Rauch, Cyril and Leigh, James A. (2014) Theoretical evaluation of wall teichoic acids in the cavitation-mediated pores formation in Gram-positive bacteria subjected to an electric field. Biochimica et Biophysica Acta - General Subjects, 1850 (4). pp. 595-601. ISSN 0304-4165

Biochimica et Biophysica Acta 1850 (2015) 595–601

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Theoretical evaluation of wall teichoic acids in the cavitation-mediated pores formation in Gram-positive bacteria subjected to an electric field

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ARTICLE INFO

Article history:
Received 9 September 2014
Received in revised form 13 November 2014
Accepted 3 December 2014
Available online 11 December 2014

Keywords:
Gram-positive bacteria
Biophysics
Electroporation

ABSTRACT

Background: Electroporation is a method of choice to transform living cells. The ability of electroporation to transfer small or large chemicals across the lipid bilayer membrane of eukaryotic cells or Gram-negative bacteria relies on the formation of transient pores across the membrane. To exist, these pores rely on an insulator (the bilayer membrane) and the presence of a potential difference on either side of the membrane mediated by an external electric field. In Gram-positive bacteria, however, the wall is not an insulator but pores can still form when an electric field is applied. Past works have shown that the electrostatic charge of teichoic acids, a major wall component; sensitizes the wall to pore formation when an external electric field is applied. These results suggest that teichoic acids mediate the formation of defects in the wall of Gram-positive bacteria.

Methods: We model the electrostatic repulsion between teichoic acids embedded in the bacterial wall composed of peptidoglycan when an electric field is applied. The repulsion between teichoic acids gives rise to a stress pressure that is able to rupture the wall when a threshold value has been reached. The size of such small defects can diverge leading to the formation of pores.

Results: It is demonstrated herein that for a bonding energy of about ~ 1 − 10 k_BT between peptidoglycan monomers an intra-wall pressure of about ~ 5 − 120 k_BT/nm^3 generates spherical defects of radius ~ 0.1 − 1 nm diverging in size to create pores.

Conclusion: The electrostatic cavitation of the bacterial wall theory has the potential to highlight the role of teichoic acids in the formation pores, providing a new step in the understanding of electroporation in Gram-positive bacteria without requiring the use of an insulator.

1. Introduction

As a tool, the electroporation technique has been used over the last two decades to deliver gene to cells [1] or in animal or plant tissues [2–5], to promote drug uptake by cells [6] and to implement food safety measures via electroporation-related sterilization mechanisms that are independent of temperature [7].

The ability of electroporation to transfer small chemicals (e.g., drugs) or large protein complexes (e.g., genes) across the bilayer membrane of cells rely on the formation of transient pores [8]. The mechanism of transient pores formation in eukaryotic cells and Gram-negative bacteria is now well understood and has been modeled in depth using physics [9,10]. However, as the structure of Gram-positive and Gram-negative bacteria differ significantly, it is difficult to transfer and apply the set of results obtained from Gram-negative to Gram-positive bacteria. Consequently, how an electric field can create transient pores is still incomplete in the case of Gram-positive bacteria and electroporation protocols are usually developed through lengthy trial and error procedures. Moreover, it is important to point out that a single and uniform electroporation protocol for all classes of bacteria and cells has not yet been found and that the different methods and tools used to enhance electroporation in Gram-positive bacteria, reviewed in [11], create a natural precedent in underlying the lack of general understanding concerning electroporation in Gram-positive bacteria.

Recent works have demonstrated nonetheless that the formation of pores in Gram-positive bacteria relies on the electrostatic charge carried by the teichoic acids that are major constituents of the wall of Gram-positive bacteria [12] and that the bacterial lipid membrane located underneath the wall can stabilize the pore once the later is formed [13].

The central role of teichoic acids for bacteria has been underlined in (i) the regulation of the bacteria morphology and division, (ii) bacteria ion homeostasis, (iii) the protection from host defense and antibiotics, (iv) the adhesion to the host, (v) the colonization of the host and (vi) the horizontal transfer of genes [14,15]. Unsurprisingly, teichoic acid is now a target of choice for new antibiotics [16]. Finally, the negative charges carried by teichoic acids [14] make them an essential component of the bacterial wall to interact with an external electric field [12].

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http://dx.doi.org/10.1016/j.bbagen.2014.12.004

Theories describing pore formation in eukaryotic cells or Gram-negative bacteria consider that the increase in conductivity across the outer bilayer membrane is associated with pores arising from a competition between an interfacial energy mediated by the external electric field and a tension line once the pore is formed [17]. Naturally, these theories have to consider the bilayer membrane as an insulator initially, so that an interfacial energy can be defined.

