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Dynamic Model Visualizing the Process of Viral Plaque Formation

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INTRODUCTION

Understanding the concept of viral plaque and the process of viral plaque formation is essential for navigating viral biology and grasping the foundations of key laboratory methods in virology. Plaque assays are utilized routinely in Microbiology and Virology teaching laboratories to quantify viruses and as a tool to introduce and/or reinforce the technique of serial dilutions (2). The hands-on model described here creates an opportunity for students to experience the process of viral plaque formation while engaging multiple senses and creating a lasting impression. After all, a loud pop in the classroom never fails to attract attention!

In Microbiology and Virology courses viral plaques are often presented to students as the way one can visualize viruses/bacteriophages. A viral plaque is defined as a physical entity: “a clear area on a lawn of bacteria or a monolayer of cells, where viruses have destroyed the cells” or functionally; as “the progeny of one virus” (3). Both definitions are received well by students and help them to grasp the idea that counting plaques is essentially the same as counting viruses in their sample (assuming that one virus entering the cell is sufficient for productive infection). In contrast, many times the process of plaque formation itself remains obscure. Many students fail to appreciate that viral plaques are actually a “laboratory-made” phenomenon allowing us to observe and study the growth of lytic viruses. (No plaques are found in nature or liquid culture!) The latter often presents a challenge for the interpretation of experimental data related to viral growth and drug discovery using plaque reduction assay.

PROCEDURE

To facilitate student understanding of the process of plaque formation, I have developed a dynamic model that draws students’ attention and promotes active discussion of various aspects of viral biology. Small balloons (water balloons are just the perfect size) are blown up and attached to a cork tile using push pins to build a bacterial lawn (Fig. 1(A)). Each balloon is modeled as a single cell and the cork tile is modeled as a Petri dish. The infection of the very first cell from the bacterial lawn is visualized by attaching a flag tag (in pink) to a cell/balloon of your choice (Fig. 1(B)). The infected cell is subsequently popped, clearing a small area of the cork tile and exposing push pins that were hidden under the popped balloon, thus visualizing the onset of plaque formation. The push pins are modeled as the progeny of the virus that initially infected the popped cell. Some of the newly propagated viruses infect neighboring cells (Fig. 1(C)) that are subsequently lysed. Over time, a larger area is cleared from bacterial growth, which we routinely perceive as a viral plaque. A snapshot of a growing plaque is depicted in panel D of Figure 1.

The modeling process is most effective when executed using a classroom overhead projection camera, allowing all students to follow the demonstration on the classroom screen. It is critical to pause after the first flag depicting a virus is attached to the host cell (Fig. 1(B)) and emphasize what biological processes are taking place. Once students hear the first balloon popping they tend to get super-excited and it becomes harder to review the relevant biology content in detail. It is important to stress that the viruses depicted by flags and push pins are genetically identical (unless a mutation took place), so that the two modes of presentation do not create misconceptions. This is an unavoidable flaw of the setup related to the physical properties of the used manipulatives. Instead of using flags, one can use a marker to label the infected cells; however, that process occasionally results in instant balloon-popping.

In my upper level Virology course, I use the described dynamic model together with the video of bacteriophage T4 “exploding” Escherichia coli in liquid culture available...
from the Cells Alive video library and the time-lapse video of Vaccinia virus plaque formation published by Doceul and colleagues in 2010 (1). That gives students the opportunity to visually contrast and compare the outcomes of viral cell lysis in suspension culture and a cell monolayer. One can consider the developed teaching model as a reenactment of the time-lapse video of plaque formation with the bonus feature of having a visual for the infecting viruses. While the Vaccinia virus video is fantastic, very few undergraduates have experience with mammalian tissue culture and the process of plaque formation is often perceived as movement of cells as opposed to cell lysis. Using the teaching model and the scientific videos in combination offer the students the best gateway for understanding the process of viral plaque formation. Throughout my course I refer to the teaching model when discussing viral evolution (mutant viruses can be modeled with a push pin or flag with different color), the principles of plaque reduction assay as a tool for drug discovery, and the importance of plaque purification in preparation of viral stocks.

Overall, the execution of the dynamic model and videos takes very little class time and is straightforward to incorporate in lecture or laboratory settings. Logistically, the model is easy to assemble, cost effective, and requires minimal storage space. The Vaccinia plaques video is freely available on the Virology blog run by Dr. Vincent Racaniello, Columbia University, as part of a very useful mini-article focused on the nature of viral plaques (www.virology.ws/2010/02/03/now-playing-viral-plaque-formation).

ACKNOWLEDGMENTS

The author declares that there are no conflicts of interest.

REFERENCES