

Brief Report

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Brief Report

Possible Rethink of the Bacterial Peptidoglycan Layer as a Non-Porous Layer Rather Than a Perforated Mesh

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Abstract

In Gram-negative bacteria, the peptidoglycan layer is often thought to be a thin layer (even monolayer) that is in-between the two phospholipid bilayer of the bacterial double membrane cell envelope. However, preliminary evidence from scanning electron microscopy observations of the shape and morphology of *Escherichia coli* DH5 α cells subjected to autoclave treatment at 121 °C for 20 minutes suggests that the peptidoglycan layer may be thicker, and it may be a non-porous layer rather than a perforated mesh. Specifically, the traditional view of the *E. coli* peptidoglycan layer as a thin or monolayer layer punctuated with ion channels and transporter does not explain the ability of *E. coli* cells to retain its cellular structure after autoclave treatment. On the other hand, the new proposed model of a thicker peptidoglycan layer which is non-porous may help explain the observation of intact *E. coli* cells (without any holes) after autoclave treatment. Future work may examine the compressive and tensile strength of *E. coli* cells both before and after autoclave treatment to gain a deeper understanding of its mechanical properties as well as draw inference on the structure of the peptidoglycan layer.

Keywords: *Escherichia coli*; peptidoglycan layer; non-porous layer; perforated mesh; monolayer

Subject areas: biochemistry; microbiology; biophysics; cell biology; mechanical engineering

Autoclave is the standard and reliable way for killing bacterial cells. Typically operated at elevated temperatures of 121 °C for 15 to 20 minutes, it is validated to kill most of the microbial cells in the sample. This work describes an accidental observation that *Escherichia coli* DH5 α cells treated with standard autoclave decontamination still retain an intact morphological structure, and crucially, with no holes in the cell wall as examined by scanning electron microscopy.

In terms of sample preparation, the cell sample was taken from a 15 hour activated *E. coli* culture of 100 mL volume in a 250 mL conical shake flask with aerobic cultivation at 230 rpm shaking, that has undergone autoclave decontamination at 121 °C for 20 minutes. After autoclave treatment, the residual liquid was decanted off, and the remaining cell sample was put into a 15 mL polypropylene sample tube, and the sample was freeze dried for 24 hours to remove the residual liquid from the cell sample. The autoclaved freeze dried cells were then coated with platinum on a platinum sputter for 30 seconds, and viewed under a JEOL 5600 LV scanning electron microscope with 15 kV accelerating voltage.

Figure 1 shows a series of scanning electron micrographs of *E. coli* DH5 α of increasing magnification showing *E. coli* cells with no visible holes after autoclave treatment. In addition, the *E. coli* cells still retain their rod-shaped structure with smooth cell wall and surface. Hence, this contradicts conventional notion that autoclave treatment will cause cell wall breakage and puncture due to the high pressure environment in autoclave sterilisation.[1] Such a result suggests that the peptidoglycan layer in between *E. coli* double membrane cell wall is able to sustain a higher pressure than anticipated.

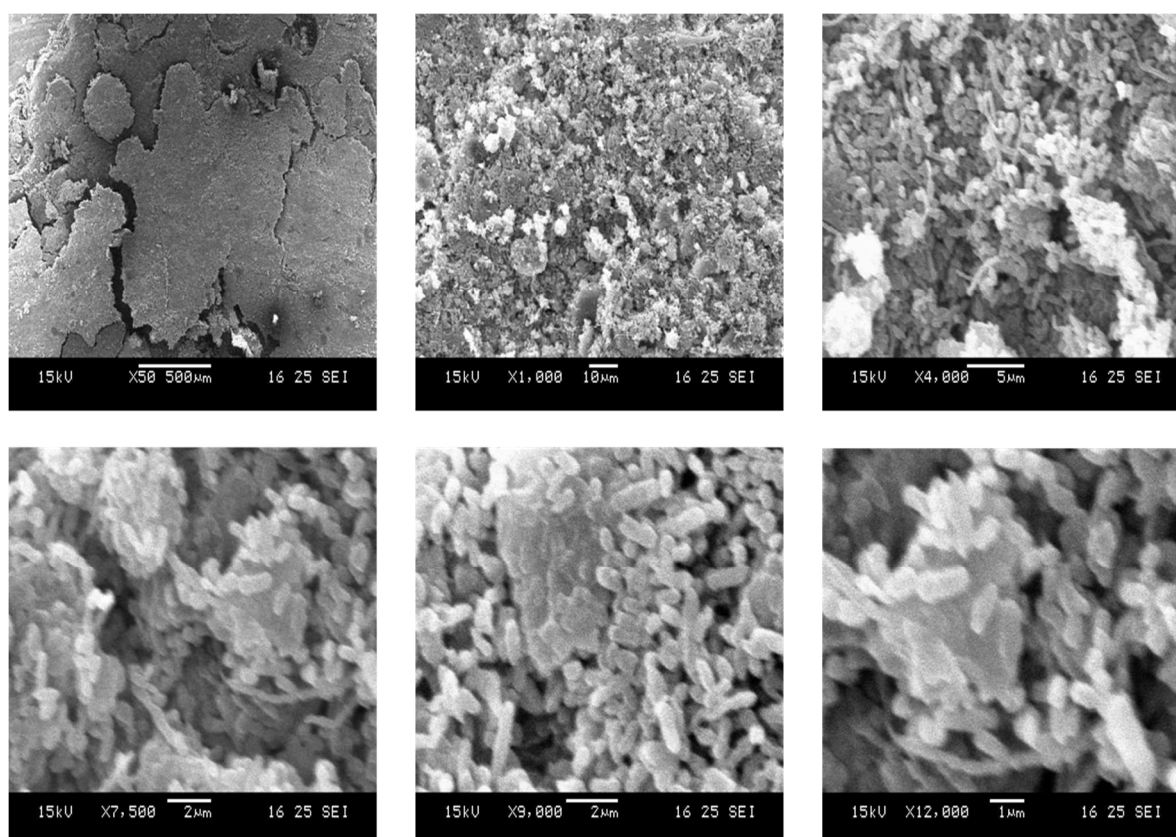


Figure 1. Scanning electron microscopy images of autoclaved *E. coli* cells at increasing magnification showing clusters of rod-shaped *E. coli* bacterial cells with intact cell structure and morphology, and crucially without any holes in the cell wall ultrastructure.

One possibility for this observation is that *E. coli* DH5 α may possess a thicker peptidoglycan layer than commonly expected. This peptidoglycan layer is also likely to be non-porous and possess ion channels and transporters for transferring metabolites and biomolecules. Such a notion is in stark contrast to the textbook depiction of the peptidoglycan layer of Gram-negative bacteria as a perforated mesh with loosely held intertwined cross-linked filaments.[2–4]

In conclusion, this work describes an accidental observation that *E. coli* DH5 α cells still retained their rod-shaped morphological structure with no holes in the cell wall after autoclave treatment at 121 °C for 20 minutes. Such an observation suggests that the peptidoglycan layer in *E. coli* cell wall is able to withstand higher pressure than previously thought, and is likely to be thicker than the single layer perforated mesh depicted in textbooks. Future work could validate this accidental observation, as well as compare the mechanical strength and properties of *E. coli* with and without autoclave treatment.

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