In Gram-positive bacteria, however, this stance regarding an interfacial energy cannot hold as the bacterial wall is permeable to ions and is therefore not an insulator. This suggests therefore that pores arise from a change in energy defined inside the volume of the wall that may expand to form pores at the wall surfaces.

The bacterial wall is a polymeric gel made of peptidoglycan units interacting via covalent bonds. Inside the wall, a number of molecules exist among which the teichoic acid composing 60% of the cell wall and whose charge is inherently negative due to phosphate groups composing the polyelectrolyte [14]. Binding of free cations to teichoic acids is also thought to minimize repulsion between nearby phosphate groups, which can affect polymer structure and therefore cell wall integrity [18,19]. The best example of such an interaction is when the bacterium wall is incubated at low tonicity provoking its swelling and a concomitant reduction in the zwitterions (i.e., cations) interacting with the wall surface (see Table 1). It seems therefore that swelling results from an excess of negative wall charge, very probably driven by wall teichoic acids repulsion. Augmenting the repulsion between teichoic acids is well known to weaken the wall. Indeed, the bacteria electro-competency step that consists of incubating bacteria at low tonicity to drop the medium conductivity also makes their wall more susceptible to electric fields. Taken together, these observations suggest therefore that when the electrostatic equilibrium between the wall and the surrounding medium is altered, the wall is affected. Finally, the overall teichoic acid charge can also be modulated via addition of positive D-alanyl residues reducing its binding capacity for cations [20]. In particular, the inactivation of the *dlta* gene that has been shown to inhibit the addition of D-alanyl residues makes Gram-positive bacteria more susceptible to the external electric field [12]. This biological observation is in line with a role of wall teichoic acids in electroporation.

It is therefore not unreasonable to think that the negative charge of teichoic acids may be involved in generating pores when an external field is applied.

This can be explained as follows: consider a negatively charged teichoic acid embedded in the wall and surrounded by free counterions. Under an external electric field, provided that the later is strong enough, it would not be surprising to see most of the free ions interacting with the teichoic acids to leave the bacterial wall, thereby unmasking the negative charges of teichoic acids. If the wall density of teichoic acids is enough, this could, in turn, increase the repulsion between them. As teichoic acids are embedded in a peptidoglycan gel their repulsion should result in the creation of a very high wall mechanical tension that could rupture the peptidoglycan gel locally once the tension has reached a threshold level. This mechanism is similar to cavitation and it is this mechanism that the present work aims to model.

Table 1
Example of surface charge density of Gram-positive bacteria as a function of the external concentration of electrolytes (data from [23,24]).

Strain	Wall thickness (nm)	Surface charge density (C/m^2)	Electrolyte concentration (M)
Corynebacterium sp. Strain DSM 44016	66	0.61	0.1
	78	0.51	0.01
	108	0.35	0.001

2. Renormalization of the electrostatic charge of teichoic acids in a peptidoglycan matrix.

Let us consider a free teichoic acid in solution. These polyelectrolytes are negatively charged, and as a result, if the solution contains also free electrolytes, the cations from the solution should gather around the teichoic acid to balance its negative charge. This "screening" will happen over a certain length scale, λ, that is defined in part by the concentration of free electrolytes in solution (Fig. 1A). In particular, if the concentration of electrolytes is low, the length scale λ should increase. This means that two free and identical teichoic acids will not repulse each other if their separation distance is larger than: 2λ, even so they have the same negative charge (Fig. 1B).

Consider now a set of teichoic acids that are not free in solution but fixed because embedded in a peptidoglycan matrix, i.e., the bacterial wall. Assuming spatially fixed teichoic acids is correct so long that the time scale considered here and needed to change the value of λ are much shorter that the time scale required for an acid to diffuse out of the matrix.[1] Their separation distance is now fixed by their density in the wall. Let us note ρ, the density of teichoic acid in this case. The average distance that separates two teichoic acids in the bacterial wall is now ~ $2/\rho^{1/3}$. This means that the two teichoic acids will start to repulse each other if their bacterial wall concentration is such that $\rho \geq \rho_c \sim 1/\lambda^3$ (Fig. 1C). As a result, the screening can be imperfect in cases where the density of teichoic acids is too high or the concentration of electrolytes is too low, or both. In these conditions, it is possible to redefine an effective charge Q for teichoic acids: $Q \sim Q_0 \exp(-c\rho^{-1/3}/\lambda)$ (Appendix A), where c is a constant that refers to the shape of the teichoic acid (e.g., $c = (4\pi/3)^{-1/3}$ for a spheric shape) and Q_0 is the true charge of teichoic acids when no counter-ions are present. Using $\rho_c \sim 1/\lambda^3$, the charge can be rewritten simply as $Q \sim Q_0 \times e^{-(\rho_c/\rho)^{1/3}}$. With this new renormalized charge, it is now possible to deduce the new physical properties of the wall.

