

ELECTRIC FIELD ANALYSIS OF COATED TITANIUM ELECTRODES ON MICROBIAL INACTIVATION UNDER PULSED ELECTRIC FIELD TREATMENT

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Abstract

The viable pathogens in the liquid food can be efficiently inactivated using Pulsed Electric Field (PEF) treatment through electroporation. Electroporation depends on applied electric field for causing minute pores on the cell membrane. The electric field application from electrodes play an important role in getting the required inactivation and it varies based on the dimensions and design of electrodes inside the chamber. Parallel plate electrodes provide more uniform electric field in the inactivation area, where all the microbes will be uniformly treated under the same field value. This paper analyses the electric field distribution under two circumstances (i) To find a suitable surface material for titanium electrodes, for future noninvasive PEF applications (ii) Comparative performance of platinum coated and alumina coated titanium electrodes, when a viable pathogen is placed in the inactivation area. Under first circumstance, the electric field distribution through four surface materials such as glass, alumina, quartz and plexiglass was compared and analyzed. The electrodes have a diameter and thickness of 40 mm and 3.5 mm respectively. From the first analysis, it was found that alumina exhibited greater percentage (82%) of electric field in the inactivation area than with other materials. This signifies the suitability of alumina as a coating for titanium electrodes for successful noninvasive PEF applications. Secondly, the inactivation performance of alumina coated titanium electrodes was compared with platinized titanium on *Escherichia coli* models. From the second analysis, alumina coated electrodes gave equivalent performance with platinized titanium on bacterial inactivation which reconfirmed alumina's suitability for non invasive PEF applications. The electric field simulations were implemented using ANSYS MAXWELL v 15.0.

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Introduction

Pulsed electric field (PEF) treatment is widely recognized as the successful nonthermal food preservation technique, involving application of high electric field pulses to liquid food placed between two electrodes. PEF treatment has the major advantage of retaining food's quality attributes such as aroma, taste, color, freshness, texture and especially the nutritional value with low energy consumption when compared to heat treatment methods (Canovas *et al.*, 1999). Thus, PEF can effectively inactivate food borne pathogen present in the liquid food through electroporation phenomenon (Weaver, 2000). There are many electrode configurations available where the corresponding electric field distribution inside the treatment chamber will have a greater impact on microbial inactivation. The parallel plate electrodes were used initially which provided more uniform field distribution, subjecting all the microbes under the same electric field application in the treatment area. Previous literature studies used many electrode materials, out of which stainless steel was used as a state of art material in PEF application due to its excellent electrical properties (Qin *et al.*, 1994). Due to frequent electric field application, the electrodes will be subjected to extensive usage, leading to surface corrosion. This results in formation of pits on the electrode surfaces and emission of metallic ions into the testing liquid during the treatment, giving the liquid food a metallic taste (Gad *et al.*, 2014). Consequently, the safety of the liquid food cannot be guaranteed for consumption after the treatment. Hence the electrochemical stability and toxicity should be considered as two significant factors when choosing electrode material for PEF applications. Previous literature studies reported comparative performance of many electrode materials with respect to metallic ion release and concluded that titanium emitted less metallic ions than stainless steel, though stainless steel was regarded as the state of material in PEF

applications (Gad *et al.*, 2014). Recent research suggested using noninvasive electrodes which involved using high permittivity coating materials on the electrode surface so that direct contact of liquid food with the electrode can be prohibited (Novac *et al.*, 2012; Qin *et al.*, 2016). By using noninvasive way of PEF application, the electric field should reach the liquid food and at the same time the electrode surface should not be in direct contact with the food. By using this methodology, the drawback of electrolysis can be prevented. In this work, initial analysis was made on selecting a suitable surface material for electrodes, which should have the property of permitting maximum electric field through it. Later, the material was chosen as a coating for substrate electrodes and its comparative performance was studied with metal coating on bacterial inactivation. Thus, the objective of this work focusses on noninvasive PEF application using coated titanium electrodes for inactivation of a viable pathogen.

