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Full Length Research Paper

Perforate on CHO cell membranes induced by electromagnetic pulses irradiation observed by atomic force microscopy

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Atomic force microscopy (AFM) has been used to visualize the morphological change on the surface of Chinese hamster ovary (CHO) cell membranes before and after electromagnetic pulses (EMP) irradiation. The results show that there were different sizes and shapes of membrane perforate (width ranging from 0.39 - 0.66 μm; depth of 392.95 nm) after 200 pulses of EMP irradiation at 400 kV/m. The results suggested that EMP irradiation can directly cause CHO cell membranes to perforate. The numbers of cell membrane perforates increased with an increase in applied EMP field intensity and an increase in the number of EMP pulses. EMP induced membrane perforate may play a very important role in EMP biological effects.

Key words: Electromagnetic pulse (EMP), atomic force microscope, CHO cell, cell membrane.

INTRODUCTION

Along with the development of electromagnetic wave technology, the denomination and intension of spatial electromagnetic radiation are increasing quickly. From televisions and computers to microwaves, our living and working environments are filled with devices that use electromagnetic radiation (Ahlbom et al., 2004). Electromagnetic pulse (EMP) is a special kind of EMF irradiation which is produced as short high-voltage pulses with an extremely fast rise with a spectral bandwidth up to 1.5 GHz. The unique properties of EMP have raised concerns about its effect on living systems and possible health hazards to humans (Adair, 1995; Merritt et al., 1995). Until now, most research on EMP-induced effects was focused on behavioral and physiological changes after EMP exposure (Frei et al., 1995). In addition, our previous work also showed that EMP influences major biological systems including nervous, endocrine and reproductive system (Seaman et al., 1999; Bao-Feng et al., 2007; Lihua et al., 2008). A number of studies have suggested that cell membranes may be a target of EMP irradiation (Lai et al., 1998).

Over the past 2 decades, advances in specify instrumentation have greatly contributed to cellular and molecular biology tools available to investigate nanoscaled biomolecular structures. Applications of atomic force microscopy (AFM) to biology have provided unique opportunities to visualize and manipulate functional biological structures with nanometre resolution in physiological conditions (Trache and Meininger, 2008; Engel et al., 2000). Force measurement with AFM has become a tool of choice to probe and manipulate a wide variety of cellular events (Kuznetsova et al., 2007; Radmacher et al., 2007; Wang et al., 2007). Indeed, the opportunity to precisely address nanoscaled structures on individual cells and to measure forces in the piconewtons range has provided unprecedented mechanistic detail of cellular processes.

Previous studies have shown that cell membrane damage can be induced by EMP irradiation (Lihua, 2001).

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In the present study, we applied EMP irradiation of varying intensity and pulse applications to CHO cells and observed changes to the cell membrane morphology using AFM. Our studies show that EMP can directly cause CHO cell membranes perforate to find some direct evidences for the mechanism that CHO cell was damaged by EMP.

MATERIALS AND METHODS

Cell cultures

Chinese hamster ovary (CHO) cells were maintained in RPMI 1640 (GIBCO, invitrogen corporation, CA) supplemented with 10% fetal bovine serum (Biosource, USA) supplemented with penicillin (100 units/ml) and streptomycin (100 µg/ml). 105 cells were seeded in 35 mm dishes use for the experiments when the cells were in the time of exponential phase.

Irradiation treatment of CHO cells

EMP emulator (manufactured by Mian Yang Ninth Academy) and GTEM-Cell (made by Southeast University, Jiangsu, China) were used in present study. The following electric field intensities and pulses time were used in this study: 50, 100 and 200 kV/m (200 pulses) and 400 kV/m (10, 100 and 200 pulses). The interval time between pulses was 4 seconds. The irradiation groups were irradiated in the GTEM-CELL and the control was shame-irradiated in the GTEM-CELL.

