Building and Breaking the Cell Wall in Four Acts: A Kinesthetic and Tactile Role-Playing Exercise for Teaching Beta-Lactam Antibiotic Mechanism of Action and Resistance †

John Popovich1, Michelle Stephens1,2,‡, Holly Celaya1,2,‡, Serena Suwarno1,2,‡, Shizuka Barclay1,2,‡, Emily Yee1,2,‡, David A. Dean2,3,‡, Megan Farris1,2,‡, and Shelley E. Haydel1,2,4,*

1School of Life Sciences, Arizona State University, Tempe, AZ 85287; 2Barrett, The Honors College at Arizona State University, Tempe, AZ 85287; 3School of Molecular Sciences, Arizona State University, Tempe, AZ 85287; 4Biodesign Institute Center for Immunotherapy, Vaccines and Virotherapy, Arizona State University, Tempe, AZ 85287

“Building and breaking the cell wall” is designed to review the bacterial cell envelope, previously learned in lower-division biology classes, while introducing new topics such as antibiotics and bacterial antibiotic resistance mechanisms. We developed a kinesthetic and tactile modeling activity where students act as cellular components and construct the cell wall. In the first two acts, students model a portion of the gram-positive bacterial cell envelope and then demonstrate in detail how the peptidoglycan is formed. Act III involves student demonstration of the addition of β-lactam antibiotics to the environment and how they inhibit the formation of peptidoglycan, thereby preventing bacterial replication. Using Staphylococcus aureus as a model for gram-positive bacteria, students finish the activity (Act IV) by acting out how S. aureus often becomes resistant to β-lactam antibiotics. A high level of student engagement was observed, and the activity received positive feedback. In an assessment administered prior to and two months after the activity, significant improvements in scores were observed (p < 0.0001), demonstrating increased understanding and retention. This activity allows students to (i) visualize, role play, and kinesthetically “build” the cell envelope and form the peptidoglycan layer, (ii) understand the mechanism of action for β-lactam antibiotics, as well as how gene acquisition and protein changes result in resistance, and (iii) work cooperatively and actively to promote long-term retention of the subject material.

INTRODUCTION

Many upper-division microbiology students have difficulty understanding the fundamental microbiological concept of how cell components work together to form a functioning organism (1). Traditional lesson plans that teach microbial cell complexity can often be challenging for students to visualize and are less effective for retention of information than having students see and act out the processes (2–6). While learning new concepts, such as the mechanism of action of antibiotics, active learning activities allow students to engage in an interactive process of knowledge construction by building on ideas posed by other participants (7). Despite the common idea that individuals learn better with their preferred teaching methods, effective learning occurs despite personal preferences or beliefs (8, 9). Incorporating role play and active learning to teach difficult concepts improves student understanding, enhances learning gains, and allows students to immediately apply the content as decision-making participants (10–12).

We present a kinesthetic and tactile learning activity designed to allow students to visualize and participate in building the structure of the cell envelope and ascertain its function. The ultimate goal of the activity is to promote long-term retention of the concepts associated with bacterial cell structure, function, β-lactam activity, and mechanisms of β-lactam resistance. Students will be able to build and rely on their own mental images, allowing them to understand the concepts and apply them in later study.

The activity was performed in four acts, with approximately 30 students, during a 45-minute session (Table 1), but instructors can adjust the lesson to fit their audience by changing the number of characters in each act or by selecting only one or two acts during instruction. The first two sections

†Supplemental materials available at http://asmscience.org/jmbe

‡These authors contributed equally to the manuscript.
of this activity focused on understanding the cell membrane and cell wall, while emphasizing peptidoglycan structure and formation. Although the majority of the information in the first half of the activity was remedial in that the students had been exposed to the material in an introductory microbiology course, prior experience revealed that most students will struggle with recalling the information. These acts served as a foundation for the second half of the activity, where new material was introduced. Act III focused on the mechanism of action of \( \beta \)-lactam antibiotics, their ability to inhibit the function of transpeptidases, and the antibacterial effect of \( \beta \)-lactam-mediated transpeptidase inhibition. Act IV demonstrated how some bacteria, such as methicillin-resistant \textit{Staphylococcus aureus}, become resistant to \( \beta \)-lactam antibiotics by acquiring and expressing structurally-altered transpeptidases, which penicillin cannot bind.

The instructor-led scaffolding questions provided students with opportunities to formulate ideas together. This kinesthetic and tactile exercise offered a clear, large-scale visualization of processes within the bacterial envelope, encouraged cooperation and interaction with other students, and provided a change of pace from traditional lecture-style learning. This activity was performed over many semesters, and students often expressed positive feedback about the experience. Testing for improvements in the understanding of fundamental concepts after the activity determined its efficacy.