3. Repulsive electrostatic energy in the bacterial wall

If we assume that $\rho \geq \rho_c$, the teichoic acids will repulse each other and an energy can be defined (Fig. 1C). Let us further assume that a teichoic acid will only be affected by its closest neighborhoods; the repulsive energy between two teichoic acids separated by an average distance, $2c\rho^{-1/3}$, is as follows: $Q_0^2 \times e^{-2(\rho_c/\rho)^{1/3}}/4\pi\varepsilon_0\varepsilon_r 2c\rho^{-1/3}$.

As each teichoic acid feels a repulsion from close neighborhoods only and that the number of neighborhoods that are electrostatically "visible" per teichoic acid is ρ/ρ_c; the total repulsive energy felt by one teichoic acid is $\left(Q_0^2/4\pi\varepsilon_0\varepsilon_r 2c\rho^{-1/3}\right) \times (\rho/\rho_c) \times e^{-2(\rho_c/\rho)^{1/3}}$.

To determine the repulsive energy that is present in the entire bacterial wall, the energy of a single teichoic acid needs to be summed up over all the teichoic acids present in the wall. As the number of teichoic acids present in the wall is ρV_{wall}, where V_{wall} is the volume of the wall, the repulsive energy inside the bacterial wall at the lowest order (i.e., for pair interaction only) is

$$E_{\text{elec}} \sim \alpha_0 \rho^{7/3} \times e^{-2(\rho_c/\rho)^{1/3}}/\rho_c \quad (1)$$

with $\alpha_0 = Q_0^2 V_{\text{wall}}/16\pi\varepsilon_0\varepsilon_r c$. A factor 1/2 is introduced in Eq. (1) to avoid counting twice the same pair interaction between teichoic acids. Eq. (1) assumes that the two surfaces of the wall have a negligible impact in the repulsive energy as otherwise a surface term should be introduced. This means that the validity of Eq. (1) is likely to be optimal for thick bacterial walls, namely, when the ratio surface to volume of the wall tends toward zero ($S_{\text{wall}}/V_{\text{wall}} \to 0$).

[1] Imposing an electrical field over a very short period of time should warrant this.