Atomic force microscopy assays

Cells were fixed in 2% glutaraldehyde (Sigma, USA) for 30 min and then immediately observed following EMP irradiation. First looking for the available cells under microscopy to fix the cutted dish piece on the microstation of atomic force microscopy (AFM) (SPM-9500J3, SHIMADZU, JAPAN), the cells were scanned by AFM probe with consecutive contact-mode by SPM-online2.45 software (SPM-9500-J3, SHIMADZU, Japan) control. At least 3 cells were scanned in each sample and the parameters were analyzed for 3 dimensional image reconstruction by SPM-offline2.2 software (SPM-9500J3, SHIMADZU, JAPAN).

RESULTS

Consecutive contact mode-AFM with standard silicon cantilevers were used to evaluate the surface topography and roughness of CHO cell membranes. In the control group, the surface morphology of CHO cell membrane is slightly undulated (Figure 1A and 1B). After 200 pulses of EMP irradiation (50 kV/m), the surface of the cell membrane is slightly undulated. There is some introcession on the surface but no significant differences with the control group (Figure 2A and 2B). After 200 pulses at higher EMP irradiation (100 kV/m), the surface of the cell membrane is more smooth with slight undulation and introcession, but, again, there is no significant difference with the control group (Figure 2C and 2D). After 200 pulses at continually higher EMP irradiation (200 kV/m), some significant differences begin to emerge compared to the control group. This includes some introcession that is irregular in size and starts to resemble perforations on the surface of the cell membrane (Figure 2E and 2F). After 10 pulses at higher EMP irradiation (400 kV/m), the surface morphology of CHO cell membranes become nonuniformly or unregularly and undulated, but perforation phenomenon was not apparent (Figure 3A and 3B). After 100 pulses of EMP irradiation (400 kV/m), the surface morphology of CHO cell membranes becomes more smooth and the perforation phenomenon is still not apparent (Figure 3C and 3D). After 200 pulses of EMP irradiation (400 kV/m), there were some perforations where the shape and size is irregular and different compared to the control (Figure 3E, 3F and 3G). The diameter of perforation ranges from 390 to 660 nm and the depth is 392.95 nm. The number of cell membrane perforations decreased when both the field intensity and number of pulses applied were decreased. In contrast, the number of cell membrane perforations increased when both the field intensity and pulses were increased. Our results showed that EMP-induced cell membrane perforation was immediate after exposure to the irradiation treatment.

DISCUSSION

In the present study, we investigated the effects of EMP irradiation on CHO cell membranes using AFM. Cell membranes were subjected to various EMP irradiation conditions including increases in the field intensity or the number of pulse applications. First, we varied the strength of the field intensity in order to determine under what condition we could see changes in membrane morphology.

With 200 pulses of EMP irradiation at 50 and 100 kV/m, the surface of the cell membrane is slightly undulated. There is some introcession on the surface but no significant difference is observed compared to the control group (Figure 2 A, B, C and D). With 200 pulses of EMP irradiation at 200 kV/m, significant differences emerge compared to the control group (Figure 2 E and F) including some introcession that is irregular in size and starts to resemble perforations on the surface of the cell membrane.

Next, we varied the number of pulse applications applied at 400 kV/m. After 10 and 100 pulses of EMP irradiation, the surface morphology of CHO cell membranes become irregularly undulated but there perforation phenomenon was not apparent (Figure 3 A, B, C and D). After 200 pulses of EMP irradiation, there were some perforation in which the shape and size is irregular and different (Figure 3 E, F and G). The diameter of these perforations is at least 390 to 660 nm and the depth is 392.95 nm. Based on these data, the numbers of cell membrane perforations decreases with both reduced EMP field intensity and when there is a reduction in the number of

