The Effect of Antibacterial Silver Eleectrodes and the Nature of lon Emission in the Outer Side of Inhibition Zone

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Purpose: The silver anode is highly microbicidal in its inhibition zone when an electric current is applied to living bacteria in a culture medium. After the treatment with electricity, the remaining bacterial population was investigated biochemically and histologically with the electron microscope. The silver ion emission from the electrodes into the medium was quantitatively estimated under various electrical polarizations.

Methods: In this study, P. Aeruginsa was inoculated on four Endo agar plates. The first plate was used as a reference, the second was exposed to a 15 uA constant direct current, the third was driven with a square wave of 15 uA amplitude and the fourth contained a nonpolar siver moiety. After the 51 hours of incubation, inhibition zones appeared around all electrodes except the cathode of DC excitation. All parameters of this study were chosen so as to avoid the possibility of dereterious effect on humans.

Results: It was found that the antibiotic susceptibilities of all samples changed after silver electrode application. The vacuolizations, pilli defects and certain electron-dense granules were foundin the bacterial cells. The silver emission curves plotted.

Conclusion: The silver ion emission was found to be more invasive with square wave than wiyh direct current. Ann Med Sci 1996;5:52-57

Keywords: Silver anode, altermating current, *Pseudomonas aeruginosa*.

The silver anode,generally, has been accepted as antibacterial, antiviral, antifungal and even antitumoral agent.[2,4,7,8,13,17,18,19,20,21,23,24,25] It is well known that, in culture medium, many bacterial strains fully die in the inhibition zone of the silver anode, but the activity outside the inhibition zone and near the cathode have not been thoroughly investigated.

The silver anode is more bactericidal than gold, stainless steel and copper anodes. [11,17,18,20,23] It has been found that a low electric current in the renge of 5-20 uA can be applied without any irreversible damage to eucaryotic cells[4] . Silver electrodes have been successfully employed in clinic cases as anti-infectious (anode) and osteoactive (cathode) agents.[2,3,4,18,19,20,21,23,24,25]

Basically, silver ions are emitted from the anode into the medium (or tissue). However, the antibacterial effect is not completely dependent on the emission of free silver ions, since the same bacterial inhibition can not be obtained by an equal amount of silver used without electricity.[6]

Silver deactivates the bacterial enzymes which are important to the life of a bacterial cell. [8] Silver prevents the

oxidation of glucose, glycerol, fumarate, succinate, *D*- and *L*-lactate, but free sulphydryl groups and NADH are oxidized by the silver. ATP and cytoplasmic components leak out of the cells.[9,12,16] The respiratory and energy metabolism of bacteria collapses.[7,24] However, DNA structure is not distorted.[12]

This paper reports an investigation to determine identical and morphologic changes in bacterial cells and the nature of silver ion emission from the electrodes.

Materials and Methods

Bacteria and Medium: The strain of *Pseudomonas aeruginosa* was isolated from clinical material and a pure culture was obtained. The inoculum was prepared in a broth medium containing exponential phase bacteria (2.8x 10^3 CFU). Four ml of inoculum was added to a flask containing 76 ml of Endo agar at a temperature of 41 ^{o} C. Solution was mixed and equally distributed into four standard petri dishes. The first dish which did not contain an electrode served as a reference. Two dishes contained a pair of pure (99.9%) silver electrodes (2.2x0.8x3 cm) to be used for direct current (DC) and square wave (SQW) excitation. The distance between each electrode in a pair was 73 mm.

Finally, a nonpolar silver electrode was employed in the last dish as a sham electrode(SE) (Fig.1a,b).