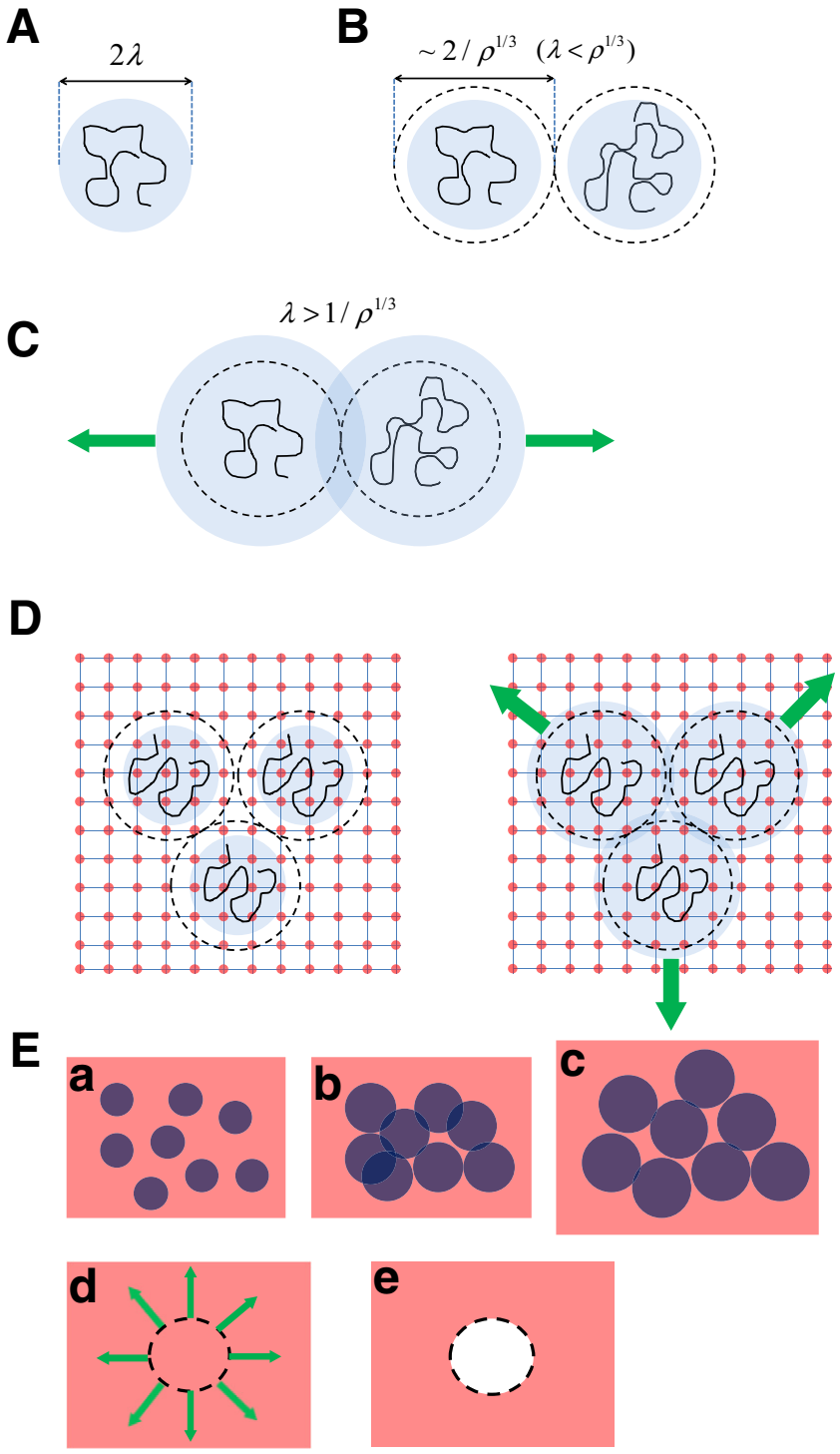

Fig. 1. (A) let us consider a single teichoic acid. The teichoic acid is a negatively charged polyelectrolyte and as a result positive counter-ions and negative co-ions surround the acid to neutralize its global negative charge. This neutralization or screening requires a certain distance λ to happen. This means that teichoic acids separated by a distance higher than λ will not interact together, i.e., repulse each other because they have similar negative charge. (B) Naturally two teichoic acids are unable to interact electrostatically if their separation distance $1/\rho^{1/3}$ is higher than the charge screening distance λ: $\lambda < 1/\rho^{1/3}$. (C) We note, however, that if the charge screening distance increases beyond the distance given by the concentration of teichoic acids, the two teichoic acids can interact electrostatically and repulse each other and as a result move far away from each other. (D, left) Consider now three teichoic acids not free in solution but embedded in a matrix (meshes in blue and nodes in red). In this case, $\lambda < 1/\rho^{1/3}$ and therefore no attraction is possible between teichoic acids and the system is stable. (D, right) Assume now that the screening length λ increases such that $\lambda > 1/\rho^{1/3}$. The teichoic acids will then repulse each other but they cannot move far away from each other as they are embedded. A resulting pressure should ensues that will be applied to the matrix to impose a separation between teichoic acids. This pressure can potentially promote the rupture of the peptidoglycan matrix. It is possible to predict some generic behavior concerning the bacterial wall. (E-a) Assume that teichoic acids (in blue) are embedded in the matrix (in red). (E-b) If the screening length increases and overlap the teichoic acids repulse each other. (E-c) To minimize this excess of energy linked to teichoic acids repulsion, the matrix can increase its volume (swell) so that the distance between teichoic increase (i.e., their concentration drop) and becomes similar to the charge screening distance λ. (E-d) As the matrix increases its size, a residual internal pressure (in green) appears. Let us focus on a small element of volume of the wall (dashed circle). The internal pressure can only disappear if the volume of the gel drops consequently reducing the volume of the matrix. (E-e) This is possible if ruptures appear in the matrix. This phenomenon can be understood as a cavitation.

Eq. (1) demonstrates that the wall is under tension due to the repulsion of teichoic acids. However, the tension is not the same whether we consider a change in the amount of teichoic acids in this case affecting the variable ρ, or a change in the external concentration of ions affecting the variable ρ_c. This suggests that the sensitivity of the system differs as a function of the stress imposed.

Nucleation of one defect in the bacterial wall: cavitation

When a strong enough electric field is applied in a solution containing bacteria, most of the counter-ions surrounding the negatively charged teichoic acids should be dragged out of the wall and follow the external electrical field imposed. This is expected to uncover the negative charges of teichoic acids and as a result increase the repulsive force between them to generate a large stress in the wall (Fig. 1D). This large repulsive stress can break the peptidoglycan matrix to nucleate volume imperfections in the bacterial wall (i.e., sort of spherical defects) (Fig. 1E). By creating volume in the bacterial wall, such imperfections would dilute the teichoic acids and drop their repulsion to rebalance the stress in the bacterial wall.

