bacteria growth since there was no difference in bacteria number of free and encapsulated Bio-K+® after incubation in broth (Figure 2a). This result was confirmed by quantifying the metabolic activity of the bacteria using an AlamarBlue assay. The metabolic activity of encapsulated Bio-K+® grown with and without tobramycin was identical, thereby confirming that encapsulation protected Bio-K+® from tobramycin (Figure 2b). Alginte encapsulation also prevented Bio-K+® from immediately escaping the beads. Bio-K+® ultimately diffused out of the bead at ~24 hours in culture and was inactivated by the surrounding tobramycin. We believe that active Bio-K+® was confined and protected within the alginate beads while able to interact with the environment.

A possible explanation for the protection mechanism of encapsulated Bio-K+® is the induction of a diminished metabolism through alginate encapsulation. The efficacy of most

antibiotics, including tobramycin, depends on a high bacterial proliferation rate. When obligate aerobic pathogens such as PA reside within a thick biofilm and thus encounter oxygen limitation, they switch into a dormant state with arrested metabolism, making them less susceptible to antibiotics such as tobramycin.[24], [25], [26] Alginate beads also exhibit rapidly decreasing oxygen concentration towards the center and reach anoxia 100 μm below the bead surface.[27] However, in contrast to PA, Bio-K+® and lactobacilli in general are facultative anaerobes[28], which means that they can switch from oxidative phosphorylation to fermentation in the absence of oxygen and remain metabolically active even after alginate encapsulation. In this work, there was no difference in bacteria count or metabolic activity between free and encapsulated Bio-K+® cultured in broth (Figure 2b), suggesting that alginate encapsulation did not impede growth or metabolic activity. We therefore investigated an alternative protection mechanism of alginate encapsulation focused on the interactions between alginate and tobramycin.

Alginate's ability to interact with tobramycin and reduce its efficacy was confirmed in a series of experiments. First, MRS broth containing tobramycin was pre-treated with empty alginate beads (not containing Bio-K+®) for 24 hours at 37°C. This was followed by the inoculation of free Bio-K+® and an additional 24-hour incubation. Pre-treatment with empty alginate beads did not affect bacteria growth but negated the effect of tobramycin (Figure 2c). We then sought to visualize the interaction of alginate and tobramycin. This was accomplished by incubating fluorescently labeled tobramycin (Tob-Cy5) with alginate beads and imaging their cross-section with confocal microscopy at multiple time points. The diffusion of Tob-Cy5 was compared to that of Cy5 alone. Beads incubated with Cy5 alone showed complete diffusion throughout the entire cross-section within 15 minutes. This was in stark contrast to Tob-Cy5, which only accumulated on the periphery at time

points up to 24 hours. Encapsulated Bio-K+® can therefore not be targeted by tobramycin, which is only able to reach the bead's periphery (Figure 2d).

It is unlikely that the pore size of alginate beads is the limiting factor of diffusion. The pore size of a 2% Ca^{2+} alginate gel is reported to be around 5 nm, allowing for free diffusion of small molecules.[29] Even Cy5 (616.19 Da), with a slightly larger molecular mass than tobramycin (467.5 Da), could freely diffuse into the alginate bead within a short time frame (Figure 2d). The slight molecular weight difference between Tob-Cy5 (931.8 Da) and Cy5 (616.19 Da), as determined by Matrix Assisted Laser Desorption/Ionization (MALDI), does not explain the large differences in diffusion. However, the electrostatic characteristics of the two molecules likely contribute to differences in diffusion. Tobramycin has a 5+ charge at physiological pH[29] as compared to Cy5 which only has a 1+ charge[30]. This 4+ difference in charge can lead to chelation of tobramycin through the coordination of several alginate (a polyanionic polymer) chains due to the presence of multiple charges.[20], [31] This finding is further supported by previous studies on antibiotic diffusion through alginate-containing biofilms. It was demonstrated that tobramycin (cationic, MW = 467.52 Da[29]) was sequestered on the periphery of the biofilm, whereas ciprofloxacin (neutral, MW = 331.35 Da[32]) diffused readily.[33] We also used *Bacillus coagulans,* another probiotic with higher antibiotic susceptibility, to screen for the protective capacity of alginate beads against other antibiotics from different families. Against positively charged antibiotics azithromycin, clindamycin, vancomycin and tobramycin, encapsulated *Bacillus coagulans* showed higher survival than free ones, whereas there was no difference in the case of neutral cephalexin and negatively charged tetracycline (Supplemental Figure 1). The protection of encapsulated Bio-K+® against tobramycin is therefore likely due to the electrostatic interaction of anionic tobramycin and polycationic alginate.