Materials and Methods

Mechanism of Inactivation: Electroporation

PEF treatment involves application of high electric field shots (20 kV/cm to 80 kV/cm) in microseconds or nanoseconds of pulse duration to inactivate microbes present in the liquid food. When these ultrashort electric field pulses reach the cell plasma membrane, a potential difference or transmembrane potential will be generated across the membrane. Due to the increase in potential difference, the cell membrane experiences electromechanical stress. After a threshold potential of approximately 1V, the membrane cannot endure this stress leading to initiation of pores on the membrane surface and will increase in number depending on the intensity of the applied electric field. Hence it is understood that, 1V is the minimum

potential required to create pores on the membrane surface, leading to cell inactivation (Schoenbach *et al.*, 2000; Oka *et al.*, 2009). The corresponding electric field is known as critical electric field E_c below which inactivation does not occur. This condition is known as reversible cell breakdown where the pores can reseal itself and survive after pulse application (Schoenbach *et al.*, 2000; Oka *et al.*, 2009). If the induced TMP increases greater than 1V, then the radius of the pores will increase beyond the critical field value leading to a condition where the cell cannot reseal its pores and survive. This condition is known as irreversible electroporation (Schoenbach *et al.*, 2000; Oka *et al.*, 2009). In this research, the electric field distribution was analyzed for two electrode designs, in a perceived awareness that the required field application from these electrodes can cause electroporation of cells embedded in liquid medium in the inactivation area.

Electrode Designs

As parallel plate electrodes were initially used by researchers, it was found to have many advantages than other electrode configurations (Qin *et al.*, 1998) (Gachovska *et al.*, 2008). When compared with coaxial and colinear designs, the parallel plate electrodes not only provided more uniform electric field distribution between two electrodes, but also had simpler geometry and implementation, providing higher inactivation rates (Qin *et al.*, 1994). When the distance gap between two parallel electrodes is d in mm and V is the charging voltage across them, then the generated electric field can be calculated

theoretically as (Canovas *et al.*, 1999)

$$E = V/d \quad (1)$$

In PEF, the upper electrode is the high voltage electrode and the lower electrode is at ground potential which will be zero. When a peak voltage is applied, a conducting path will be established from high voltage to ground electrode where high probability of inactivation is expected to take place due to the generated electric field across the electrodes.

(i) Parallel electrodes immersed in water

Due to the excellent electrical properties, titanium has been used as parallel electrodes where the diameter and thickness are 40 mm and 3.5 mm respectively. The distance gap between two electrodes including the surface material is 5 mm. These electrodes have the following advantages (i) Unlike regular electrodes, these electrodes may not be subjected to damage due to frequent uses, as they are immersed in water during PEF application. (ii) Minimalistic dimensions of the electrode require very less amount of material to be used (iii) The electric field will not be directly applied to liquid food because these electrodes are immersed and positioned inside water containing materials. Four kinds of materials are used in this simulation and electric field distribution through these materials are analyzed. Due to the above advantages, the drawback of electrolysis can be avoided. Figure 1 shows the parallel plate electrodes modelled using ANSYS MAXWELL v 15 (About Ansys).

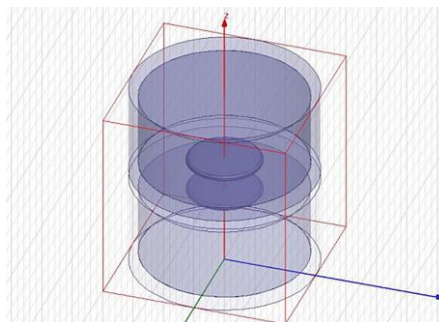
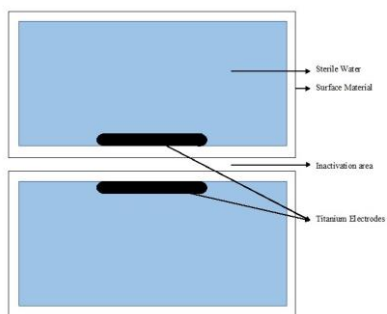