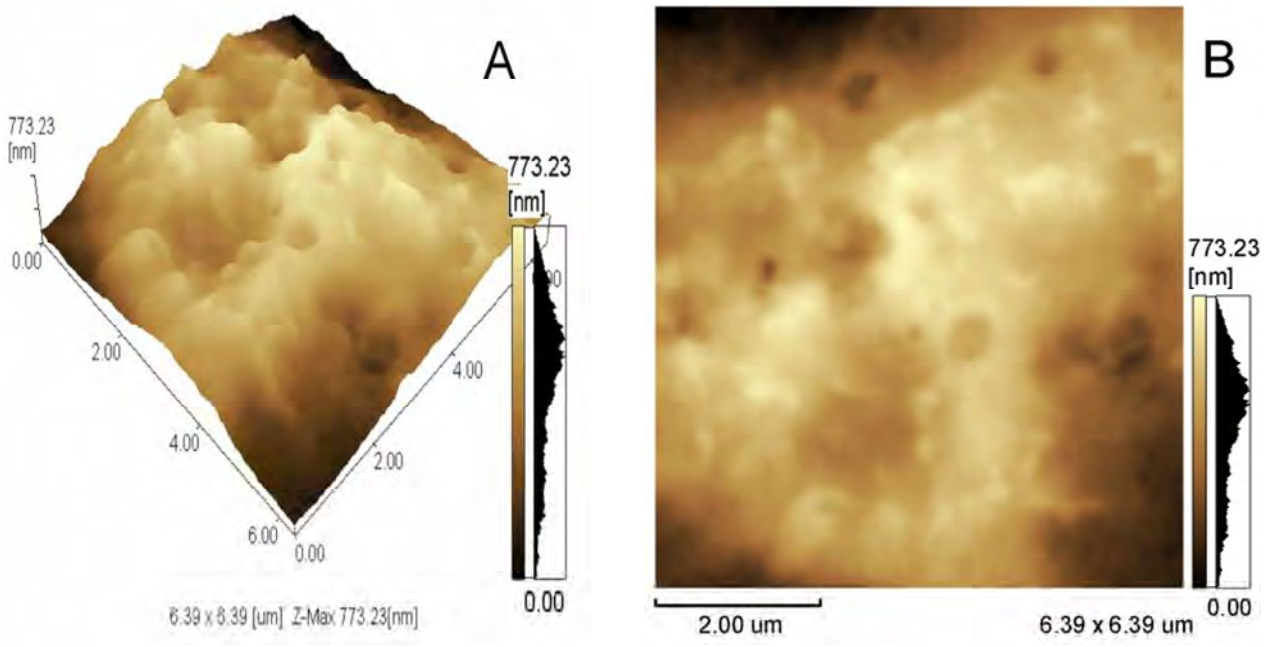

Figure 1. AFM images of cultured CHO cell membrane (control). 10^5 cells were seeding in the 35mm dishes to use for the experiments when the cells were in the time of exponential phase. The control was shame-irradiated in the GTEM-CELL. Then cells were fixed in 2% glutaraldehyde 30 min for the observation after different treatment immediately and then were scanned with consecutive contact-mode by SPM-online2.45 software control , At least 3 cells were scanned in each sample and the parameters were analyzed to do three-dimensional image reconstruction by SPM-offline2.2 software. **(A)** 3-dimensional image of the cell membrane. **(B)** Surface image of the cell membrane.

pulse applications. In contrast, an increase in the field intensity and the number of pulse applications led to an increase in cell membrane perforation. Some indirectly evidences were seen in some experiments immediately after exposure to EMP radiation (Xiao-zhe et al., 2002). These results led to the hypothesis that membrane perforate could be induced directly by EMP radiation because the total width of a double deck membrane is approximately 7 - 8 nm. In our present study, there were some perforates where the diameter of the perforation ranged from 390 to 660 nm and the depth was 392.95 nm. According to these results, we can see that EMP radiation under certain conditions can perforate the cell membrane directly and also penetrate the entire cell. Our current studies have shown that these experimental parameters are important in determining the effect of the damage in membranes is induced by EMP. EMP parameters such as pulses time and field intensity is the main reason to cause the different effects. 3 samples of cells were chosen at random and measured under each condition and the results were consistent. Unfortunately we were unable to accurately measure the percent change in perforation because of the resolution limits of AFM.