Consider a wall with a constant density of teichoic acids ρ. This assumption, i.e., constant density of teichoic acids, hold so long that the time scales considered here are much shorter than the one required for teichoic acids to diffuse out of the wall.[2] Let us note that $P_{elec} = E_{elec}(\rho_c)/V_{wall} > 0$, the pressure inside the wall associated with the electric repulsion between teichoic acids. Consider a small element of volume of the wall V_0 and assume that P_{elec} is fixed and does not change. The energy associated with any change in small element of volume of the wall V_0 is $-P_{elec}\Delta V$, where $\Delta V = V - V_0$ is the volume variation. We note that if $\Delta V > 0$, the wall energy can drop. This scenario is possible if the nucleation of a defect takes place to reduce the volume of the bacterial wall.

Let us note σ ($\sigma > 0$), the energy per unit of surface required to break the peptidoglycan bonds and to form one of these nucleation. The energy associated with the creation of one nucleation of surface, S, is σS. Adding this energy term to the former allows one to determine the chemical potential $\Delta\mu$ for a single defect namely, $\Delta\mu = -P_{elec}(V - V_0) + \sigma S$. Considering furthermore that the nucleation is spherical, one finds

$$\Delta\overline{\mu} = \Delta\mu - P_{elec}V_0 = -P_{elec}4\pi R^3/3 + 4\pi\sigma R^2 \tag{2}$$

Eq. (2) represented in Fig. 2A demonstrates that the nucleation of a spherical defect will only diverge in size if its radius R is such that $R \geq R_c = 2\sigma/P_{elec}$, where R_c is determined by $\partial_R \Delta\overline{\mu}|_{R=R_c} = 0$. As the probability $P(R_c)$ of this event is $P(R_c) = \exp[-\Delta\overline{\mu}(R_c)/k_BT]/Z$ (where k_BT is the thermal energy and $Z = \int_{R=0}^{R=+\infty} \exp[-\Delta\overline{\mu}(R)/k_BT]dR$); using $R_c = 2\sigma/P_{elec}$, one finds $P(R_c) \sim \exp[-16\pi\sigma^3/3P_{elec}^2 k_BT]$. Consequently, such an event is deemed possible only when the pressure inside the wall associated with the electric repulsion between teichoic acids reaches a critical value $(P_{elec})_c$ that verifies

$$(P_{elec})_c = \sqrt{16\pi\sigma^3/3k_BT} \tag{3}$$

Note that as $P_{elec} = E_{elec}(\rho_c)/V_{wall}$ and $E_{elec}(\rho_c) \propto V_{wall}$ (Eq. (1)), Eq. (3) demonstrates also that the dimension of the wall does not intervene at all in the nucleation of defects. Finally, it is possible to estimate R_c using Eq. (3) only by estimating σ. Consider that the mesh size of the peptidoglycan wall is ~nm [21] and that $\sigma \sim 1 - 10\ k_BT/\pi$ nm^2, one finds a critical radius of $R_c \sim 0.1 - 0.5nm$. Similarly, we find $(P_{elec})_c \sim 5 - 120\ k_BT/\text{nm}^3$. Using Eq. (3), it is possible to rewrite the probability $P(R_c) \sim \exp\left(-1/\overline{P}^2\right)$, where $\overline{P} = P_{elec}/(P_{elec})_c$. From the later relation, it is clear that $P(R_c)|_{\overline{P}\to\infty} \to 1$, meaning that a stronger electric field should increase the frequency of occurrence of spherical cavities in the wall.[3]

All along the development above, we have assumed that P_{elec} is fixed and does not change. This assumption may not be necessarily true if more than one nucleation appears as this would change the distance between teichoic acids and change the repulsion between them. In this case, the occurrence of many small defects could impede the size divergence of only few large defects. It is worth noting that to be valid this last assertion assumes that the small defects should be stable over time. However, we know, by virtue of Eq. (2), that this cannot be the case so long that $\Delta\overline{\mu} > 0$ (Eq. (2) and Fig. 2A). This means therefore that the critical threshold pressure has to be reached to degenerate defects that may thereafter stabilize the stress present in the wall by interacting together. This point is non-intuitive and merits some attention to be proven.

4. The nucleation of several stable small defects is not an option

To determine whether the occurrence of several small nucleation in the bacterial wall is an option, we consider the repulsive pressure as a function of two variables: $P_{elec}(\rho_c, \rho)$.