Figure 1. a) Titanium electrodes positioned inside water, b) Trimetric view of parallel plate titanium electrodes

In order to have control in temperature, the electrodes were immersed in water because temperature increase is a crucial factor in PEF, which should not exceed to create thermal effects on the liquid food. This setup serves two purposes (i) The best surface material suitable for noninvasive PEF applications can be chosen (ii) The setup can also be used for further experimental study.

(ii) Coated Electrodes

Electrode coating protects the substrate electrodes from corrosion during high voltage application. The major drawback of uncoated electrodes during experimentation is the release of undesirable metal ions from the electrode surface. When the testing frequency increases, this electrolysis effect will increase affecting the quality of outcome. Hence coating of electrode using an electrochemically stable material will protect the electrode not only from electrolysis but also prevents formation of undesirable pits or damage on the electrode surface. Previous studies used thick ceramic cylinders and thin titanium dioxide as coatings on stainless steel electrodes (Novac *et al.*, 2012; Qin *et al.*, 2016). However, using thick ceramic material demanded high electric field of around 200 kV/cm which may not be as suitable to apply under laboratory scale unless other cost intensive protective installations are made. Titanium dioxide coatings may pose some toxicity when used under high electric field application which needs much research. Hence in this work, 2 μm thick alumina and platinum coating were used for titanium electrodes. Alumina is a non toxic ceramic material having a permittivity of 9.2 (Ruys, 2018) whereas platinum is a highly conductive and electrochemically stable metal (Pourbaix *et al.*, 1959). A comparative performance analysis was made on the efficiency of these two kinds of material coatings on inactivating microorganisms. The area of electrodes was set to 50 $\times$ 50 mm. The distance between the electrodes was 1 mm. The setup is shown in Figure 2.

Microbe Models

The bacteria model chosen for the simulation work was *Escherichia coli* (*E.coli*).

E.coli is a gram negative bacteria which measures upto 2 μm in length and upto about 1 μm in width having cell wall thickness of 5 to 20 nm (Clifton *et al.*, 2015; Bionumbers, 2018). Some strains of this bacteria can cause diarrhea, nausea, abdominal cramps and fatigue (Food safety, 2018). This microbe will be found in water, soil and plant varieties and also on the intestines of living beings. If water is contaminated by this bacterium, then it is necessary to purify the water before drinking. Previous literature stated that the minimum field required to inactivate this microbe is 16 kV/cm (Moonesan *et al.*, 2011). However, most of the studies used greater than 20 kV/cm to break this microorganism. In order to analyze the electric field requirements, two different dimensions were fixed for *E.coli* for examining the potential of two types of coated titanium electrodes. Though both the microbes were rod shaped, their sizes differed, resulting in different field requirements.

Ansyz Maxwell v 15

The parallel electrodes and the bacteria were modelled using ANSYS Maxwell v 15 (Ansys, 2018). It is an electromagnetic simulation software, which uses finite element method to solve static, frequency domain, and time varying electromagnetic and electric fields. In this simulation work, ANSYS version 15.0 was used to analyze the electric field behavior of titanium electrodes, surface materials and then the bacteria for the applied voltage. Here, the electric field variation was monitored from

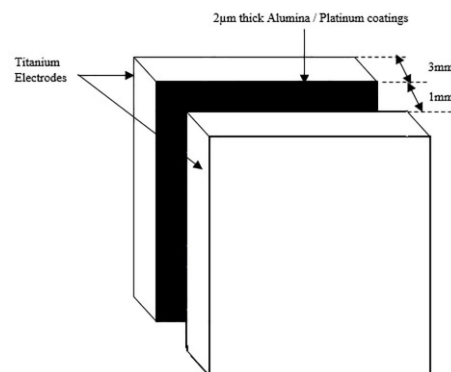


Figure 2. Parallel plate coated electrode setup

high voltage to ground electrode to provide an electrostatic solution.

