3D Printing of Human Microbiome Constituents to Understand Spatial Relationships & Shape Parameters in Bacteriology

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ABSTRACT

Effective laboratory and classroom demonstration of microbiome size and shape, diversity, and ecological relationships is hampered by a lack of high-resolution, easy-to-use, readily accessible physical or digital models for use in teaching. Three-dimensional (3D) representations are, overall, more effective in communicating visuospatial information, allowing for a better understanding of concepts not directly observable with the unaided eye. Published morphology descriptions and microscopy images were used as the basis for designing 3D digital models, scaled at 20,000x, using computer-aided design software (CAD) and generating printed models of bacteria on mass-market 3D printers. Sixteen models are presented, including rod-shaped, spiral, flask-like, vibroid, and filamentous bacteria as well as different arrangements of cocc. Identical model scaling enables direct comparison as well as design of a wide range of educational plans.

Key Words: 3D printing, microbiology education, science literacy, visual, tactile.

With the advances in omics research platforms applied to microbiological systems, a deepening of our understanding of the diversity and function of the microbiome in health and disease is occurring (Izard & Rivera, 2015). Though not as appreciated as phylogenetic and functional diversity, the microbiome comprises organisms of diverse sizes and shapes that affect its function (Young, 2006). Mycoplasma genitalium and Thiomargarita namibiensis, one of the smallest and the largest known bacteria, respectively, differ in size by three orders of magnitude. In addition to the most commonly known rod, coccus, and spiral types, bacteria also take dendroid, coryneform, cylindrical, bulbiform, fusiform, filamentous, star, disk, hourglass, lemon, pear, crescent, and flask shapes. Our conceptual interest in these characteristics for over three centuries is related to our conceptual understanding of microbiome dynamics (Dobell, 1932; Young, 2006).

Standard light microscopes, common in teaching laboratories, lack sufficient resolution for demonstrating visuospatial relationships in microbiology. High-resolution imaging solutions, such as confocal microscopy and electron microscopy, are not readily accessible for teaching or the public. Concerns for the safety of students and staff, and absence of level 2 and above biosafety laboratories for handling and maintaining biohazardous microbes, limit the diversity of microbes available for active learning.

Visual representations help demonstrate phenomena that cannot be observed or experienced directly. In particular, 3D visual representations are, overall, more effective at conveying visuospatial information. In teaching gross human or animal anatomy, when compared to all other methods, physical models are better for overall knowledge outcome, spatial knowledge acquisition, and long-term retention. In STEM, printed artifacts have been essential to instruction in diverse fields such as molecular structures in biochemistry, anatomy, crystal structure in chemistry, and marine biology specimens in zoology (for review, see Ford & Minshall, 2019).

A full set of bacteria for a microbiome can be provided only via a body site or an ecosystem approach. The diversity of the microbiome is one of the richest of any living systems (Izard & Rivera, 2015). We focused on providing a morphologically diverse set, including bacterial commensals and pathogens. This is the first microbial model set provided at the same scale that can be implemented in an existing lesson plan, or easily incorporated in novel approaches.

A scale of 20,000x was chosen through an iterative process evaluating the optimum size to (1) enhance visual and tactile aspects of printed-model handling and (2) enable comparison of size and shape within the mammalian microbiome, with a focus on the human microbiome. We took into account the resolution of available mass-market 3D printers to avoid hindering dissemination and enable multiple handlings of the models without breakage. This led to the exclusion, for example, of external flagella in the model of Helicobacter pylori, periplasmic flagella in treponemal species that modify the outer shape of the cell, as well as pil and limbs. The attachment organelle of Mycoplasma sp. was included because it is part of the general morphology. Texture rendering was considered. The lack of data for most organisms, as well as the lack of structure within the range of printing resolution when a data set was available via atomic force microscopy experiments, led to the decision not to add texture to the models. An exception was made for the
Lactobacillus casei model, where users experience what bacterial texture can look and feel like.

