which is more strict comparing with the condition eq. (2.10) when $v = 2$.

c) Serfling's proof is more complex. But the proof in this note is much easier.

Using Theorem 1, we can easily obtain

Theorem 4. Suppose that function $g(a, n)$ is well defined for all $a \in \overline{\mathbb{N}}$, $n \in \mathbb{N}$ and satisfies "super additivity" eq. (2.16); $\lambda(n)$ is a nonnegative function defined on $\mathbb{N}$. If for any $a \in \overline{\mathbb{N}}$, $n \in \mathbb{N}$,

$$\mathrm{E}|S_{a,n}|^v \leqslant g(a,n)\lambda^v(n), \tag{2.18}$$

then

$$\mathrm{E}M_{a,n}^v \leqslant g(a,n)\left(\sum_{i=0}^{[\log n]}\lambda(2^i)\right)^v \tag{2.19}$$

for any $a \in \overline{\mathbb{N}}$ and $n \in \mathbb{N}$.

Proof. Using condition eq. (2.16), we easily get eq. (2.19) from equations (2.18) and (1.5).

Remark 3. Comparing this result with Moricz's Corollary 3[4], we drop off the condition in ref. [4] that $\lambda(n)$ is nondecreasing.

In a word, the method of binary digit representation is a simple and effective one to estimate the moment of the maximum partial sums, which simplifies the proofs and weakens their condition of many classical results, and extends applied ranges of the results.

Acknowledgement This work was supported by the National Natural Science Foundation of China (Grant No. 19671078), the Doctoral Program Foundation of Institute of High Education and a grant of Chinese Academy of Sciences.

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(*Received April* 8, 1998)

Experimental study and mechanism analysis on cell electroporation due to low-intensity transient electromagnetic pulses

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Abstract The phenomenon of cell electroporation due to low-intensity transient electromagnetic pulses was studied by using a broad band transverse EM-wave cell (BTEM CELL). The experimental results and preliminary mechanism analysis are presented.

Keywords: **electromagnetic pulses, electroporation, cell fusion, mechanism analysis.**

STRONG electric pulses can cause a temporary loss of semipermeability of tissue or cell membrane, and thus lead to pores on the membrane. This biophysical phenomenon is called electroporation. In this field, many investigators have focused on using single or several electric pulses of intensity in kilovolts per centimeter and of duration in microseconds to milliseconds. Weave, Zimmermann and Song have presented some mechanism analysis of electroporation and applications of this biotechnique[1—3].

In the field of electroporation, electric field intensity less than 10^4 V/m is believed to be low intensity. Up to the present, only a few investigators, such as Song, have studied the electroporation related biological effects due to low-intensity electric field. He applied the low-frequency electric field of intensity 50 to 200 V/cm to *E. coli* (JM105) cell, and successfully observed the gene transfection when the DNA/cell ratio was 50 to 75[4]. In his experiment, sine, square and triangle wave electric field generators were used with frequency from 0.1 Hz to 1 MHz. However, those results were of gene transfection after all, which were not applicable to electroporation directly.

Based on the study on biological effects of transient electromagnetic pulses[5], electric pulses of the peak intensity 20 V/cm with repeat frequency 300 Hz, width 100 ns, and the rising time 1.2 ns were used to radiate the animal erythrocytes. Since both the duration and the rising time were fairly short, they were called transient electromagnetic pulses. After irradiation, pores on the membrane were observed by using a scanning electronic microscope. It was the first time to prove the electroporation due to low-intensity electric pulses.

1 Materials and method

An MFD-1 nanosecond pulses generator was used. It was able to generate square waveform with width 2 to 100 ns at repeat frequency 1 to 300 Hz and amplitude up to 200 V. In this experiment, pulses with 100 ns duration at 300 Hz repeat frequency and 187 V amplitude were used. The broad band transverse EM-wave cell (BTEM cell) was used to simulate the free space radiation condition. The detailed characteristics of the BTEM cell and its applications can be found in refs.[5,6]. In the experimental system, a thermal control device was added to keep the temperature inside the BTEM cell at 37 ℃. Each experiment was repeated twice, and each time two samples were used either in the control group or in the radiation groups.