EMP is a form of high energy non-ionizing radiation. It has energy concentration, wide band almost cover all of the electromagnetic band. EMP radiation causes damage to cells by non-thermal effects targeted at the cell membrane (Loginov, 1991). The resting potential of the cell membrane suddenly rises to 100 mV, caused by enhanced transmembrane potential by EMP, resulting in changes in the macromolecule in membrane inconvertible configuration alteration. These events lead to nonreversible membrane shock and damage to the tissue. It damages the cell membrane by electroporation through additive effects of long exposure time at low field intensity EMP (Bao-yi et al., 1997, 2006). AFM approach has been widely applied to study cellular membrane and to map the distribution of membrane receptors in several recent studies (Iscru et al., 2008; Lamontagne et al., 2008). AFM can measure forces in the piconewtons range has provided unprecedented mechanistic detail of cellular processes (Muller and Engel, 2002; Pamir et al., 2008). The quantity, duration and formation of transmembrane potential directly affect cell function and structure. Cell membranes play an important role in cell function and regulation. Damage to cell membrane/morphology can result in the loss of many important physiological functions. Cell membranes have strong and important relationships with material transportation, energy conversion, information transfer, cell surface recognition, cell movement and differentiation. Many studies over the decades have shown that alteration in cell membrane in an indicator of cell damage and alterations in cell membrane structure can directly affect proper functioning of the cell to induce other effects (Bao-Feng et al., 2007; Lihua et al., 2008; Williams et al., 1980; Lu et al., 1999).