Let us consider a number, n, of defects all with an average volume, v. Each of these small volumes, if they exist, will add to the total volume of the bacterial wall available for teichoic acids interaction. Consequently, the volume of the wall is now modulated as follows: $V_{wall} = V^0_{wall} + nv$, where V^0_{wall} is the initial volume of the bacterial wall without any defects in it. Consequently, the initial density of teichoic acids ρ will now be transformed into $\rho/(1 + nv/V^0_{wall})$. Noting $\rho_{nuc} = nv/V^0_{wall}$, the density of nucleated defects in the bacterial wall, using the development above the energy of the bacterial wall can be written as

$$\Delta E = -P_{elec}\left(\rho_c, \frac{\rho}{1+\rho_{nuc}}\right)\rho_{nuc}V^0_{wall} + \chi\rho_{nuc} \tag{4}$$

where $\chi = \sigma V^0_{wall} \times s/v$ and where s is the average surface of nucleated defects. Assume that $\rho_{nuc} \ll 1$ and let us develop Eq. (4) to the second order in the variable ρ_{nuc}:

$$\Delta E \sim \frac{V^0_{wall}}{v}\Delta\overline{\mu}\rho_{nuc} + \left.\frac{\partial P_{elec}}{\partial\rho}\right|_{(\rho_c,\rho)} V^0_{wall}\rho\rho^2_{nuc} \tag{5}$$

As $\partial P_{elec}/\partial\rho > 0$ (Eq. (1)), the only stable physical solution to Eq. (5) is $\rho_{nuc} = 0$ so long that $\Delta\overline{\mu} > 0$ (Eq. (2)). This development shows that although the bacterial wall may generate small defects, they will not be stabilized in the wall over time (as $\Delta\overline{\mu} > 0$) but will collapse their volume and disappear. This bacterial wall bubbling (or bacterial wall cavitation) regimen will remain until the electric field is strong enough to create defects with diverging sizes. If this scenario happens, the chemical potential $\Delta\overline{\mu}$ can change sign ($\Delta\overline{\mu} < 0$), resulting in a new equilibrium regarding the density of spherical defect inside the wall given by $[\partial\Delta E/\partial\rho_{nuc}]_{\widetilde{\rho}_{nuc}} = 0$, namely, $\widetilde{\rho}_{nuc} \sim -\Delta\overline{\mu}/2v\rho[\partial P_{elec}/\partial\rho]_{(\rho_c,\rho)}$. However, as there is no physical insight to fix the value of $\Delta\overline{\mu}$, it is difficult to estimate the value of: $\widetilde{\rho}_{nuc}$.

5. Electroporation of the bacterial wall

We consider now the possibility of creating a pore through a similar mechanism as described above. In order to do so, let us consider a tube

[2] This point is important as the wall density of teichoic acids is supposed to change in response to the external electric field imposed if is applied for long enough. So, only short perturbations are considered here.

[3] That is because according to the Arrhenius Law the frequency is proportional to the probability of occurrence.

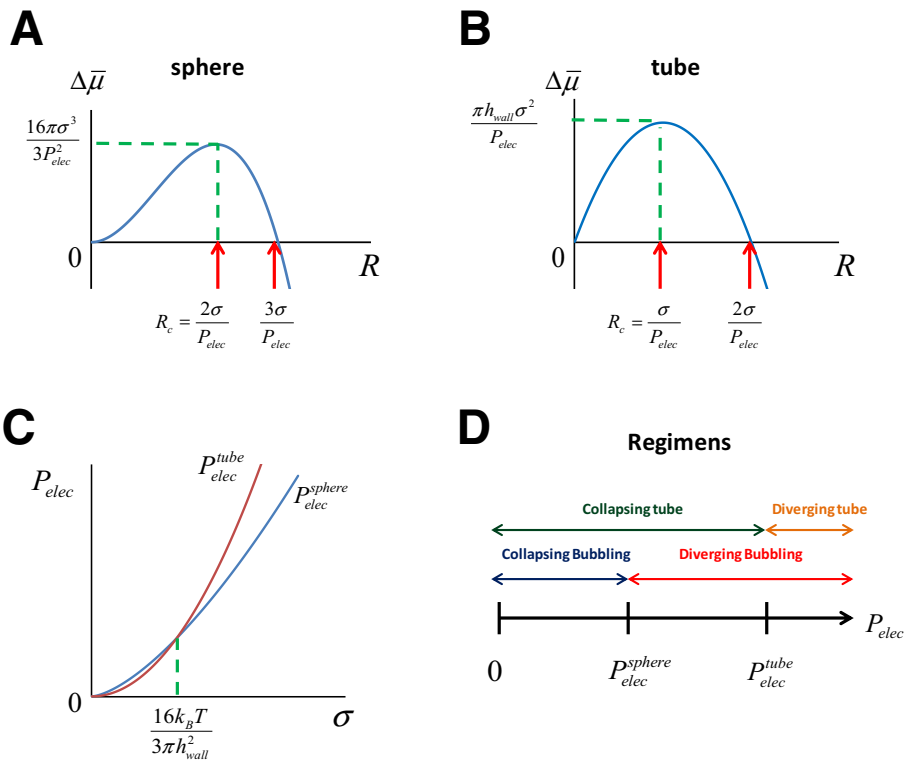

Fig. 2. (A) Representation of Eq. (2). (B) Representation of Eq. (6). (C) Representation of the electric pressure for the sphere and tube. Note that their intersection is at a value of the surface tension σ, that is very low: $\sigma \sim k_B T/h_{wall}$. It is therefore expected that the repulsive pressure to create a tube will always be higher than the one to create a sphere. (D) Different regimens expected based on the repulsive pressure values determined.