Material Properties

Titanium is used as the substrate electrode in the simulation in both the electrode setups. Titanium is a durable and less dense material which has been used in aerospace applications. The metal has also been used in cooking utensils for its excellent nontoxic properties. Titanium also has a highly reactive property when exposed to oxygen or moisture as it readily reacts with oxygen. It has a stable and adherent oxide layer which protects the metal from damages. The anticorrosive property comes again from the protective oxide layer on the metal surface. Hence titanium is best suited for PEF applications than other metals. The resistivity of titanium ranges from 4.2×10^{-7} - 5.2×10^{-7} ohm-m (Mitchell, 2004). Previous studies tested the anticorrosive property of titanium by comparing the metal with aluminum, nickel, chromium, silver and stainless steel and found to release less metal ion release than other metals (Gad *et al.*, 2014). On the other hand, platinum is a malleable, dense and ductile metal which is electrochemically stable and has not corroded

even at high potential applications (Pourbaix *et al.*, 1959). It is a highly conductive and nontoxic material which has wide applications in jewelry, for making non corrosive wires, for medical equipment etc. Sterile water is used for immersion of electrodes under setup 1 which is a clear, colorless and odorless liquid and does not contain any bacteriostatic or antimicrobial agents. In the work, the electrodes were immersed in sterile water to have a control in temperature. Four surface materials were chosen for containing water through which the electric field will get distributed from the electrodes and reach the inactivation area. The conductivity and permittivity of the four materials are mentioned in Table 1. Here, the conductivity of all the four materials are considered as zero since they serve as dielectric materials having different permittivity values.

Properties of Cell

When a biological cell is considered, the cytoplasm of the cell is highly conductive in nature. Also, when pathogens are present in liquid food, the liquid food have a property to have charged particles or ions embedded in them which makes them conductive. The plasma membrane of the cell is a nonconductive membrane which when subjected to electric field, becomes highly permeable resulting in formation of pores. The properties of the cell and the suspension medium used in this work is shown in Table 2 (El-Hag *et al.*, 2011).

Table 1. Properties of Surface materials chosen

Material	Conductivity (S/m)	Permittivity
Glass	0	5.5 (Brodie <i>et al.</i> , 2015)
Acrylic	0	3.4 (Brodie <i>et al.</i> , 2015)
Quartz	0	3.8 (Sarafis and Nassiopoulou, 2014)
Alumina	0	9.2 (Catalogue, 1993/94)

Table 2. Properties of Biological cell and suspension medium

S.No.	Cell region	Conductivity (S/m)	Relative Permittivity
1	Cell / Plasma Membrane	10^{-5}	2.3
2	Cell cytoplasm	1	80
3	Suspension Medium	0.1	80

Results and Discussion

Though very high permittivity ceramic cylinders have been used in the previous studies for *E.coli* inactivation, the setup required much higher electric field of greater than 200 kV/cm (Novac *et al.*, 2012; Qin *et al.*, 2016). While intending for PEF application for liquid food preservation, the electric field should be maintained in a nominal range which paves way for more practical and wider applications. Hence in this research, the field was maintained nominal, which served two

purposes i.e., Both non invasive PEF application and bacterial inactivation.

Observation 1

The electric field distribution was analyzed through four surface materials using ANSYS MAXWELL v 15 as shown in Figure 3. Here, three dimensional modelling was preferred for better understanding of the electrode shape and it's electric field behavior for the applied peak voltage, which can be viewed in XYZ coordinates. A maximum thickness of 5 mm was used for four surface materials to check for the potential of each material in allowing the electric field through them.