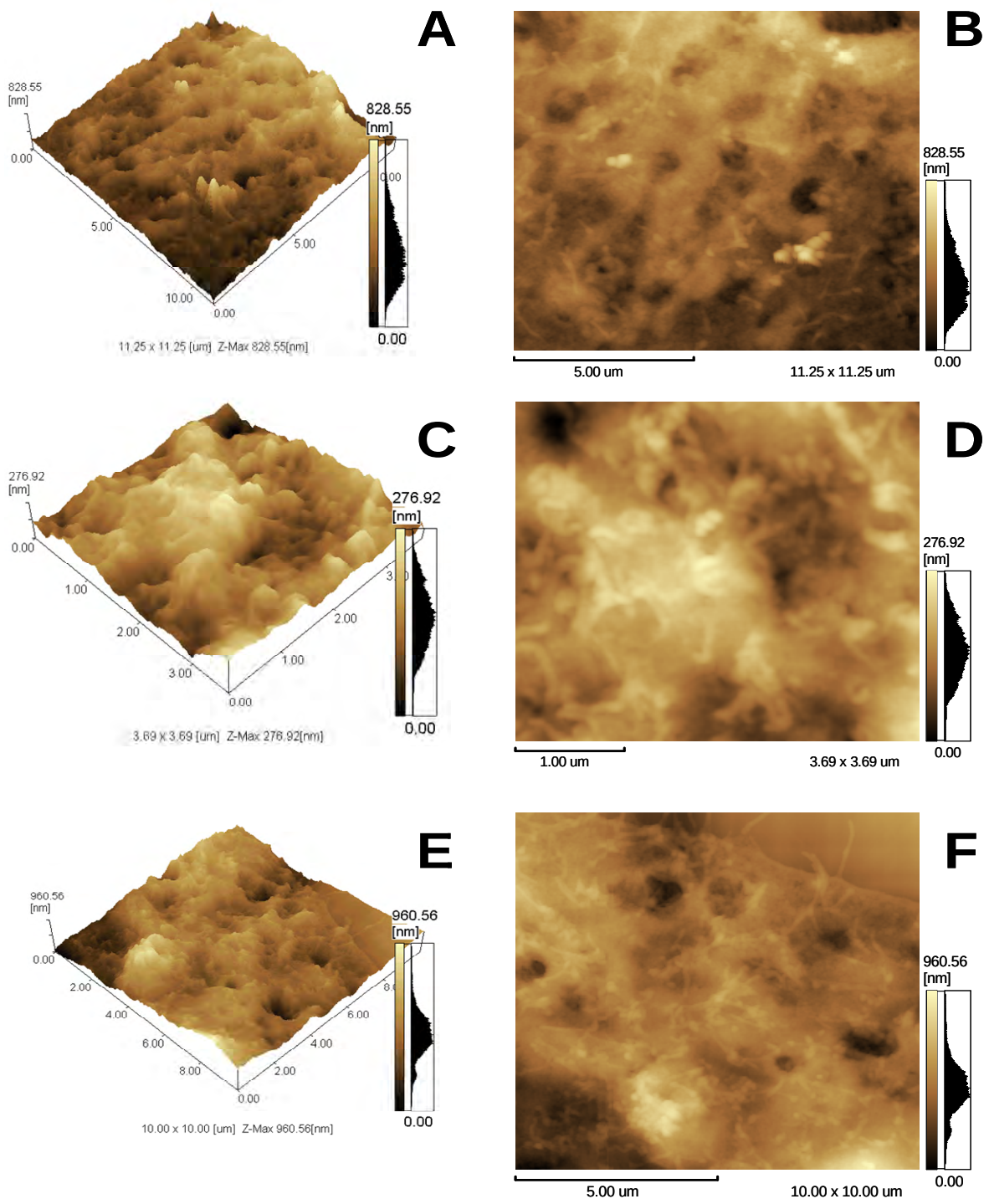

Figure 2. AFM images of CHO cells after 200 pulses of 50kV/m, 100 kV/m and 200 kV/m EMP irradiation. 10^5 cells were seeding in the 35mm dishes to use for the experiments when the cells were in the time of exponential phase. The irradiation groups were irradiated 200 pulses EMP irradiation in the GTEM-cell, and field intensity is 50 kV/m, 100 kV/m and 200 kV/m respectively. Then cells were fixed in 2% glutaraldehyde 30 min for the observation after different treatment immediately and then were scanned with consecutive contact-mode by SPM-online2.45 software control , At least 3 cells were scanned in each sample and the parameters were analyzed to do three-dimensional image reconstruction by SPM-offline2.2 software. **(A)** 3-dimensional image of CHO cell membrane after 50 kV/m EMP irradiation. **(B)** Surface image of CHO cell membrane after 50 kV/m EMP irradiation. **(C)** 3-dimensional image of CHO cell membrane after 100 kV/m EMP irradiation. **(D)** Surface image of CHO cell membrane after 100 kV/m EMP irradiation. **(E)** 3-dimensional image of CHO cell membrane after 200 kV/m EMP irradiation. **(F)** Surface image of CHO cell membrane after 200 kV/m EMP irradiation.