of length h_{wall} and internal diameter R. Using Eq. (2), the chemical potential of the tube is

$$\Delta\bar{\mu} = -P_{elec}\pi h_{wall}R^2 + 2\pi h_{wall}\sigma R \tag{6}$$

From Eq. (6) (represented in Fig. 2B) and as done above, one finds a critical pore radius $R_c \sim \sigma/P_{elec}$ that can diverge in size if

$$P_{elec} \geq (P_{elec})_c = \pi\sigma^2 h_{wall}/k_B T \tag{7}$$

Assuming $\sigma \sim 1 - 10 k_B T/\pi nm^2$, one finds $R_c \sim 10^{-3} - 10^{-2}$nm (this small value is discussed later) if $h_{wall} \sim 100$ nm. The ratio of electric pressures between the pore and the sphere defects gives $\left(P_{elec}^{tube}\right)_c / \left(P_{elec}^{sphere}\right)_c = \sqrt{3\pi\sigma h_{wall}^2/16\, k_B T}$. If one assumes that $\sigma \sim 1 - 10 k_B T/\pi nm^2$, with a wall thickness of about $h_{wall} \sim 100$ nm, one finds $(P_{elec}^{tube})_c/(P_{elec}^{sphere})_c \sim 5 - 15$. So the value of the minimal repulsive pressure to create a pore will always be larger than the one to create a sphere (Fig. 2C). This last result shows that depending on the energy associated with the electric field, different regimes can exist or coexist together (Fig. 2D). In addition, it is not excluded that a "diverging bubble" arising from the centre of the wall will connect the two surfaces of the wall creating a tube that may expand or collapse later on.

6. Discussion

Electroporation is a widely used method for the delivery of small or large molecules in eukaryotic or prokaryotic cells hence bypassing the bilayer lipid membrane and/or the bacterial wall. However, given the heterogeneity of living organisms, there is no guaranty that understanding the physical impact of electroporation in human cells can be transferred to Gram-positive bacteria, for example. As a result, there is a need to redefine the physics of electroporation in different systems when required.

We have focused on Gram-positive bacteria given the significant difference that exists between this species of bacteria and Gram-negative bacteria or eukaryotic cells. In Gram-negative bacteria or eukaryotic cells, the membrane plays the initial role of an insulator from which pores can be formed [22]. In Gram-positive bacteria, this has to be ruled out and therefore there is no theory explaining the formation of pores when an electric field is applied. We suggest that the peptidoglycan wall can rupture if the repulsion between teichoic acids is strong enough, i.e., is beyond a threshold value (see Eqs. (3) and (7) or Fig. 2A and B) and that cavitation, i.e., nucleation of defects, is only possible in bacterial wall at an energy cost much smaller (by a factor of 5 to 15) than the one required to generate a pore directly. It is important to keep in mind, however, that such a low energy cost for spherical cavities may not be efficient for an experimentalist, for it is also associated with a low frequency of occurrence.

It is interesting to note that cavitation can lead to the formation of pores as well. For this to happen, consider that a nucleation appears exactly at the center of the wall and that the repulsive electric pressure allows the nucleation to diverge in size. Once the spherical nucleation reaches both the upper and the lower sides of the wall, a pore can form. Numerically, we have estimated that the initial critical spherical size defect should be ~0.1–1 nm in agreement with the dimensions of the bacterial wall (~10–100 nm). For the pore, however, we found a much smaller value that is not realistic as pore sizes have been measured using electron microscope with values ranging from ~10 to 100 nm [13]. Such a small value that arises from the 2D plane geometry we have used and the thickness of the wall we have considered raises question with regard to the validity of the theory as far as pore formation only is involved. Indeed, it is expected that the peptidoglycan gel will demonstrate some compliancy with regard to pressure. This mechanical compliancy is not taken into consideration in the model that only focuses on the system energy/thermodynamic values. It is therefore reasonable to think that for small strains

Table 2
Thickness of bacterial wall between different strains at a given concentration of external electrolytes (data from [23]).