To demonstrate the utility of the co-administration of tobramycin and encapsulated Bio-K+®, we evaluated this approach with bacteria that are relevant to chronic wounds. Chronic wounds pose clinical complications due an enhanced inflammatory state caused by a polymicrobial infections that lead to deficiencies in the normal healing process.[34] While it takes normal wound healing two to four weeks to close a wound, chronic wounds fail to restore skin integrity over a period of 3 months.[35] The two most widely implicated bacteria in chronic wounds are *Staphylococcus aureus* (SA) and PA.[36] *Methicillin-resistant Staphylococcus aureus* (MRSA), a multi-resistant strain of SA, represents a particularly difficult treatment target. Additionally, MRSA and PA are also known to form biofilms that lower antibiotic efficacy, impair wound healing, and are insusceptible to different classes of antibiotics.[37] We therefore chose the co-culture of MRSA and PA as our model system. The predicted interdependencies of the pathogenic bacteria (MRSA and PA), probiotic (Bio-K+®), and antibiotic (tobramycin) are as follows: Bio-K+® has been shown to have antimicrobial effects on MRSA both *in vitro* and *in vivo*, an ability that is attributed to the production of organic acids, bacteriocins and biosurfactants.[38], [39], [40] In contrast to conventional antibiotics, bacteriocins are not affected by the diffusion barrier of the biofilm and can penetrate through the extracellular polymeric matrix to reach their targets.[41] Furthermore, the combination of bacteriocins and antibiotics has a synergic effect against multi-drug resistant pathogens, such as MRSA, and it is more effective in the eradication of biofilms.[42] Biosurfactants derived from lactic acid bacteria have antiadhesive properties to combat the colonization of pathogenic microorganisms and the adhesion of biofilms.[43], [44] Hence probiotics not only target pathogens in planktonic but also in biofilm state.

Bio-K+® has no antimicrobial effect on gram-negative bacteria, such as PA.[40] Tobramycin is effective on PA and Bio-K+® but has no effect on MRSA. Therefore, the protection of Bio-K+® from tobramycin is needed for successful eradication of both pathogens (Figure 3a).

As a proof of concept, free or encapsulated Bio-K+® with or without tobramycin was introduced to a co-culture of planktonic MRSA and PA. In the absence of tobramycin, both free and encapsulated Bio-K+® resulted in the elimination of MRSA and persistence of PA. Encapsulation of Bio-K+® did not measurably interfere with its antimicrobial efficacy towards MRSA. The antimicrobial agents secreted by Bio-K+® into the surrounding had little interference from the alginate beads.[45], [46] In the presence of tobramycin, free Bio-K+® had no antimicrobial effect on MRSA, while PA was completely eradicated (Figure 3b). This demonstrates the antibiotic susceptibility of Bio-K+® and PA to tobramycin, as well as MRSA's resistance (Supplemental Table 1). The alginate encapsulation method discussed above was used to protect Bio-K+® from tobramycin and allows for the preservation of Bio-K+®'s antimicrobial properties towards MRSA. The addition of encapsulated Bio-K+® and tobramycin resulted in the complete elimination of both MRSA and PA. Combinational treatment resulted in no detectable colonies on antibiotic- and probiotic-free agar plates. This result supports other reports that have demonstrated the ability of lactobacilli to prevent MRSA and PA biofilm formation.[44], [47], [48] This could be valuable approach for limiting pathogen colonization and biofilm formation in chronic wounds.

In conclusion, this work demonstrates that the co-administration of probiotics and antibiotics through biofilm-inspired encapsulation offers a promising therapeutic route for treating complex infections and overcoming AMR. The encapsulation of probiotics with alginate uses easily scalable and well-established techniques with high biocompatibility. Alginate's history of use for the

treatment of chronic wounds and its ability to absorb wound exudate and promote healing also makes it an attractive material to use in this capacity.[49] The efficacy of this approach was demonstrated *in vitro* where the planktonic form of two highly pathogenic bacteria (MRSA and PA) were completely eradicated. Future work will focus on establishing a biofilm model and studying the efficacy of this system in increasingly complex infections. We would also like to explore the incorporation of probiotics directly into wound dressings as well as assessing this approach's potential in other relevant applications, such as the co-administration of oral antibiotics and probiotics to prevent antibiotic-related diarrhea. Alginate encapsulation can be further modified by blending in smart bioresponsive polymers to allow environment specific drug release.[50] This work also lays the foundation for designing temporary modifications to bacteria through material encapsulation.