### Intended audience

Intended audiences for this activity are life science majors and any other students who require an understanding of bacterial cell structure, antibiotic mechanisms of action, and antibiotic resistance. This kinesthetic and tactile active learning exercise is suitable for any college-level course (lower or upper division) and can be modified to suit the specific needs of the class. We conducted the activity at a large research university in a course entitled Medical Bacteriology, which is an upper-division microbiology course primarily composed of juniors, seniors, and post-baccalaureate students majoring in microbiology, molecular biosciences and biotechnology, biological sciences, biochemistry, or health-related disciplines.

### Learning time

This lesson can be structured to fit within a single class period of 50 to 75 minutes, depending on the needs of the instructor and students. The lesson requires 10 to 15 minutes to administer the pre-assessment quiz (Appendix 1) and for the mini-lecture and discussion (Appendix 2), plus 35 to
55 minutes for performing Acts I to IV with discussions and explanations. Table 1 contains a teaching timeline.

Prerequisite student knowledge

Students are required to complete a pre-lecture reading assignment of Chapter 1 Structure and Function of Bacteria in the course textbook (13). The information presented in the chapter is considered remedial, as all students had completed an introductory microbiology course. Although we expected students to know most of the information in Acts I and II, their knowledge was limited. This activity can be performed with any students who have an understanding of basic microbiology (e.g., bacterial cell structure and function) and biochemistry (e.g., peptide and glycosidic bonds, enzyme function).

Learning objectives

Students will be able to:

1. Recall the structural similarities and differences between gram-negative and gram-positive bacteria
2. Discuss the molecular subunits of peptidoglycan and how enzymes link the molecules
3. Explain how the linked structure of peptidoglycan provides mechanical strength
4. Demonstrate how β-lactam antibiotics inhibit transpeptidases (penicillin-binding proteins, PBP), preventing the formation of the peptidoglycan cell wall
5. Explain how methicillin resistance is genetically acquired by Staphylococcus aureus
6. Demonstrate how β-lactam antibiotics are unable to bind to alternate transpeptidases (PBP2a) due to a modified (or altered) active site

PROCEDURE

Materials

This kinesthetic and tactile activity primarily involves teacher-led discussion and student role play, so materials are minimal. Images of required props are shown in Appendix 3. The props needed are labeled paper signs to distinguish cell parts; yarn or string to hang the paper signs around necks; and labeled, plastic, hollow balls (or similar) to serve as the antibiotics. A textbook to prepare students and instructors for the activity might be needed. We used chapter one of a Clinical Bacteriology textbook (13) as a pre-activity reading assignment.

Student instructions

Prior to class, students were expected to have read a textbook chapter on bacterial cell structure and function (13). The information presented was remedial, as students had completed an introductory level microbiology course. When class began, students were administered a pre-activity assessment quiz (Appendix 1), followed by a short, in-class presentation which included questions (Appendix 2) for engaging students and generating an interactive learning environment. At the end of the presentation, students moved to the location of the activity. We chose to perform the activity outside due to a lack of appropriate space in the classroom (Appendix 3). The instructor asked for volunteers to “play” certain molecules or proteins in the cell envelope, and students worked together to form different parts of the cell. During the activity, all students were encouraged to participate by engaging in role play, volunteering answers to questions, or helping to arrange cell parts.

Faculty instructions

Instructors should have foundational knowledge of general microbiology, bacterial cell structure, antibiotic mechanisms of action, and antibiotic resistance. Additionally, instructors should be comfortable with engaging in kinesthetic, tactile, role-playing activities with a large group of students and be responsive to an interactive, learner-centered environment. While student involvement in the activity is critical, there is a strong reliance on the instructor to lead the activity, as students receive no written instructions. The teaching timeline is outlined in Table 1. A detailed description of instructor-led questions and acceptable answers is included in Appendix 4, which also includes some common areas where students might get confused and tips to help students recall details.

Suggestions for determining student learning

An assessment quiz (Appendix 1) was given at the beginning of class (prior to mini-lecture and in-class discussion) and eight weeks after the activity. Pre-assessment quizzes were not returned to students, and students were unaware of the post-assessment quiz to be administered eight weeks later. Comparisons of the pre- and post-assessment quiz results can be examined by question and by overall score to determine retention of the material. Each assessment quiz questions assessed a student learning objective (SLO) (Table 2). In addition, the midterm exam included three multiple-choice questions that related to the activity (Table 3). Student performance on these questions can be used to determine the efficacy of the activity.