In Figure 3, the red color represents maximum electric field value which is present in the central circumferential area of the treatment region. Blue color represents minimum field to zero value varying from lightest to darkest shade respectively. The various colors

between these two extremes represent the varying intensities of electric field. These intermediate field variations can occur, due to variation in properties at the junctions between the electrode and surface material and also depends on the proximity of electrode placement. The maximum field gradually decreases to zero while reaching the sides of the chamber. When modelling the electrodes, the overlapping cannot be tolerated between associated models. So, the modelled electrodes are placed in the water and subtracted from the water body inside the containing (Surface) material. Then the properties of each material are applied and meshed.

The high voltage electrode is then assigned a nonzero excitation peak voltage where the ground electrode will be at zero potential. While the generated field has to travel through the four surface materials in each case to reach the inactivation area, the intensity of field through each surface material depends on the properties of the material used. Hence the generated field corresponding to a particular peak voltage varies and reduces in intensity when it reaches the inactivation area. In our work, all the simulations were run at peak voltage ranging from 10 kV to 50 kV with corresponding electric field distribution passing through each surface material intended for noninvasive PEF applications. The result thus obtained in solving the model can be interpreted in the form of percentage field reaching the inactivation area for all the four surface materials, which are shown in Table 3 and Table 4 respectively. For instance, from Table 3, when a peak voltage of 10 kV is applied

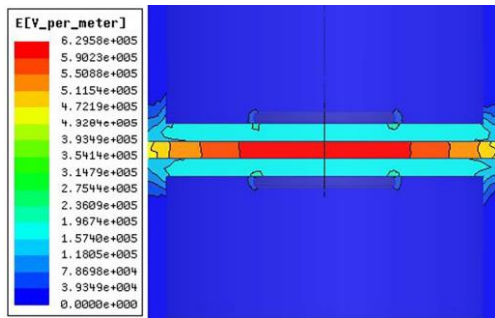


Figure 3. Electric field distribution with more uniform field in the centre

Table 3. Peak voltage applied across electrodes and the generated electric field

Peak Voltage (kV)	Distance Gap (mm)	Generated Electric Field Strength in the inactivation area (kV/cm)			
		Glass	Alumina	Plexiglass	Quartz
10	5	14.7	16	12.59	13
15	5	22	24.6	18.9	19.6
20	5	29.3	32.8	25	26
25	5	36.7	41	31.5	32.7
30	5	43.9	49.3	37.8	39
35	5	51.3	57.5	44	45.8
40	5	58.6	65.7	50	52.3
45	5	65.9	73.9	56.7	58.8
50	5	73.3	82	62.9	65.4

at a distance gap of 5 mm between two electrodes, according to Equation 1, the generated field should be 20 kV/cm without the surface material. But, since the field travels from high voltage electrode through a specific surface material thickness of 5 mm, the intensity of electric field decreases depending on the permittivity of each material, when it reaches the inactivation area. Accordingly, the resultant field values are 14.7 kV/cm for glass, 16 kV/cm for alumina, 12.59 kV/cm for plexiglass and 13 kV/cm for quartz respectively. The corresponding percentage reduction of electric field through each of the above surface material are 26.5%, 18%, 37%, and 35% respectively. Thus glass allowed approximately 73%, alumina allowed 82%, plexiglass allowed 63% and quartz allowed 65% of the electric field in the inactivation area which are given in Table 4. Here, Permittivity of each surface material plays a significant role in enabling the electric field to pass through it. The field distribution is thus observed from high voltage to ground electrode through each surface material chosen and also on the sides of the chamber. From the first observation, it was understood that in addition to glass and alumina being non toxic than other chosen materials, alumina allowed higher percentage of electric field in the inactivation area, which has confirmed the material's suitability for electrodes as the best surface material intended for future noninvasive PEF applications.