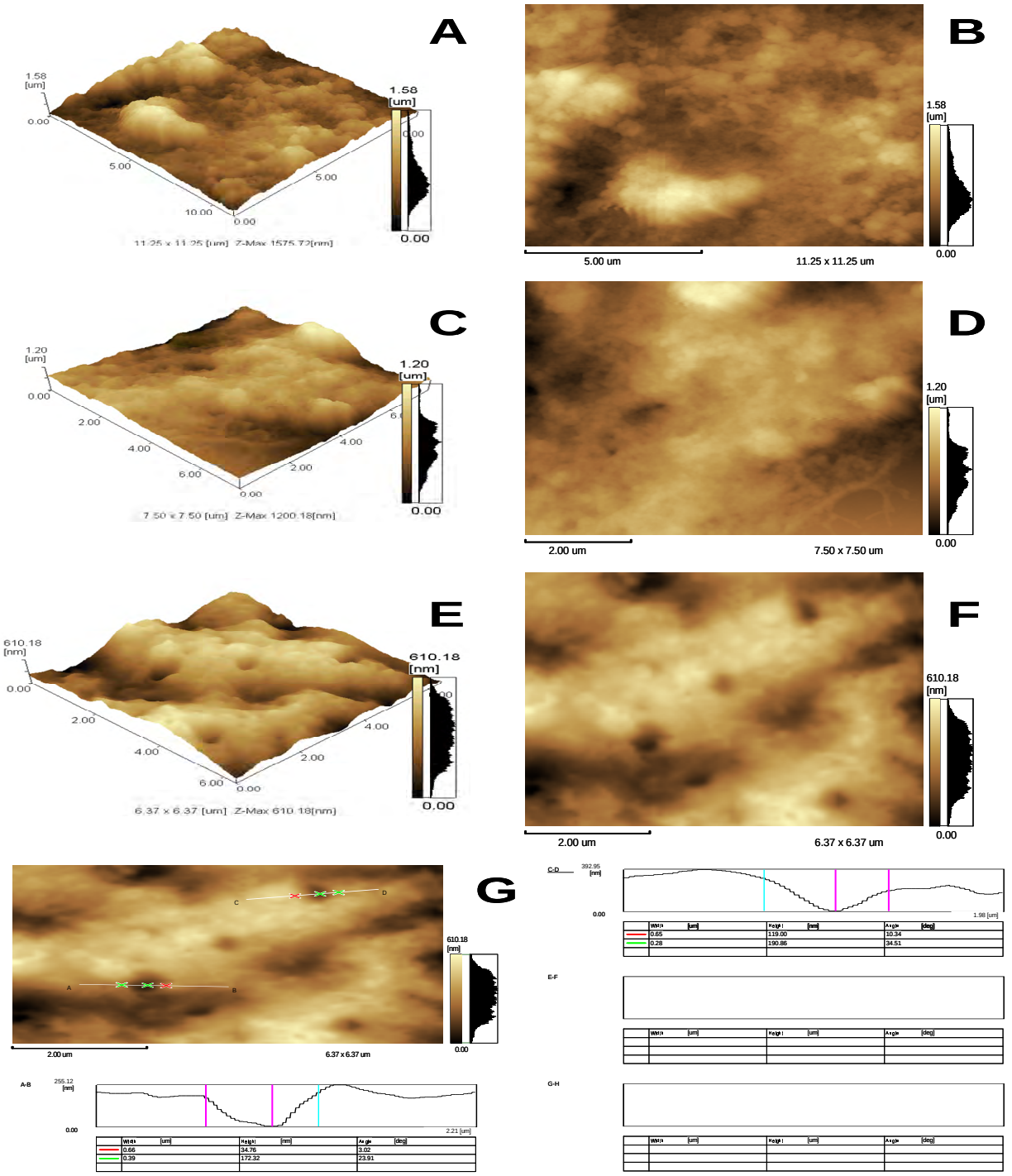

Figure 3. AFM images of CHO cells after different pulses of 400kV/m EMP irradiation. 10^5 cells were seeding in the 35mm dishes to use for the experiments when the cells were in the time of exponential phase. The irradiation groups were irradiated 10,100 and 200 pulses of 400kV/m in the GTEM-CELL. Then cells were fixed in 2% glutaraldehyde 30 min for the observation after different treatment immediately and then were scanned with consecutive contact-mode by SPM-online2.45 software control , At least 3 cells were scanned in each sample and the parameters were analyzed to do three-dimensional image reconstruction by SPM-offline2.2 software. (A) 3-dimensional image of CHO cell membrane after 10 pulses EMP irradiation . (B) Surface image of CHO cell membrane after 10 pulses EMP irradiation. (C)3-dimensional image of CHO cell membrane after 100 pulses EMP irradiation.(D) Surface image of CHO cell membrane after 100 pulses EMP irradiation. (E)3-dimensional image of CHO cell membrane after 200 pulses EMP irradiation . (F) Surface image of CHO cell membrane after 200 pulses EMP irradiation. (G) Analysis of cell membrane perforation in shape and size in SPM-offline2.2 software after 200 pulses EMP irradiation.

Our results have shown that EMP can induce CHO cell membrane perforation directly. The damage to cell membrane and the percent of perforation is more serious with increased field intensity and the number of pulses applied. This suggests that the cell membrane is a possible direct target of EMP. However, the existence of some positive findings and the limitations in the previous studies suggest further work will be needed to do in future.

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