Strain	EM measurement of wall thickness (nm)	Electrolyte concentration (M)
Corynebacterium sp. Strain DSM 44016	60	1
Corynebacterium sp. Strain DSM 6688	35	1
Rhodococcus erythropolis A177	35	1
Rhodococcus opacus C125	35	1
Bacillus brevis	75	1

the bacterial wall should be able to balance the pressure stress. This should apply to the critical pore dimension we have estimated. From this, we can conclude that (i) the energy to create a pore as such should be much higher than the one we have estimated to bypass the peptidoglycan compliancy and (ii) pores are unlikely to appear in the wall as such but, instead, rise from small spherical defects as discussed above.

Previous works have suggested that a wall pore radius >15–24 nm can lead to cell lysis due to membrane bulging [13]. If one considers that a spherical defect can connect both sides of the wall, for a wall thick enough, bulging followed by cell lysis is therefore guaranteed. In Table 2, we provide a list of cell wall thicknesses in some Gram-positive bacteria, demonstrating that lysis should be expected due to a thick cell wall (thickness > 24 nm). This may explain why the rate of bacterial death is so high during electroporation.

7. Conclusion

We suggest that pore/hole formation in the bacterial wall under an external field should arise from a cavitation mechanism that relies on an increase in wall pressure mediated by the repulsion between wall constituents.

Acknowledgements

This work was funded by the University of Nottingham.

Appendix A

Let us consider the Poisson equation linking the electric potential $\Psi\left(\vec{r}\right)$ to the charge density $\rho\left(\vec{r}\right)$:

$$\Delta\Psi\left(\vec{r}\right) = -\frac{\rho\left(\vec{r}\right)}{\varepsilon_0\varepsilon_r} \tag{A1}$$

where ε_0 and ε_r are the vacuum and relative electrical permittivities, respectively, and Δ is the Laplacian operator.

We consider a system composed of teichoic acids and counter-ions where the teichoic acids are fixed and counter-ions move freely. We assume initially that all the teichoic acids are screened by counter-ions so that they do not repulse each other.

We consider now the application of an external electric potential $V_{\text{ext}}\left(\vec{r}\right)$. This external potential can remove counter-ions by increasing their energy by a factor $Z_i V_{\text{ext}}\left(\vec{r}\right)$, where Z_i is the charge of the particle species "i". Now let us assume that the external potential is constant over a length scale similar to the size of a teichoic acid.[4] In this case, we note $V_{\text{ext}}\left(\vec{r}\right) = V_{\text{ext}} = cst$, and we replace $Z_i V_{\text{ext}}\left(\vec{r}\right)$ by $|Z_i V_{\text{ext}}|$. By using the absolute value of the external field energy, we consider that the reservoir attracts ions, namely, that it is not always necessary for a counter-ion to remain close to the teichoic acids. In this context, the charge density can be rewritten as follows:

$$\rho\left(\vec{r}\right) = \sum_i Z_i C_i^{\infty} e^{-\frac{Z_i\Psi\left(\vec{r}\right)+|Z_i V_{\text{ext}}|}{k_B T}} \tag{A2}$$

where C_i^{∞} is the concentration of the free ionic species "i" in the solution (far away from the teichoic acids).

Finally, if one assumes that the interaction between teichoic acids and their counter-ions are sufficiently weak to warrant a linearization of Eq. (A2), one finds

$$\Delta\Psi\left(\vec{r}\right) \sim \sum_i \frac{Z_i^2 C_i^{\infty}}{\varepsilon_0\varepsilon_r k_B T} e^{-\frac{|Z_i V_{\text{ext}}|}{k_B T}} \Psi\left(\vec{r}\right) \tag{A3}$$

Noting $\frac{1}{\lambda^2} = \sum_i \frac{Z_i^2 C_i^{\infty}}{\varepsilon_0\varepsilon_r k_B T} e^{-\frac{|Z_i V_{\text{ext}}|}{k_B T}}$, we find that λ defines the screening length involved in the interaction between counter-ions and teichoic acids. Finally, if we consider the teichoic acids as spherical particles for simplicity, one can easily show that the potential from teichoic acids should follow a Yukawa-type potential where the charge of teichoic acids should vanish with the distance: $Q \sim Q_0 \exp(-r/\lambda)$.

[4] Consider a teichoic size of $l \sim 1$ nm and the thermal energy $k_B T$. A thermal force can be defined by $k_B T/l$. To determine the "thermal gradient potential," we use the electric force $Z_i \Delta V_{\text{ext}}/\Delta x$. Equating the relations, we find that $\Delta V_{\text{ext}}/\Delta x = k_B T/Z_i l \sim 10^7$ V/m. This value is similar to the external electric fields applied for electroporation.

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