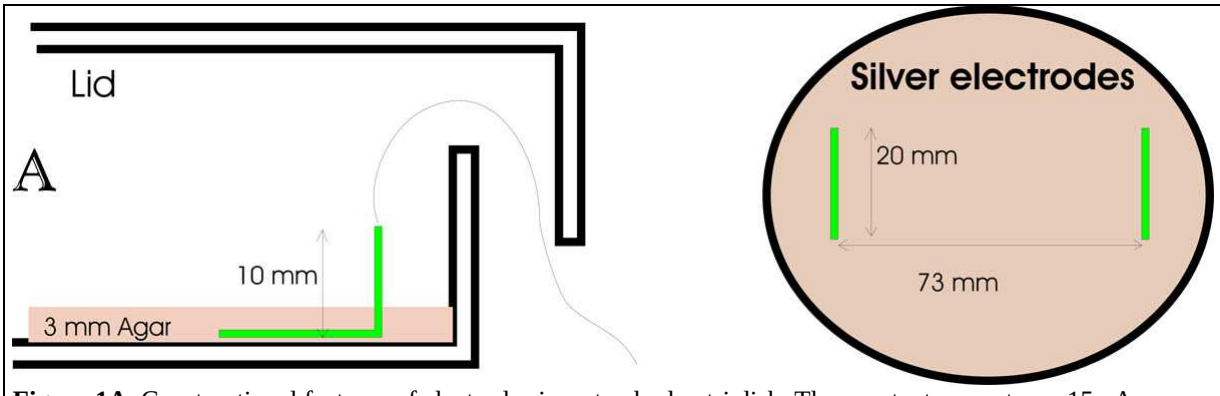

Figure 1A. Constructional features of electrodes in a standard petri dish. The constant current was 15 uA, thickness of agar was 3 mm, the volume of the medium was 20 ml and each electrode surface area was 0.4 cm^2.

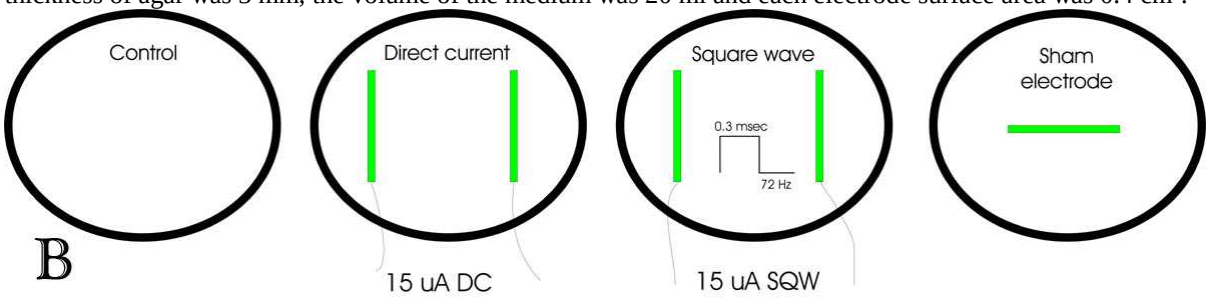

Figure 1B. Experimental setup. P. Aeruginosa was inoculated on to four media. The first was control, the scond was exposed to the direct current, the third was exposed to the square wave, and the fourth contained a nonpolar silver electrode (sham electrode).

Electrical parameters: The average impedance of the medium was found to be 443-+4.5Ω (at 10 Hz, 100 Hz, 200 Hz). A 15 uA direct current was applied to ane pair of electrodes. A SQW (0.3 msec, 72 Hz) was applied to another pair. In this experiment, the frequency and pulse duration used was approximately equal to those of the human heart. The current was stabilized at 15 uA in both electrical applications by the Nihon Kohden Sen3301 Stimulantor, SS102J isolator. Total charge density was 1.296 Coulombs/hour under these conditions.

After the cultures were incubated for 51 hours at 37 ^{o}C, intibition zones were

found around all of the electrodes except for the DC cathode. Bacterial colonies were taken from the inhibition zone boundary of DC anode, SQW anode, SQW cathode, SE and from the control plate and around the DC cathode. The samples were tested by the following:

Chemical Tests: Standard biochemical and physiological tests used for Gram negative rods were performed on all samples.

Antibiotic susceptibility tests: These were performed on all samples with use of commercial standard discs.

Electron Microscopy: The samples of bacterial colonies except those around the SE were separately placed in 3 ml of a glutaraldehyde (5%) buffered solution at Ph 7.4. These solutons were sent to the histology department for electron microscopic analyses. The routine preparation technique was used.

Quantitative silver measurement: Many equal portions of agar were picked from the surfaces of the four agar media. Each agar sample was placed in 3 ml deionized water. Their distance from the electrode(s) was noted with the wxception of those taken from the control plate. The amount of silver in the agar samples was quantitatively determined using atomic absorption spectrophotometry.