Observation 2

Previous studies used TiO₂ coating on stainless steel electrodes which required only half the number of impulses to inactivate around ~3-log₁₀ of microbial reduction (Qin *et al.*, 2016) when compared to uncoated electrodes. Since TiO₂ has the concern for toxic effects, further study was made on choosing a material which should be non toxic, should enable maximum electric field through it and should also be effective on microbial inactivation. Hence, from observation 1, alumina was finalized for further analysis as electrode coating for titanium electrodes. In this study, the performance of alumina coated titanium electrodes was compared with platinized titanium electrodes for their

efficiency on microbial inactivation. The distance between two electrodes was kept at 1mm and the electric field simulations were run from the peak voltage of 1kV to 10 kV. The *E.coli* model was placed under the influence of electric field is shown in Figure 4. Using ANSYS Maxwell, a three dimensional *E.Coli* model was designed with dimensions matching real cell parameters (Bionumbers, 2018). The properties of cell are given in Table 2 (El-Hag *et al.*, 2011). When this microbe model was placed in the centre between two electrodes, the electric field was monitored through the cell body which can be observed in the form of coloured lines. The induced transmembrane potential across the cell membrane was calculated as (Kotnik *et al.*, 2000)

$$\phi_T = \phi_i - \phi_o \quad (2)$$

where ϕ_i is the potential generated on the inner membrane surface and ϕ_o is the potential on the outer membrane surface.

Table 4. Percentage of field reaching the inactivation area for each surface material

Material	Percentage of field reaching the inactivation area
Plexiglass	63
Quartz	65
Glass	73
Alumina	82

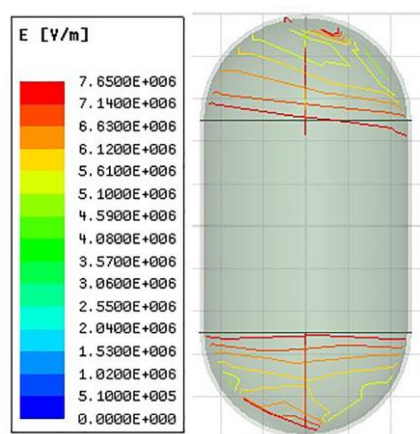


Figure 4. Field distribution through the cell

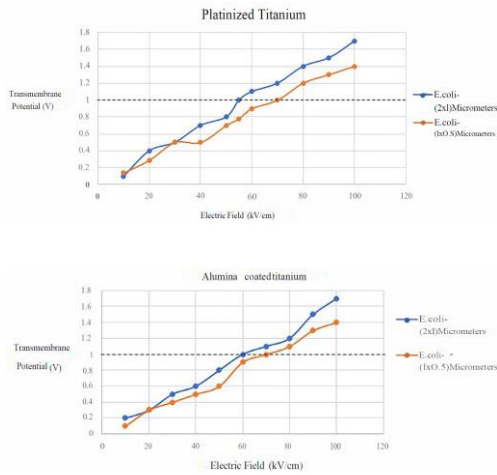


Figure 5. a) Induced transmembrane potential of 1 V at 55 kV/cm and 70 kV/cm field strength for larger and smaller *E. coli* cells
b) Induced transmembrane potential of 1V at 60 kV/cm and 70 kV/cm field strength for larger and smaller *E. coli* cells

From the simulation (Observation 2) two types of analysis can be performed.