Experimental Section

Bacteria culture: Bio-K+® capsules containing *L. acidophilus CL1285®, L. casei LBC80R® and L. rhamnosus CLR2®* were commercially purchased (Laval, Canada) and grown at 37 °C in Difco lactobacilli MRS broth or on Difco lactobacilli MRS agar (Becton Dickinson, Franklin Lakes, New Jersey). *Methicillin-resistant Staphylococcus aureus* (ATCC #43300, Manassas, Virginia) and *Pseudomonas aeruginosa* (ATCC #27853, Manassas, Virginia) were grown at 37 °C in BBL brain heart infusion broth or on selective BBL mannitol salt agar and selective BBL cetrimide agar (Becton Dickinson, Franklin Lakes, New Jersey) respectively. *Bacillus coagulans* (ATCC #7050, Manassas, Virginia) were grown at 37°C in Difco nutrient broth or on Difco nutrient agar (VWR, Radnor, Pennsylvania). All bacteria strains were stored in 25% glycerol, 25% water, and 50% broth at -80 °C

while in the exponential phase prior to use. To track the bacterial growth, optical density of the bacteria culture was measured at 600 nm using a SpectraMax Plus 384 Microplate Reader.

Antibiotic susceptibility assay: The broth microdilution method[51] was performed for all bacteria strains to determine the minimal inhibitory concentration (MIC) of tobramycin (Sigma-Adrich, St. Louis, Missouri).

Alginate encapsulation: Bio-K+® was grown to OD 4.9. The bacteria pellet was isolated from the broth solution after centrifugation and resuspended in PBS (1 ml) before being added to 2.5% (w/v) sodium alginate (W201502 ALDRICH) in a 1:50 bacteria to alginate ratio. The mixture was thoroughly vortexed to create a homogenous solution that was then added to a 5-ml syringe. The syringe was attached to a 31-gauge needle that was connected to a voltage generator by an alligator clip, and 5 kV was applied across the needle. Using a syringe pump, the bacteria-alginate solution was introduced as droplets at 500 µl/min into a 0.1 M calcium chloride ($CaCl_2$) bath, which cross-linked the bacteria-alginate solution into small beads. The small alginate beads (1 ml) was then collected in a cell strainer of 40 µm pore size (VWR), washed with Millipore water and added to (3 ml) 2.5% (w/v) alginate. After vortexing, the bead-alginate solution was pipetted into the 0.1 M $CaCl_2$ bath with a 1000-µl pipette to form large beads with the small being incorporated within. The large beads were also collected in the cell strainer of 40 µm pore size and kept suspended in $CaCl_2$ solution until incubation. To visualize the bacteria within the small bead, Bio-K+® was stained using a LIVE/Dead BacLight bacterial viability kit (L7012, Invitrogen) prior to encapsulation.

Alginate bead dissolution: Alginate beads were dissolved in a 55 mM sodium citrate dihydrate solution (Sigma Aldrich) and shaken on an orbital shaker at 300 rpm for 15 min. Bacteria

were isolated after centrifugation (4000 rcf, 5 min) and resuspended in PBS (1 ml). Bacteria were plated on the appropriate agar plate using the drop plate method and counted the next day. The serial dilution with number of visible colonies around 20-200 was used to calculate back the CFU/ml in the initial solution.

AlamarBlue assay: AlamarBlue (Bio-Rad) (15 µl) was added to sample (150 µl) placed in a 96-well black Corning plate, followed by a 1 h incubation at 37°C. Fluorescence signal was read with the Plate reader Tecan safire at 530 nm/590 nm excitation/emission.

Antibiotic removal through alginate: MRS broth (8 ml) with 256 mg/L tobramycin was incubated with empty cross-linked alginate beads (3 ml) (2.5%) for 24 h at 37°C. After incubation, 10^7 CFU/ml Bio-K+® was added to the alginate-treated tobramycin solution as well as the control tobramycin solution without alginate. Bio-K+® was incubated for another 24 h and plated accordingly.