Results

Biochemically, there were no significant differences in the results of the biochemical tests between initial and silver electrode treated bacteria with both DC and SQW application as compared to unteated bacteria, although motility and urease activities of the treated bacteria were slightly decreased. However, the antibiotic susceptibility of the bacteria changed after silver electrode application (Table.1).

Table 1. The changes of antibiotic susceptibilities of P. Aeruginosa before and after silver electrode treatment under various electrical current applications

Inhibition zone (MM) Antibiotic pattern	Control	DC Anode	DC Cathode	SQW Anode	SQW Cathode	Sham Electrode
Amicasin	8	17	14	0	14	25
Amoxilin+Clavulanate			fully resistant			
Ampicilin+Sulbactam			fully resistant			
Ceyhtasidime	20	25	20	0	22	0
Ciprofloxacin	30	28	26	15	30	40
Clindamycin			fully resistant			
Nethilmycine	16	15	14	10	17	24
Eritromycine			fully resistant			
Enoxacine	30	32	30	15	32	40
Imipenem	24	25	20	10	25	36

Table 2. The details of ion emission from electrodes (redundant data omitted, * intrapolated data)

	Inhibition zone (mm)	Silver Emission zone(mm)	MIC of Silver (µg/ml)	Silver ion concentration measured from anode to cathode (ugxml /medium)								
				5	6	7	10	12	20	30	65	68mm
DC anode	12	14	2.16	280.3	236.17	224.55	26.32	0.9	0	0	0	0*
DC cathode	0	0	-									
SQW anode	15	22	1.40*	178.09	102.21	49.55	2.71	1.76	1.1	0	3.09	7.74
SQW cathode	6	11	1.50*									
SE	6	7	2.32*	6.96	2.32	0	0	0	0	0*	0	0*
Control	-	-	-	0	0	0	0	0	0	0	0	0