The computer-aided design (CAD) was performed using FreeCAD version 0.17 (open source FreeCAD project) or Autocad Fusion360 version 2.03803 (Autodesk, San Raphael, CA). The models were generated on the basis of published descriptions or published microscopy images, using ImageJ version 1.51 for measurement (Supplemental Table 1; see Supplemental Material with the online version of this article). During the design process, a number was included on the surface of the model for future identification when mixed with other cells. Prior to printing, the numbered and unnumbered models were exported as stereolithography (.stl or STL) files (additional files 1–32 in Supplemental Material). The printing compatibility test has been performed on different platforms, such as Stratasys Mojo Desktop 3D (Stratasys, Eden Prairie, MN), Ultimaker 3 Extended (Ultimaker, The Netherlands), and Formlabs Form 2 (Formlabs, Somerville, MA) printers. All models were scaled at 20,000× for printing. To reduce the amount of printing material, multiple prints were made at the same time, and/or infill density was lowered. To avoid model collapse while printing, the infill density was set between 50% and 70%, based on model and printer characteristics.

In the case of printing with the Formlabs Form 2 system, the STL file was first imported into PreForm version 2.18 software (Formlabs, Somerville, MA). Support structures were automatically generated and included in model designs to prevent deformation of models. A 0.7 mm support-structure touchpoint size was used for all the models except Mycoplasma pneumoniae. In this case, a 0.4 mm touchpoint size was used to prevent breaking of the model during support separation. Models were fabricated using photopolymer resins (Formlabs) at 0.05 mm resolution. Printed models were first submerged in isopropanol for two 10-minute cycles to remove uncured resin, and then hardened in a Form Cure oven (Formlabs) for 30 minutes at 60°C. Support structures were separated from the models with pliers. Remaining stubble from the supports, if any, was filed and sanded by hand using precision files and 320 grit sandpaper.

The chosen panel included pathogens, commensals, disappearing bacteria, and beneficial organisms. A total of 16 printed models representing 14 different species of bacteria were generated and scaled congruently (Supplemental Table 1 and Supplemental Figure 1). Distinct species were included for a subset of the shapes: Escherichia coli and Lactobacillus casei for bacilli; Helicobacter pylori, Treponema denticola, and T. succinifaciens for spiral-shaped; and three species of streptococci (Streptococcus salivarius, S. pneumoniae, and S. pyogenes), Aerococcus urinae, and Sarcina ventriculi for cocci. Additional shapes include filamentous (Simonsiella muelleri), flask-like cells (Mycoplasma sp.), and vibrioid (Vibrio cholerae). Two disappearing bacteria were included: Helicobacter pylori, associated with a chronic infection of the gastric mucosa that might lead to ulcer and cancer, and T. succinifaciens, now present only in traditional societies, are seeing their prevalence affected by antibiotic use and changes in lifestyle.

The students welcomed a tactile and practical demonstration of the scale and shape concepts (Figure 1). Student feedback on the lesson included “The models make the confocal data on biofilms more real,” “They illustrate that immense shape differences occur despite the minuscule size,” and “It gives a good perception of actual microbial range of scale within a body site.” The teaching objectives were to make tangible the learned concepts on biofilm cell organization, microbial diversity within a biological niche, and the effect of scale in mixed biofilms.

The models presented here are intended for comparative analysis at the same scale. However, our models can be scaled differently, modified to include finer structural details (both inside and outside of the cells), or be incorporated in more complex representations such as polymicrobial systems and biofilms. Models of bacteria available in online resources can be resized to enable similar comparison and analysis.

## Supplemental Material

- **Supplemental Table S1.** Description of shapes and dimensions of bacteria models with associated references.
- **Supplemental Figure S1.** CAD 3D models of bacteria with different morphologies.
- **Supplemental Data.** Thirty-two stereolithography (.stl) files representing the 16 generated models, both numbered and unnumbered.

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## References

Dobell, C. (1932). The first observations on entozoic protozoa and bacteria. In Anthony van Leeuwenhoek and His “Little Animals” (pp. 217–256). New York, NY: Dover.
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