Co-incubation of probiotics with pathogens: Free or encapsulated Bio-K+® was incubated in MRS broth (8 ml) with or without tobramycin (256 mg/L) in a 6-well plate. MRSA (OD 4.0) and PA (OD 3.1) were grown in BBL brain heart infusion broth, from which (13 ml) was collected, centrifuged and resuspended with PBS (10 ml). For co-incubation (5 ml) from each was added. The 6-well plate was sealed with parafilm and incubated for 24 h at 37 °C on an orbital shaker at 100 rpm. The solution containing the pathogens was centrifuged and resuspended with PBS (1 ml). The plating and counting of pathogens was performed in the same way as for Bio-K+® except that MRSA was grown on selective BBL mannitol salt agar and PA on selective BBL cetrimide agar.

Fluorescence labeling of alginate: Alginate was fluorescently labeled with the fluorochrome Alexa Fluor 568 hydrazide (Life technologies corporation DBA Invitrogen). Alginate was dissolved in PBS to give approximately 60 mM carboxylic groups. EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, Sigma) and Sulfo-NHS (N-hydroxysulfosuccinimide sodium salt, Fluka) were added at 6 mM each. Alexa Fluor 568 hydrazide was added at a concentration of 0.12 mM to the alginate solution, and the reaction mixture was stirred at room temperature for 18 h. The solution was transferred to an ultrafiltration tube with an Amicon Ultra Centrifugal Filter (3 kDa MWCO) and underwent several centrifugations and washing steps until the filtrate was free of non-conjugated fluorochrome.

Tobramycin conjugation: Tobramycin cyanine5 (Tob-Cy5) was synthesized by reacting tobramycin (3.2 mg) with cyanine5 NHS ester (Cy5, Lumiprobe, Maryland) (5 mg) in DMSO overnight at room temperature. The ratio tobramycin:Cy5 was 1:1.2. Triethylamine was used to adjust the pH to 9.0. The reaction mixture was dialyzed through a 1 kDa MWCO dialysis membrane to exchange DMSO against water, followed by separation on a C-18 reverse phase column by HPLC (Gilson GX-271). Separation was achieved using a gradient of acetonitrile/water where the acetonitrile content was increased from 0% to 80% over 63 min. The UV monitor was set at 210 nm and 280 nm. The separated products were verified for their molecular weight using Matrix Assisted Laser Desorption/Ionization (MALDI). The pure Tob-Cy5 product was isolated and lyophilized.

Diffusion experiment: Conjugated Tob-Cy5 and native Cy5 were adjusted to the concentration of tobramycin (256 mg/L) used in the experiment by applying the Beer-lambert law. Empty large alginate beads were incubated in a solution of Tob-Cy5 or Cy5 at 37 °C for 15 min, 1 h or

24 h. A Nikon Spinning-disk Confocal Microscope with TIRF Module was used to image the beads. All images were taken with an Apo 4x, NA 0.2 Nikon objective, with the same z step of 25 µm.

SEM: Glass coverslips were coated with poly-L-lysine by incubating them in poly-L-lysine solution (Ted Pella Inc., MW = 150,000 – 300,000 Da) for 5 min at room temperature, followed by drying at room temperature for 1 h. On the corners of the coverslip 10 µm of a Bio-K+® culture was loaded and left there for 1 h. Fixation of Bio-K+® was performed with a solution of 5% sucrose, 3% paraformaldehyde and 2% glutaraldehyde for 1 h at room temperature. The sample was stepwise dehydrated with 50%, 75%, 90%, 95%, and 100% ethanol followed by critical point drying. Images were taken with the FEI/Philips XL30 FEG ESEM.