(i) *Impact of microbe size on cell inactivation*

The inactivation of *Escherichia coli* using Platinized titanium and Alumina coated titanium is shown in Figures 5(a) and 5(b). Two different dimensions of *E. coli* were tested for their inactivation using alumina and platinum coated titanium electrodes, where the length by width of one microbe was (2 $\times$ 1) μ m, and the other microbe had (1 $\times$ 0.5) μ m. Each microorganism was placed in the inactivation area between two electrodes and was subjected to high electric field. As stated before, the application of electric field induces a potential difference across the cell plasma membrane of minimum 1V, which is considered as the condition where pores start to form, subsequently leading to cell inactivation. In Figure 5(a), the electric field was applied through platinized titanium electrodes which induced necessary potential of 1V across the membrane at an electric field strength of 55 kV/cm in the case

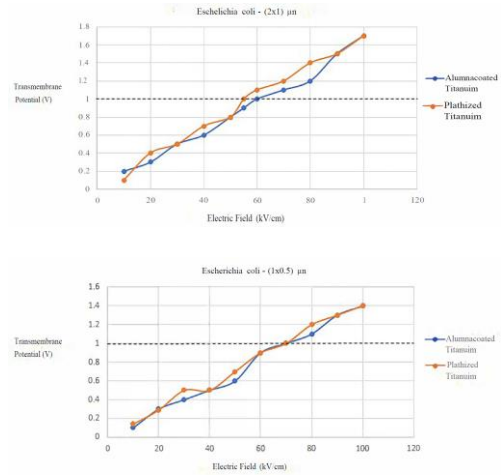


Figure 6. a) Induced transmembrane potential of 1V at 55 kV/cm and 60 kV/cm using platinum coated and alumina coated electrodes
b) Induced transmembrane potential of 1V at 70 kV/cm using platinum coated and alumina coated electrodes

of 2 μ m length *E. coli* and 70 kV/cm in the case of 1 μ m length *E. coli*. Also when alumina coated electrodes were used, the former bacterium required 60 kV/cm and latter required 70 kV/cm for inducing 1V of transmembrane potential. From this analysis, we can understand that smaller cells require more critical electric field E_c to induce 1V for inactivation, where larger cells require less critical field for their breakdown. This fact is very well realized and applicable to both platinized and alumina coated titanium electrodes.

(ii) *Impact of Platinum and Alumina coatings on cell inactivation*

While both the coating materials had the same substrate electrode, the performance of platinum and alumina coatings on titanium was compared and examined for cell inactivation efficiency as shown in Figures 6 (a) and 6(b).

From the above analysis, the alumina and platinum coatings on titanium electrodes had an impact of achieving a transmembrane potential of 1V at 55 kV/cm and 60 kV/cm

respectively for larger *E. coli* cell and at 70 kV/cm for the inactivation of smaller *E. coli* cell for the inactivation. While alumina is a ceramic material, the permittivity plays a major role in electric field application. When platinum is considered it has high conductivity and permittivity, which enables the electric field to be efficiently applied on the inactivation area. However from the analysis, it is understood that though alumina has no conductivity part, it gave equivalent performance when compared with a highly conductive material, when 2 μm coating was used. When compared with other chosen surface materials, alumina has high permittivity which enabled majority of the electric field to pass through it. Hence, the permittivity of alumina has a positive role on the microbial cell inactivation. Thus, alumina is highly recommended to be used as a coating for Noninvasive PEF applications.

Conclusions

Electric field distribution was analyzed in parallel plate electrodes under two types of model setup. Firstly, field distribution through four kinds of surface materials was analyzed to find the best suitable material for noninvasive PEF applications. From the results, alumina exhibited higher percentage of field distribution (82%) than other materials. Hence, alumina is thus regarded as the most suitable material for noninvasive PEF applications. This may be attributed to the permittivity of the material and the other advantage is alumina being nontoxic. After finding the suitable material, the performance of the material was analyzed for its potential on microbial inactivation. Under setup 2, 2 μm of alumina coating on titanium electrodes exhibited equivalent performance when compared with metal coated titanium electrodes especially for smaller cell. In other words, both platinized titanium and alumina coated titanium electrodes inactivated *E. coli* at a critical field of 70 kV/cm for (1 $\times$ 0.5) μm cell. Practical analysis of the two types of proposed electrode setup will aid in deciding the choice of specific setup

for PEF applications, thus increasing the efficiency of pulsed electric field treatment.

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