(ⅰ) Electroporation observation under a scanning electronic microscope. Chick erythrocytes were used in this experiment. A 10 mL mixture of chick blood and Alsever's liquid was rinsed three times by D-Hanks and once by isotonic low-conductivity liquid. After detaching, the chick erythrocytes were diluted to 5 mL, and divided into six groups each with 0.1 mL. All groups except the control group were put into the BTEM cell to accept irradiation of 20, 40, 60, 80 and 100 *min* separately.

Each time after irradiation, 0.5 mL 1% glutaraldehyde was added, and it was supported by Formvar. Then glutaraldehyde-osmic acid was used for cell fixing, and acetone-isoamyl acetate four concentration grades dewatering (50%, 70%, 90% and 100% four grades) was performed. Following the dioxide critical point drying and gold plating, the scanning electronic microscope was used to observe chick erythrocyte electroporation.

(ⅱ) Field-induced cell fusion. The rabbit blood and chick blood were mixed with Alsever's liquid, and the following process was the same as the steps above. Then each group except the control group was irradiated for 20, 40, 60, 80 and 100 min separately. Each time after irradiation, 1 460 medium was added to culture the cells for 120 min. After flaking, it was dyed with Giemsa for 10 min. Then the microscope was used to observe the cells. From the cell's size and with or without nucleus it was easy to distinguish chick erythrocyte from rabbit's. The detailed steps and the reagent batch formula can be found in reference [7].

2 Conclusion and discussion

(ⅰ) Cell electroporation. The pores on the erythrocyte membrane can be observed directly by the scanning electronic microscope. The ratio of electroporation was greater than 2% with pores from 20 to 500 nm. At different irradiation duration between 20 and 100 min, all the pores can be seen. However, there was no obvious relation between the irradiation dosage and the electroporation ratio. The peak value with the ratio up near to 3% can be got at 100 min. Fig. 1 gives the cell electroporation photos from the scanning electronic microscope. While in the control group, no electroporation was found.

Figure 2 illustrates some processes of cell membrane electroporation. Fig.2(a) shows the protuberance formed by cytoplasm before the membrane pore appeared; in fig.2(b), cytoplasm was blowing from

the cell to form a pore on membrane; in fig.2(c), it was the depression when the membrane was healing.

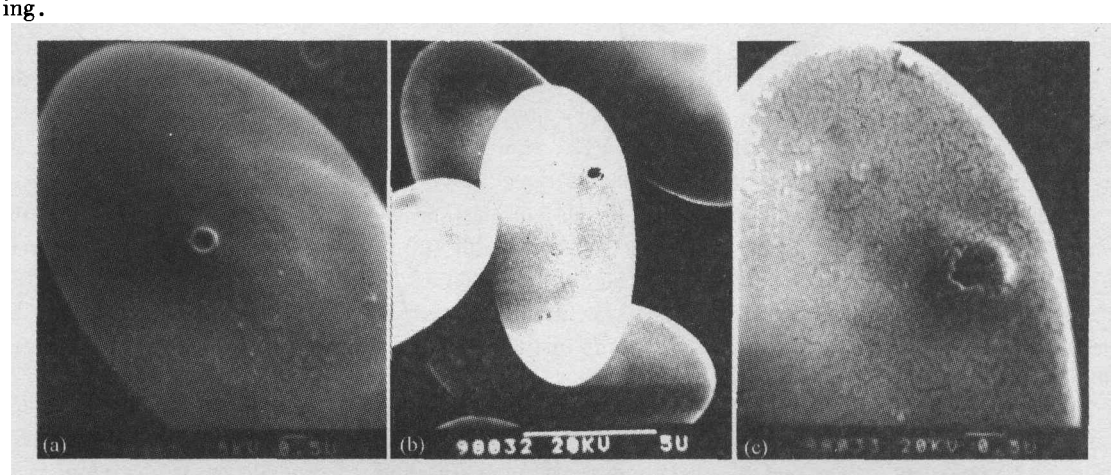

Fig.1. Cell electroporation photos.