Supporting Information

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

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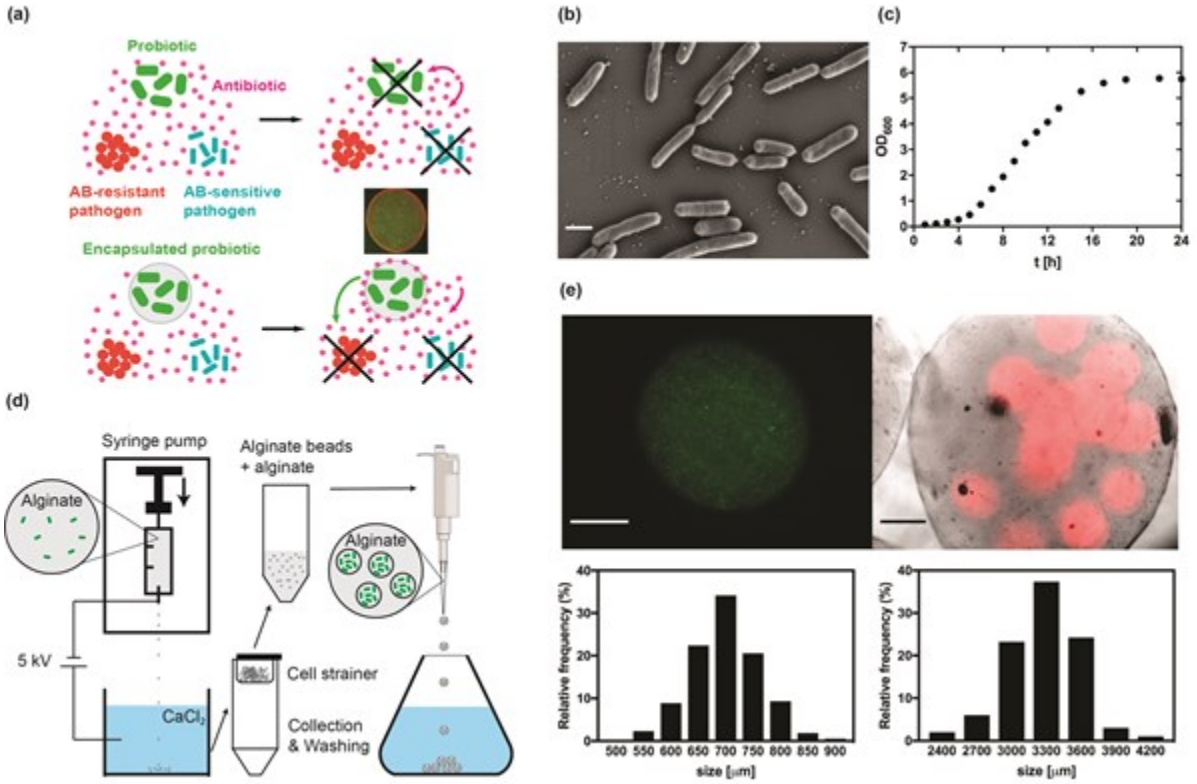

Figure 1. Characterization of Bio-K+® encapsulation. a) Scheme illustrating the benefit of antibiotic-probiotic co-administration in pathogen clearance. b) SEM of Bio-K+® (white bar = 1 µm). c) Growth curve of Bio-K+®. d) Fabrication schematic for a two-step alginate bead encapsulation. e) Fluorescence microscopy and size histograms of small (left, white bar = 200 µm) and large (right, black bar = 5 mm) alginate beads. Bio-K+® (green) labeled by Live/Dead BacLight staining is encapsulated inside the small beads. Small beads formed by fluorescently-conjugated alginate (red) are encapsulated inside the big bead.

