Interdisciplinary Research Brought to School—Connecting Chemistry and Biology through Nanotechnology †

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INTRODUCTION

Nanoscience is a cutting-edge, highly interdisciplinary field of research. The experiment presented here is a good example of bringing attention to this interdisciplinary research at school. By synthesizing silver nanoparticles and subsequently testing their antimicrobial effect in a yeast culture, students gain insight into different research methods commonly used in nanoscience (for a brief background on nanosilver see Appendix 1).

From a biology education point of view, this experiment could prompt discussion of the structure and growth conditions of eukaryotic single-cell microorganisms like yeast, as well as their relevance for food production as part of human culture. In addition, students learn how to prepare simple nutrient media and how to work aseptically. From a chemistry education point of view, students should discuss the size relations of different silver-containing species (e.g., silver nanoparticles, silver atoms, and Ag⁺ ions), as well as the role of citrate in the synthesis of silver nanoparticles. On a more general level, the experiment may encourage students to discuss the risks and benefits of using nanotechnology, and particularly nanosilver (see, e.g., (1)).

The experiment is suitable for students in grade 9 and above. The teaching unit could be integrated into lessons in several ways. Ideally, teachers would use a jointly-taught chemistry and biology unit to highlight interdisciplinary work in current research by actually doing interdisciplinary work at school. It also could be taught as an integrated natural science subject or be part of a project on nanotechnology. Existing citrate environment, these clusters of silver atoms could prompt discussion of the structure and growth conditions of eukaryotic single-cell microorganisms like yeast, as well as their relevance for food production as part of human culture. In addition, students learn how to prepare simple nutrient media and how to work aseptically. From a chemistry education point of view, students should discuss the size relations of different silver-containing species (e.g., silver nanoparticles, silver atoms, and Ag⁺ ions), as well as the role of citrate in the synthesis of silver nanoparticles. On a more general level, the experiment may encourage students to discuss the risks and benefits of using nanotechnology, and particularly nanosilver (see, e.g., (1)).

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PROCEDURE

In the main part, the experiment follows a procedure proposed by Muskin et al. (2), but the second part was adapted by using fresh yeast from a commercial source instead of E. coli bacteria to avoid safety issues, as well as to make it easier to use in school. The teaching unit includes three parts. In the first part, students synthesize silver nanoparticles using a citrate process. Depending on age and skill level, this takes about 30 to 45 minutes. In the second part of the teaching unit, the students prepare a yeast culture to test the antimicrobial activity of the particles produced and compare their effectiveness with other antimicrobial agents, such as disinfectants (Fig. 1). The preparation of the nutrient medium and test setup takes about 60 minutes, and the incubation (and observation) time of the samples is about 72 hours at room temperature. The third part, the subsequent interpretation of test results, takes another 15 to 30 minutes.

Part I – Production of silver nanoparticles

The silver nanoparticles are synthesized following a standard procedure (3, 4) in which sodium citrate acts both as a reducing agent and as a stabilizer. Adding sodium citrate (Na₃C₆H₅O₇) to the silver ions (Ag⁺) reduces them to elementary silver, which is gathered into clusters. In the existing citrate environment, these clusters of silver atoms are surrounded by a shell of citrate ions, which prevents further growth of the clusters and stabilizes them. These silver atom clusters are called silver nanoparticles.

To synthesize the nanoparticles, students heat up a silver nitrate solution in a water bath and cook it for 10 minutes. Then, a few droplets of trisodium citrate solution are added, and the mixture is boiled for another ~15 minutes, until the solution becomes yellow in color, indicating the presence of nanoparticles. Subsequently, the mixture is cooled to room temperature. Different shades of the transparent yellow color indicate different amounts of citrate ions surrounding the silver clusters and hence the size of the silver nanoparticles. A detailed illustrated step-by-step manual is provided in Appendix 2, Part 1.
Part II – Preparation of the yeast solution and setup of the experiment

In the second part of the experiment, the antimicrobial effect of the silver nanoparticles is tested in a culture medium. As a reference sample, an antimicrobial detergent (disinfectant/chlorine cleaner) is applied as a positive sample, and sterile water is used as a negative sample.

About 24 hours prior to the lesson, the agar plates for the experiment need to be prepared by the teacher. In the lesson, students prepare the nutrient solution containing fresh yeast. This solution is plated on the agar plates. Small discs of filter paper are soaked with the different samples and positioned in the four sectors of the culture medium. See Figure 1 and the detailed instructions in Appendix 2, Part 2.

After the agar plates are closed and sealed, they are placed in a room-temperature location to be incubated for about 72 hours. Twice a day, the students photograph the plates and measure the diameter of the inhibiting zones, which become visible after about one day. This analysis could also be done using software tools, such as apps for measuring (e.g., “Photo Ruler ABC” for iOS, https://itunes.apple.com/us/app/photo-ruler-abc-measure-your-world/id645239737?mt=8 or “Camera Ruler” for Android, https://play.google.com/store/apps/details?id=com.zhenqian.cameralruler) or for creating time-lapse movies (see https://youtu.be/yttZZZxcG9w and setup described in Appendix 4).

Part III – Interpretation of results

The measurement of the inhibiting zones shows that the size of each zone is unchanged from the first time it becomes visible until the end of the experiment. This is due to the suppression of the yeast growth in the area of influence. The negative sample (water) should not have an inhibiting zone at all.

After students collect the data, the results for different samples and points in time could be merged and analyzed in an Excel sheet (see Fig. 2 and Appendix 3). This is a good opportunity to discuss the usual scientific process of taking several measurements on the same sample to obtain more reliable results.

SAFETY ISSUES

For standard laboratory safety procedures, the ASM BSL-1 guidelines (5) were used. These guidelines include training the teacher and students in handling BSL-1 organisms, conducting the experiments in a teaching laboratory, wearing nitrile gloves when needed, wearing safety glasses at all times, and disinfecting all surfaces before and after usage. The notes of the Nuffield Foundation (6) were applied for training in aseptic technique. After the experiment, the cultured plates should be sterilized using oven bags in a pressure cooker (7) or a baking oven (210°C, 3 h) and then put in the general trash.

CONCLUSION

This experiment was successfully conducted in several classes during the IRRESISTIBLE project. Although the growth process took place under different conditions depending on the school (time of year, weather, location), in almost all groups a decent result for the yeast growth was achievable, and for the positive and negative control sample, a clear result was visible. In a few schools, the result for the nanosilver samples was not very clear, which could be traced back to using lower grade or old educts for the synthesis of the nanosilver particles. Although some students complained about the amount of work to be done, others mentioned that it felt like a “real research project” due to its high complexity, sequential experiments, and data analysis. Some students considered the time-lapse video (which was done in a few groups only) to be a “cool result.” The focus of the IRRESISTIBLE teaching module Nano and Health is on Responsible Research and Innovation (RRI). However, even without the surrounding module, the experiment itself offers several points for starting a discussion on RRI, e.g., regulation and laws (RRI aspect “governance”) or the usefulness of getting hands-on experience in science (RRI aspect “science education”).

SUPPLEMENTAL MATERIALS

Appendix 1: Background on nanosilver
Appendix 2: Manuals for experiments
Appendix 3: Excel sheet for experiment analysis
Appendix 4: Building a simple video box for creating time-lapse videos
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