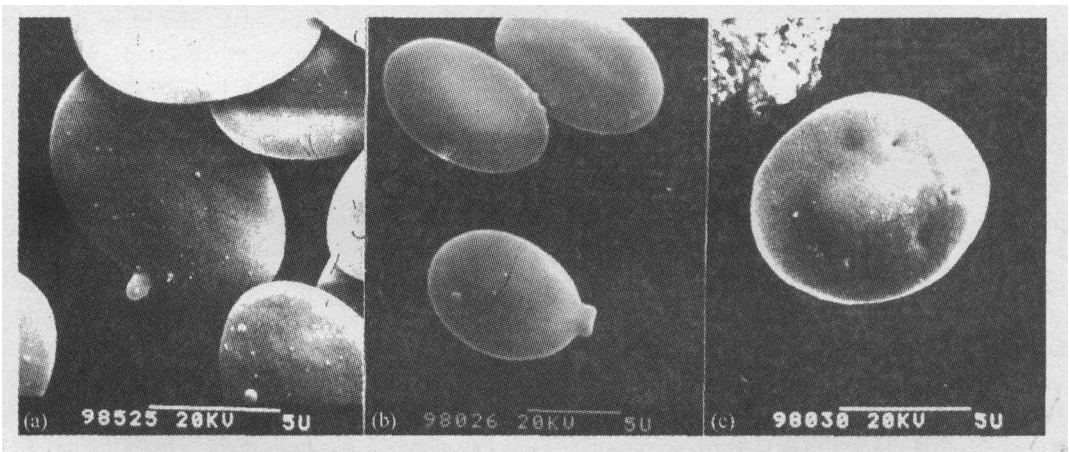

Fig.2. Electroporation process.

(ii) Field-induced cell fusion. Compared to the electroporation, the ratio of cell fusion was fairly low. It was less than 5‰. The erythrocyte cells fusion in chick-chick, rabbit-rabbit and chick-rabbit can all be observed. Fig. 3 shows the erythrocyte fusion between chick and rabbit.

(iii) Discussion. In BTEM cell, the peak electric intensity was 20 V/cm at the position where the biological samples were located. Based on the facilitated spherical cell model, the transmembrane potential can be estimated[8].

When an oscillating electric field is used, the maximal transmembrane potential generated for the spherical cell can be calculated by

$$\Delta\Psi_m = \frac{1.5RE}{\sqrt{1 + (\omega\tau)^2}}, \quad (1)$$

where

$$\tau = \frac{RC_m}{r_i + r_e/2}. \quad (2)$$

In the above equations, R is the radius of the cell, E is the applied field intensity, ω is the angular frequency of the electric field, τ is the dielectric time constant

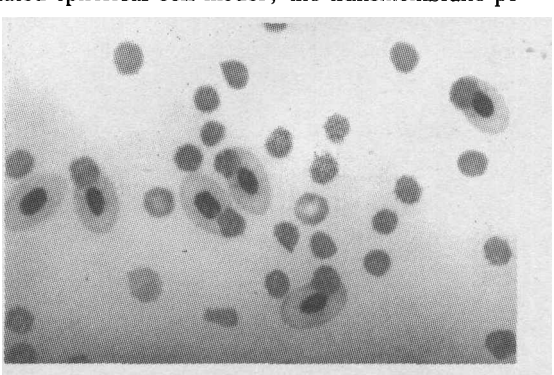

Fig.3. Field-induced cell fusion.

of the cell membrane, C_m is the membrane capacitance, and r_i and r_e are the resistivity of the cytoplasmic fluid and that of the outside respectively. Under the low frequency condition ($\tau \ll 1$), eq. (1) is simplified to

$$\Delta\Psi_m = 1.5RE. \quad (3)$$

This equation can also be used to estimate the maximum transmembrane potential at high frequency. Since the electromagnetic pulses were used in this experiment, frequency domain analysis was required to calculate the transmembrane potential induced by the electric pulses. If the radius of the cell R is 10 μm, the maximum transmembrane potential 18 mV can be obtained, which is far less than 500 mV the electric breakdown threshold of cell membrane. So low-intensity electric field cannot directly lead to cell membrane electroporation by the electric breakdown. In this point, the electroporation mechanism between low-intensity and high-intensity electric field is quite different.

On the cell membrane many transient pores were generated and decayed spontaneously since the random thermal motion. The pores were very small, and only small inorganic molecules can cross it. Ions cannot cross the membrane directly because it has a high-energy barrier to them. According to the model of interaction between electric field and cell membrane[9,10], the increase of transmembrane potential can enlarge the small pores on membrane. Under the repeat action of pulses, the micro-pores became larger and larger. Based on our calculation, the larger the pore was, the less energy was needed for an ion to cross the membrane[11]. While the micro-pores were large enough, many small molecules and ions can cross the membrane and enter the cell by osmotic pressure, leading to cell swelling which increased the surface of the membrane. Surface tension generated by the swelling membrane made the micro-pores larger and larger until a pore was presented and cytoplasm was blowing outside to balance the osmotic pressure. Since part of the cytoplasm was ejected, there would be a depression after the pore healed.