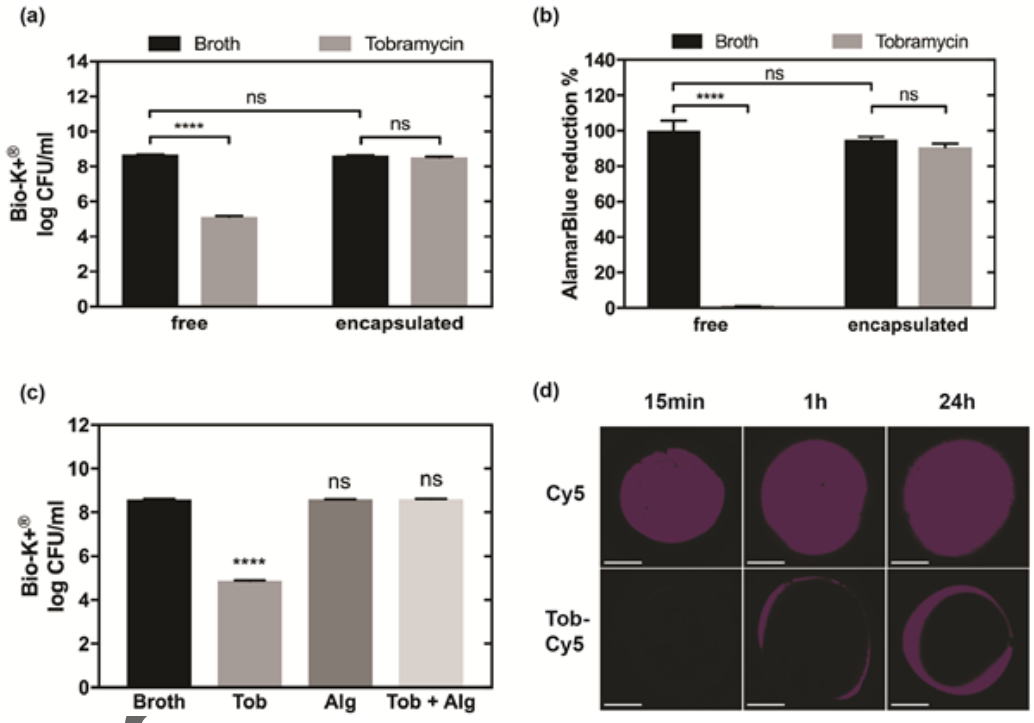

Figure 2. Alginate (Alg) encapsulation protects Bio-K+® against tobramycin (Tob). a) Effect of alginate encapsulation on CFU of Bio-K+® after incubation in broth and tobramycin. b) Effect of alginate encapsulation on metabolic activity of Bio-K+® after incubation in broth and tobramycin as quantified by AlamarBlue assay. c) Pretreatment of tobramycin-containing broth with empty alginate beads (Alg) nullifies the effect of tobramycin and does not affect bacteria growth. d) Fluorescence microscopy of alginate beads incubated with Cy5 or Tob-Cy5 during 15 min, 1 h and 24 h. (white bar = 10 mm) **** denotes statistical difference ($P < 0.0001$) using Student's t-test between broth and tobramycin groups

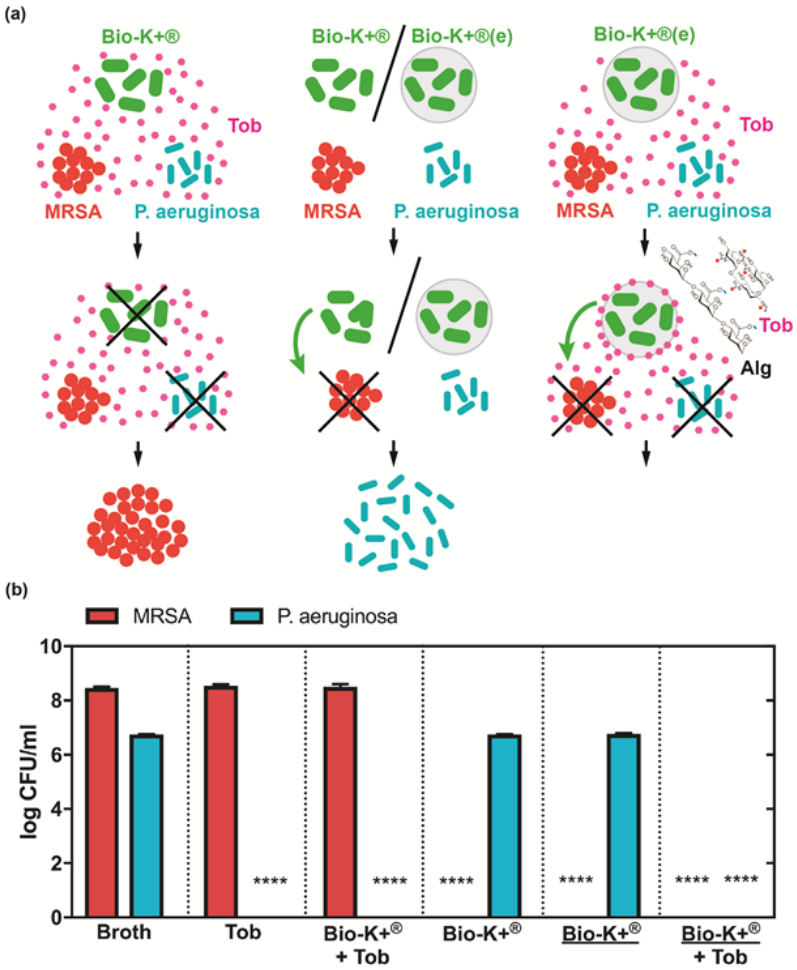

Figure 3. Encapsulated Bio-K+® (Bio-K+®) inside alginate (Alg) beads combined with tobramycin (Tob) achieves complete pathogen eradication. a) Depiction of co-incubation of Bio-K+® with MRSA and PA. b) MRSA and PA survival after co-incubation with Bio-K+® and tobramycin. **** denotes statistical difference ($P < 0.0001$) using Student's t-test between broth and tobramycin groups.

Bioflim-inspired encapsulation confers temporal antibiotic resistance to probiotics and allows for their co-administration with antibiotics. This approach offers a simple and promising therapeutic route for treating complex infections.

Keywords: probiotics, biofilms, chronic wounds, antimicrobial resistances, alginate encapsulations

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Biofilm-inspired encapsulation of probiotics for the treatment of complex infections