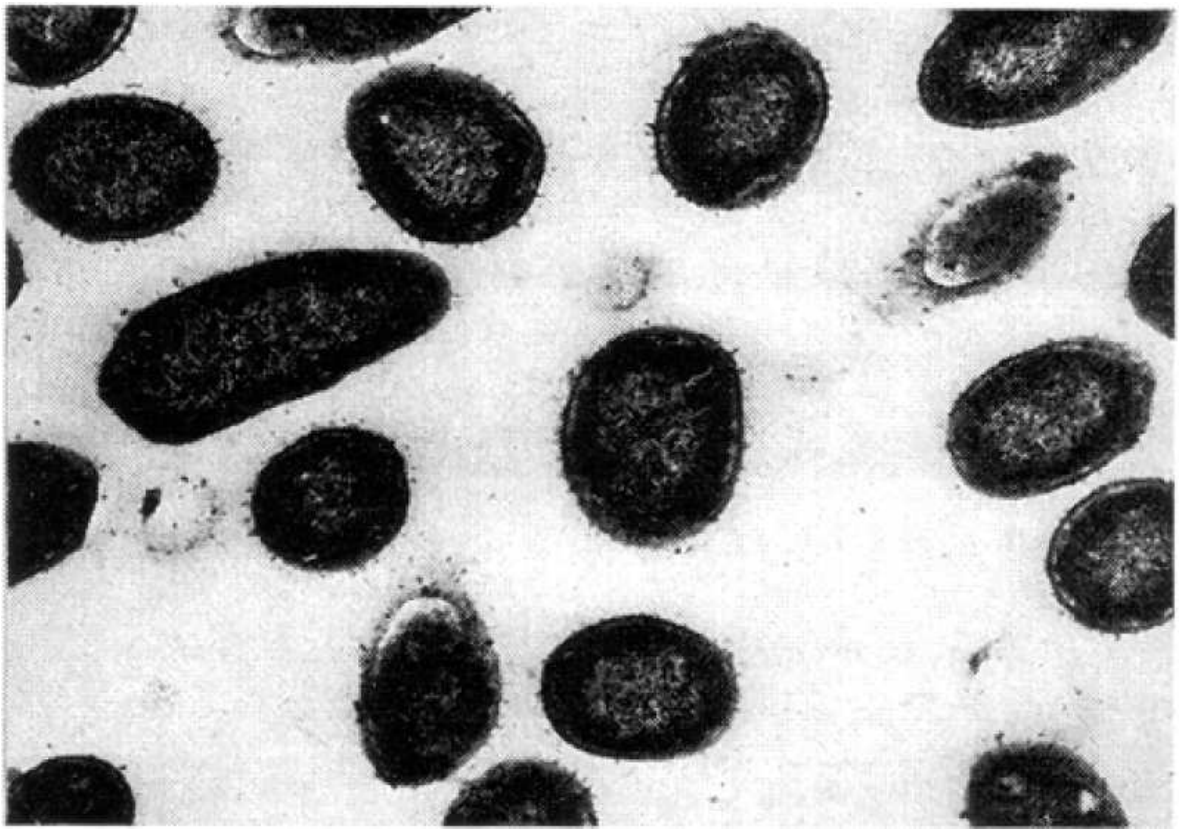

Figure 2A. The original cells (40000x)