The ratio of cell fusion was fairly low, possibly because the mechanism of cell fusion was different from that of cell electroporation.

Since the low-intensity electric field was used, almost all cells were able to survive. It means this electric field is safe to cells and can be applied to biomedical engineering techniques. For example, it is useful in gene transfection and can be used to increase the drug uptake by cells. The results can also explain some athermal biological effects caused by transient electric pulses[12]. In the on-going experiment, we find that these low-intensity electric pulses can increase the toxicity of anticancer drug to cancer cells. Therefore, we are going to determine whether it is related to cell electroporation.

The research of cell electroporation due to low-intensity transient electric pulses is in progress. In this new field, the mechanism is not very clear, and further research is required.

Acknowledgement This work was supported by the National Natural Science Foundation of China (Grant No. 69771020).

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(*Received March* 23, 1999)

Synthesis of cage-like octa(trimethylsiloxy)silsesquioxane

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Abstract Cage-like octa(trimethylsiloxy)silsesquioxane $[(Me_3SiO)SiO_{1.5}]_8$ has been synthesized via the trimethylsilylation of cubic tetramethylammonium silicate octamer $[(Me_4NO)SiO_{1.5}]_8$ with chlorotrimethylsilane. The silicate octamer can be selectively formed by the reaction of tetraethoxysilane $Si(OEt)_4$ with aqueous tetramethylammonium hydroxide in equal molar ratio. Elementary analysis, FT-IR, ^1H, ^{13}C, ^{29}Si NMR are used to characterize these silsesquioxanes.

Keywords: **cage-like, silsesquioxane, silicate octamer, trimethylsilylation.**

THE silsesquioxanes with general formula $(RSiO_{1.5})_n$ ($n \geqslant 4$, R = hydrogen, alkyl, aryl or organofunctional groups) are a sort of polyhedron compounds which are also called cage-like silsesquioxanes due to these structures resembling cages. When $n = 8$ in $(RSiO_{1.5})_n$ the cage-like silsesquioxane forms a cube with 8 Si atoms at the vertex and the contiguous Si atoms connected by one O atom (T_8 for short). Recent investigation indicates that the structure of the cubic octamer corresponds to one of secondary building units in framework of crystalline silica or zeolite and the polymers based on T_8 silsesquioxanes are expected to have high hardness and high thermostability. Therefore, it is suggested that polymerization of the T_8 silsesquioxanes with organofunctional groups by proper methods leads to formation of organic-inorganic hybrids with molecular level and defined structure[1]. These materials attract wide attention owing to their potential application to structural ceramic, nanocatalysts, flinty paint, membrane separations, medical materials, etc.

Syntheses of alkyl and aryl substituted T_8 silsesquioxanes are not quite difficult and have been reported[2]. Though progress has been made in the preparation of hydrogen and organofunctional groups substituted T_8 silsesqiuxanes in recent years[3, 4], the yield is very low and it is impossible to afford enough amount to meet the need of polymerization. Thus we are interested in the preparation of organosiloxy substituted cage-like silsesquioxanes $[(R_3SiO)SiO_{1.5}]_8$, and here we report a synthetic procedure of octa(trimethylsiloxy)silsesquioxane $[(Me_3SiO)SiO_{1.5}]_8$ as a model compound and its characterization.

1 Experimental

(ⅰ) Materials and instruments. Tetraethoxysilane, hexane and N, N-dimethylformamide (DMF) were commercial agents. Tetramethylammonium hydroxide was used in 10% aqueous solution, chlorotrimethylsilane was redistilled before use. ^1H, ^{13}C NMR spectra of the synthesized compounds were recorded on a UNITY-500 NMR instrument and the ^{29}Si NMR spectrum on a UNITY-200. TMS was used as internal standard, $CDCl_3$ as solvent for $[(Me_3SiO)SiO_{1.5}]_8$, CD_3OD for $[(Me_4NO)SiO_{1.5}]_8$ and chromium acetylacetonate as relaxation agent. FT-IR spectrum was obtained on a NICOCET 5-SX IR spectrometer.