Sample data

This activity is usually performed in the first week of class, meaning students may be unfamiliar with each other and are often hesitant to volunteer answers at the start of the activity. An engaging and entertaining instructor, who offers encouragement for students to discuss amongst themselves before answering, will help alleviate student anxiety and enhance the cooperative learning environment as the activity
progresses. Students responded positively to the activity and consistently reported more effective learning through this kinesthetic and tactile active learning exercise. Often, the most time-consuming aspect involves the movement of students into the correct position prior to proceeding through each act. To promote a student-centered learning environment, instructors should encourage students to work together to position themselves and provide guidance and feedback when necessary. Appendix 5 includes photographic representations of appropriate student assembly.

**Safety issues**

There are no safety issues with this activity. Assessment of this activity was performed in accordance with Arizona State University Institutional Review Board (IRB) approval (STUDY00002936).

**DISCUSSION**

**Field testing**

This activity was performed in an upper-division microbiology class, often consisting of 30 to 45 students, during the fall semesters of 2015, 2016, and 2017. A prerequisite for this class was an introductory microbiology course, so students had previously been exposed to the material. In the fall 2017 semester, 27 participating students completed an anonymous student satisfaction survey immediately following administration of the post-assessment quiz. A significant

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### TABLE 2.
Assessment quiz scores and learning gains.

| Question | Percent Correct<sup>a</sup> | Percent Correct<sup>a</sup> | P Value | SLO Assessed |
|----------|-----------------------------|-----------------------------|---------|--------------|
|          | Pre-Assessment              | Post-Assessment              |         |              |
| 1        | 67                          | 88                          | 0.00030 | 1            |
| 2        | 92                          | 94                          | 0.62    | 1            |
| 3A       | 5.8                         | 9.9                         | 0.27    | 1            |
| 3B       | 8.7                         | 74                          | <0.0001 | 2            |
| 3C       | 8.7                         | 75                          | <0.0001 | 2            |
| 3D       | 66                          | 67                          | 0.88    | 1            |
| 4        | 78                          | 83                          | 0.36    | 1            |
| 5        | 41                          | 48                          | 0.38    | 3            |
| 6        | 77                          | 93                          | 0.0011  | 4            |
| 7        | 29                          | 69                          | <0.0001 | 5.6          |
| Total Points | 4.7                          | 7.0                          | <0.0001 | All          |

<sup>a</sup>Values include results from fall 2015, 2016, and 2017 semesters.

### TABLE 3.
Scores on mid-term multiple-choice exam questions related to the activity.

| Question<sup>a</sup> | Answer (Other Selected Answers) | Correct<sup>b</sup> (%) | SLO Assessed |
|-----------------------|----------------------------------|--------------------------|--------------|
| A bacterial cell contains a unique bond between N-acetylmuramic molecules in the cell wall. Which of these normally functioning enzymes must be altered to maintain cell wall rigidity? | Transpeptidases (catalases; hyaluronidases; acetyl transferases) | 102/106 (96.2%) | 2 |
| Based on the strain of bacteria in [previous question], which of the following might be compromised, altered, or nonfunctional? | Penicillin (protein secretion; protein synthesis; aminoglycosides; DNA replication) | 85/106 (80.2%) | 4 |
| A disk susceptibility test is performed on this bacterium, and large zones of inhibition are found around vancomycin and cephalosporin. Based on this information, you assume that the cell has… | Thick layers of N-acetylmuramic acid and N-acetylglucosamine (a lipopolysaccharide outer membrane; a capsule; no proteins present in cell membrane) | 90/106 (84.9%) | 1 |

<sup>a</sup>Complete questions from fall 2015, 2016, and 2017 semesters are included in Appendix 7.

<sup>b</sup>Values include results from fall 2015, 2016, and 2017 semesters.
level of satisfaction \( (p = 0.0091) \) was associated with the activity, indicating that the positive feedback and perceived activity learning gains were not due to random variation (Fig. 1). All students agreed that the activity should be implemented next year, and over 90% of students reported that they enjoyed the activity (Fig. 1). Student-based criticisms of this activity included distractions due to performing the activity outside, lack of a handout that contained important terms or questions, and lack of participation by some students.

### Evidence of student learning

Students showed significant improvement and learning gains on the identical post-assessment quiz that was administered eight (fall 2015, 2016) or nine (fall 2017) weeks after the date of the activity (Fig. 2A). On the pre-assessment quiz, students scored an average of 4.6 out of 10 points, while the average of 6.9 points on the post-assessment quiz was significantly higher \( (p < 0.0001) \) (Fig. 2B). It is important to note that the students did not have prior knowledge of the assessment quizzes or the specifics of the active learning exercise. They also did not receive feedback from the pre-assessment quiz before they took the post-assessment quiz. Furthermore, the post-assessment quiz average was 6.8 points when the student co-author scores were eliminated (Fig. 2), indicating that the student co-author results did not skew the data analysis. When the percentage of correct answers was assessed for each question, post-assessment quiz scores significantly improved for questions 1, 3B, 3C, 6, and 7 (Fig. 3, Appendix 1). Pre-assessment scores for questions 2 and 4 were 90.9% and 76.6%, respectively (Fig. 3), indicating that the majority of students retained previously-acquired information related to differences between prokaryotic and eukaryotic cells and structural similarities and differences between gram-negative and gram-positive bacteria.