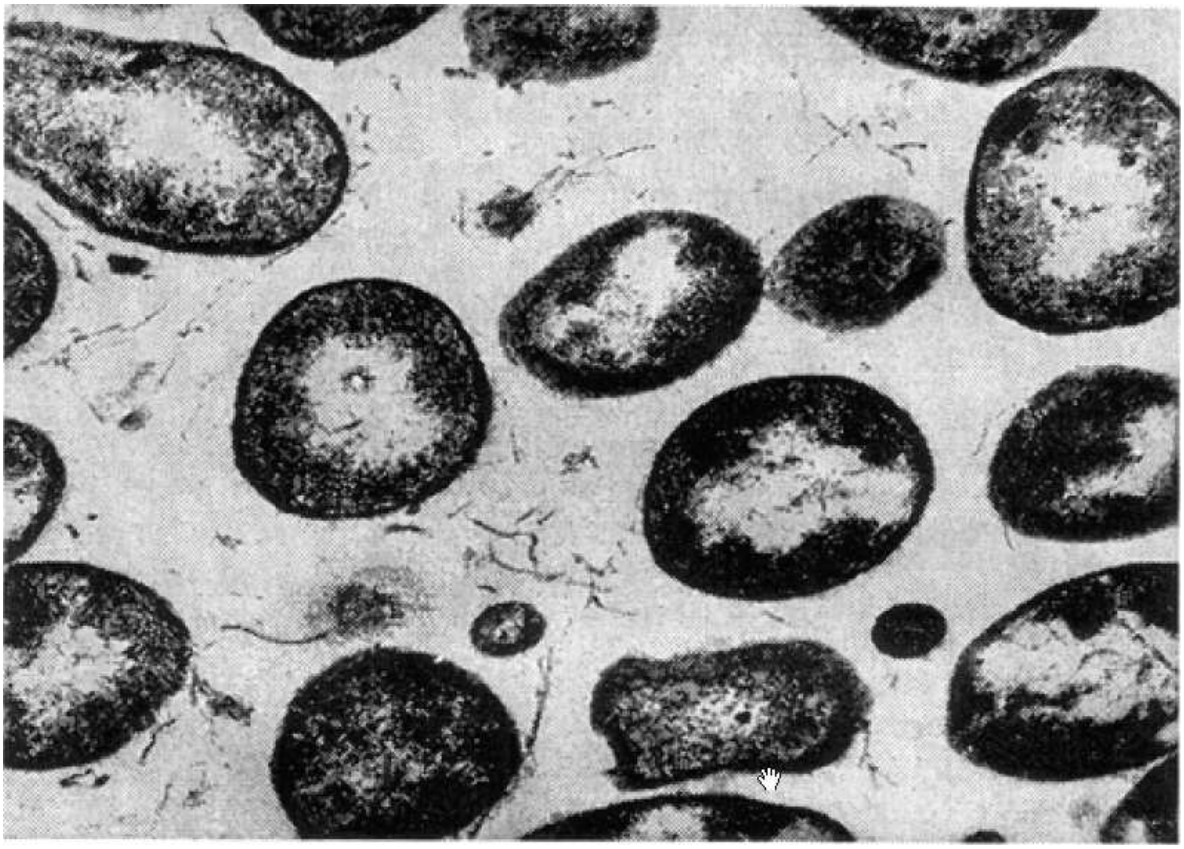

Figure 2B. Around the DC anoe the vacuolization is typical. The cytoıplasmic contents have disintegrated, the celullare membrane and cell wall have kept their original forms, but the pili defects are present;pili material is seen in the background of photograph. Also extracelullar vesicles are present. The vacuolization and pili defects may be important for both immunological and clinical perspectives (60000x)

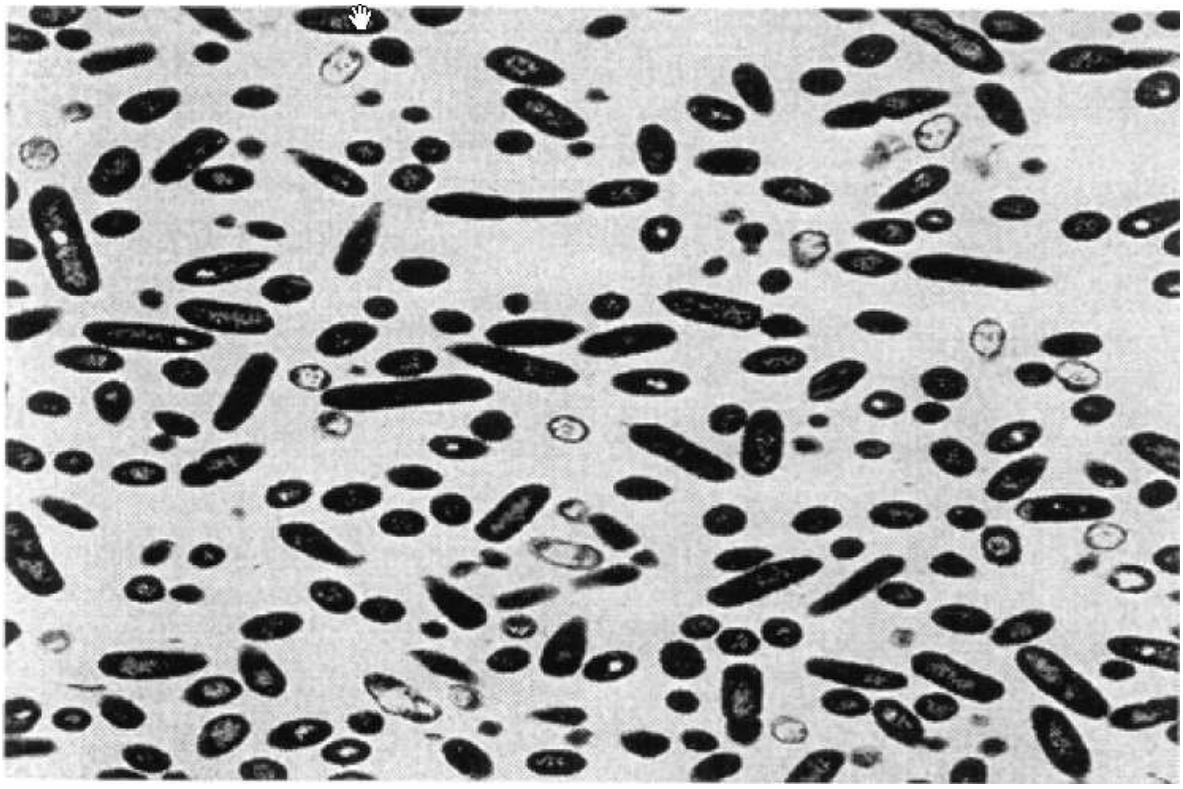

Figure 2C. Around the DC cathode the cells show pleomorphism (8800x)

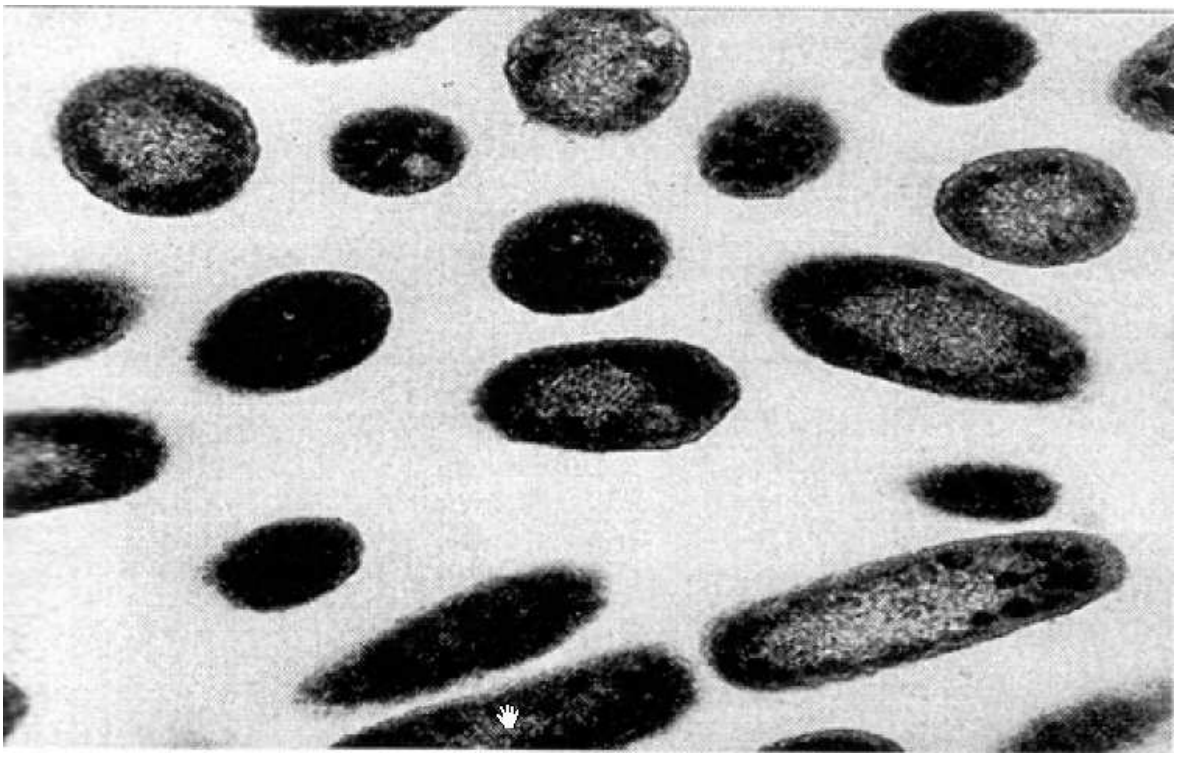

Figure 2D. Many electron dense granules appeared in the cells around the SQW anode (40000x)