In addition to the assessment, a midterm exam was given to students three and a half weeks (fall 2015) or three weeks (fall 2016, 2017) after the initial activity was performed. Three multiple-choice questions on the exam were directly related to the activity (Appendix 7). Table 3 shows a paraphrased version of the questions that were asked, the answers chosen by students, the number of students who answered each question correctly, and the SLO assessed by each question. The average overall exam scores were 83.1%, 81.1%, and 85.4% in the fall 2015, 2016, and 2017 semesters, respectively. The percent correct for one of the questions was considerably higher (96.2%) than the exam average, and percent correct for two questions were similar to the exam averages (84.9% and 80.2%) (Table 3), revealing learning gains associated with activity-related content.

The quizzes and the three exam questions also assessed student retention of material addressed by the kinesthetic and tactile learning activity and the associated SLOs. Based on the pre- and post-quiz assessment results, students accomplished SLOs 2, 4, 5, and 6 with significant performance improvements and retention (Table 2). Moreover, more than 80% of students answered exam questions related to SLOs 1, 2, and 4 correctly (Table 3), indicating retention of material due to the activity. As a key component of undergraduate microbiology education, SLO 1 was tested extensively with varying levels of improvement (Tables 2 and 3). The results showed that although understanding is demonstrated, prior knowledge of material relating to this SLO may have skewed the results for quiz questions 2 and 4 (Table 2). While students had been introduced to lipid bilayers, lipid A, and lipopolysaccharides in previous courses and in the pre-reading assignment, no learning gains were evident with assessment quiz questions 3A and 3D (Table 2, Appendix 2). Students also did not show improvement on the question related to SLO 3 (Table 2), which was assessed with quiz question 5.

### FIGURE 1. Student satisfaction survey responses. Twenty-seven participating students rated their satisfaction of the activity and their self-reflected learning gains. Chi-squared analysis of grouped positive (strongly agree and agree) and grouped negative (neutral, disagree, and strongly disagree) responses revealed significant \( (p = 0.0091) \) satisfaction and perceived learning gains associated with the activity. For the complete survey questions, see Appendix 8.
FIGURE 2. Comparison of the pre-assessment (PRE) and post-assessment (POST) quiz scores, (A) separated by semester and (B) combined. Students were administered a quiz in a pre-/post-activity manner, with the average (±SEM) scores for each semester displayed. POSTa indicates that the post-assessment quiz scores from the student co-authors were eliminated from the data analysis. ****p < 0.0001; two-way ANOVA, Fisher’s LSD test.

FIGURE 3. Comparisons of individual questions in the pre- and post-assessments in (A) fall 2015, (B) fall 2016, (C) fall 2017, and (D) combined fall 2015, 2016, and 2017 semesters. Students were administered a quiz in a pre-/post-activity manner, with the average (±SEM) percentage for each question displayed. POSTa indicates that the post-assessments from the student co-authors were eliminated from the data analysis. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; two-way ANOVA, Fisher’s LSD test.
(Appendix 2). Despite being covered in previous courses, lysozyme enzymatic activity was not a major focus of the learning activity, so the failure of students to improve on this question may be explained by their lack of understanding of lysozyme function.

Possible modifications

If time allows after the activity, it may be helpful to have small, self-selected student groups discuss the exercise and complete an assessment that directs them to discuss the active learning experience and the SLOs. This assessment could provide insight on the efficacy of the activity for students who did not actively participate. While the majority of students enjoyed the exercise, some students provided feedback for instructor consideration. Adjusting the number of cast members proportionally based on class size would allow all students to actively participate, although this strategy may not be feasible with larger class sizes or shorter class instructional times. Instructors should consider where the activity will take place ahead of time and plan accordingly. In a traditional style lecture hall, students can stand between the rows, or the front of the room can be used as a stage. Otherwise, a large area with minimal disruptions is preferred.

SUPPLEMENTAL MATERIALS

Appendix 1: Pre- and post-assessment quizzes for fall 2015, 2016, and 2017
Appendix 2: Mini-lecture and discussion presentation
Appendix 3: Photos of required props and active learning classroom
Appendix 4: Detailed instructions for instructors
Appendix 5: Photo demonstration of the activity
Appendix 6: Schematic representation of the activity
Appendix 7: Complete exam questions
Appendix 8: Student satisfaction survey

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