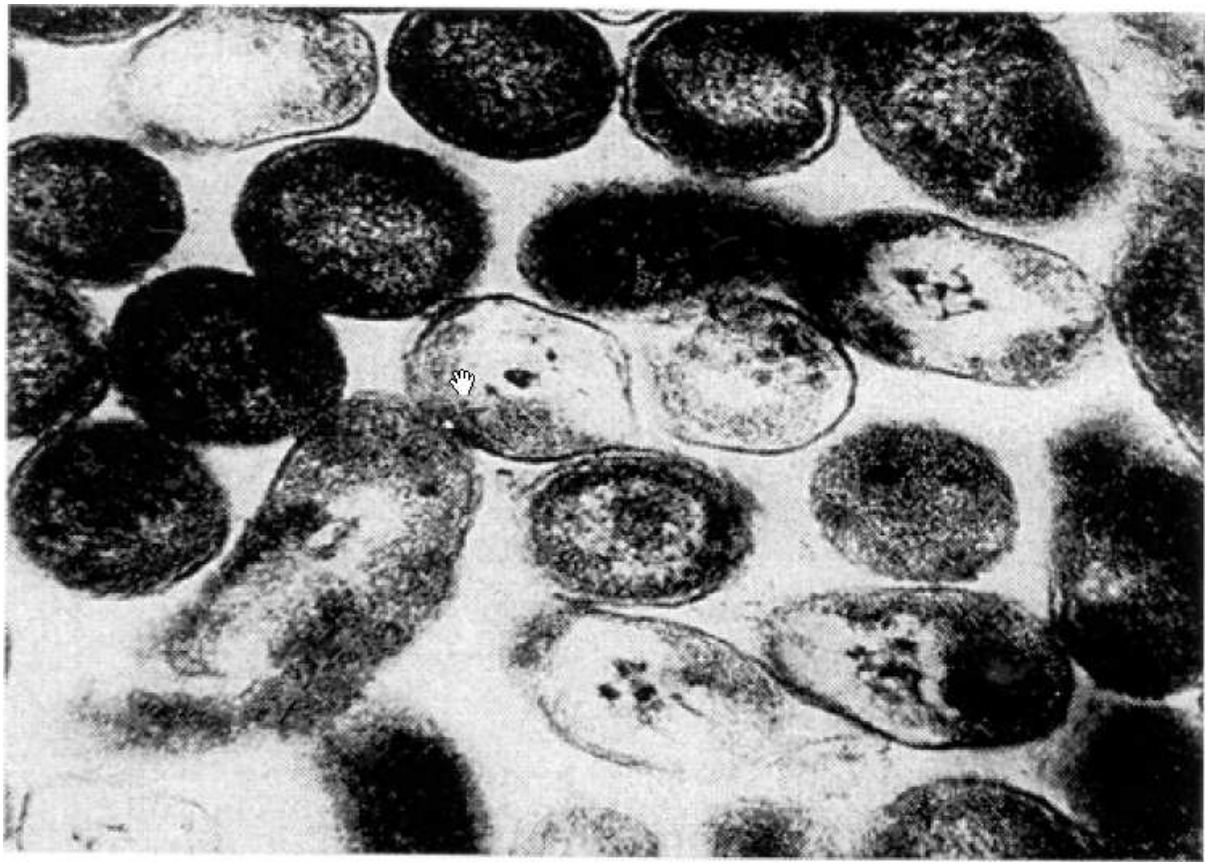

Figure 2E Around the SQW cathode the electron dense granules and vacuolisation are seen in the lesser cells but not pleomorphism (60000x)

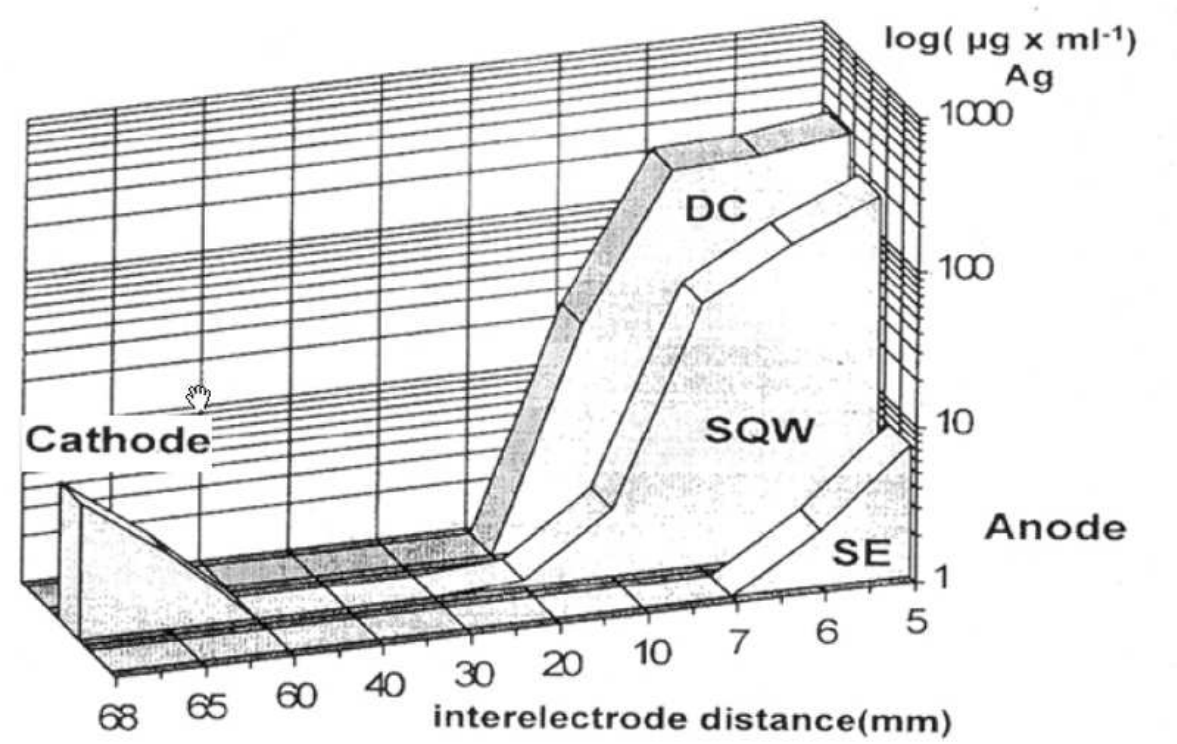

Figure 3. The silver ion emissin from the electrodes (X axis is dividen unequally for clear presentation)
D, distance from electrode (mm); y, amount of emitted silver (ug/ml)

$Y = 40355 \times e^{-0.85 \times d}$

$Y = 9355 \times e^{-0.758 \times d}$

$Y = 7 \times 10^{-9} \times e^{0.0306 \times d}$

$Y = 5700 \times e^{-1.35 \times d}$

The electron microscopic results are summarized in the Fig1A,B,C,D,E. Both the emitted silver and the electric current inhibited the bacterial growth near the electrodes, due to an inhibition zone around the electrodes. The radius of the inhibition zone which correlates with the antibacterial effect depends on both the amount of emitted silver (Table.2) and the electrical parameters.

Discussion

In the direction from the anode to the medium, the antibacterial effect disappeared as the amount of silver on the anode side, declined under the minimum inhibition concentration, and the inhibition zone boundary was sharp at this point. For all tests, the bacterial samples were taken from the inhibition zone boundary, because the bacterial cells on or outside close the boundary of the zone were affected even if they did not completely die. Some important clues of the antibacterial mechanism of silver electrodes can be seen from these cells.

In our preliminary study, the bacteria showed an identity crisis in biochemical test results after exposure to the silver anode application. However, in this study, *P.aeruginosa* did not show a definite genus specific deviation, but the SQW anode treated cells developed antibiotic resistance ($p=0.011$). This sample was monitored throughout four subgenerations and they finally redeveloped antibiotic sensitivity (data not shown). The DC anode and SE effected the antibiotic susceptibility test results more than the other electrodes.

Acording to Takade[22], when *P. aeruginosa* is grown under adverse conditions, it is able to accumulate the intracytoplasmic granules containing polyphosphate structures. As it can be seen in Fig.2D, we detected certain granules in the silver electrode treated cells. The development of these granules may be bue to a nutritional defect followed by the application of the silver electrodes.

On the other hand, a negligible amount of silver ions were spontaneously released from the surface of SE into the medium. They caused the oligoynamic effect on the bacteria.

On the surfaces of both anodes, silver emission was enhanced by the positive polarization which aided the positively loaded silver left on the anode surface. The emitted silver ions were attracted through the medium along a voltage gradient by the cathodes. This became continuous with DC application, but it only appeared during the pulse duration with SQW application. For this reason, when the anode was driven with the SQW, the amount of emitted silver was found to be less but more invasive than that from the DC anode. This suggests that, the emitted silver ions could easily migrate through the pores of agar gel medium by the twitching effect of the SQW oscillation.

No silver emission was found around the DC cathode. The emission of positively loaded silver was inherently occupied by the negative polarization of the DC cathode. However, the SQW cathode does have a certain capability for ion emission despite to the fact that the negative polarization is present. At the SQW cathode, both the radius and amount of silver emission was larger than that of the SE. It is possible that,the reverse electrode polarization accurred during the interpulse time.

In conclusion, bacterial inhibition appeared around all silver electrodes except DC cathode are proportional to the amount of silver emitted from electrodes. However, for elinical treatments, the DC polarization of silver electrodes seems to be more feasible than the others.

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