Study of the optimizing karyotype by applying AC voltage

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Abstract

Background: The main problem in the conventional karyotype slide is twisted and folded chromosomes that make it challenging to analyze karyotype slide. Most of the time karyotype sorting software mistake chromosome with each order. In this study, the alternating current (AC) voltage has been conducted in order to drag the chromosome on karyotype slides. This method can facilitate the analysis of chromosomes structure by researchers, and karyotype sorting software can perform better and show a more accurate result.

Methods: In this study, the AC ramp voltage has been applied to drag the chromosome on karyotype slides. AC voltage interact with chromosomes in the slide so the force of electric charge can untwist and unfold chromosomes partially.

Results: Karyotype chromosomes that were under AC voltage showed somewhat lined, and arranged chromosomes compare to conventional karyotype chromosomes.

Conclusions: This method makes the study of the chromosomes structure very easy and more reliable. Hopefully, with the design and development of custom signal generators, the results of the study will be better and more accurate.

Keyword

Cytogenetics, Karyotype, Chromosome Slide, Ramp Voltage, AC Voltage

Introduction

Chromosome karyotyping evaluates the number and structure of a person's chromosomes to diagnose disease and detect abnormalities. It is usually performed on a blood sample, bone marrow aspirations, amniotic fluid aspirations or chorionic villus biopsy. (1)

The final karyotype report is generally created by select sorting and reporting software (ex: Ikaros karyotyping system) Today, karyotype in genetic clinics and laboratories in the world is one of the most popular diagnostic methods. So, it is important to have improved and optimized the karyotype protocols. (2)

Most of the time in karyotype slide, long Chromosomes are bend and twist. This problem can reduce the accuracy of karyotype analysis. In this study, the AC ramp voltage has been used as the external force to stretch chromosome in karyotype slide. (3, 4)

The primary chemical components of chromosomes are DNA, RNA, histone proteins and non-histone proteins. The principal elements of chromosomes are DNA, and the DNA backbone contains phosphates bands. These phosphates bands are negatively charged. This negative charge makes the whole DNA molecule to appear negatively charged like a mild acid. So the construction of chromosomes has a negative electric charge. This study aimed to investigate the interaction between chromosomes and AC ramp voltage, and it is interaction to stretch chromosomes in karyotype slides. (5)

Successful Giemsa standing chromosomes and aligned pro-metaphase chromosomes are essential for high-quality karyotype analysis. One of the significant problems with the chromosome sorter software is the folded and collapsed chromosome that mistakes the software in the correct identification of the chromosomes. (6)

Methods

In this study, standard karyotype protocol has been done, and Buffer media in lymphocyte conductivity consisted of deionized water adjusted to 114 ± 1 ppm/ml by TDS meter (Figure 1A). Two drops of cell/media applied on karyotype slide (Figure 1B). AC[1] ramp voltage (Figure 2A) was generated by a signal generator (Twintex-TFG320) (Figure 2B) for straightening and move the chromosomes in the slide. Final AC ramp voltage inserted with a 100_{um} fine needle into the karyotype slide (Figure 2C). (7)

Figure 1 A: TDS meter B: Two drops of cell/media on karyotype slide

[1] Alternating Current

The construction of chromosomes has a negative electric charge, and AC ramp voltage can cause them to move towards electrode needles. This forward and backward movement can untwist and align chromosomes in a karyotype slide. The karyotype slide air dried and stained with Giemsa (Merck Millipore) (Figure 2D).

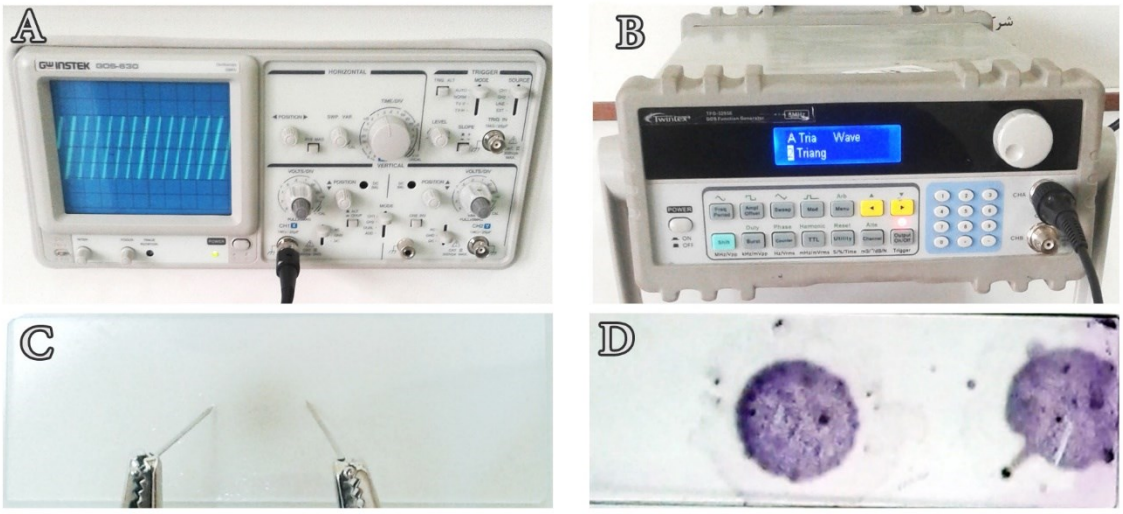

Figure 2 A: AC Ramp Voltage B: Signal Generator C: Fine Needle on Cell/Media Drop D: Giemsa Staining

There are five main properties for every AC voltage, and each of these five properties has many states. So lots of signal type can be generated, but a small range of these signals helps to stretch chromosomes in karyotype slide. Suitable electric force to stretch chromosomes depends on many factors. The simplest theoretical model for determining dielectric force to single cell surrounded by a low conductivity media is DEP[2] force model. DEP force is dependent upon the size of the particle (R^3), the shape of the particle (F_{CM}), signal frequency (∇) and signal magnitude (E^2_{RMS}) of the applied AC field (Equation 1). For small particles like chromosomes, the *Clausius-Mossotti factor* or (F_{CM}) are defined by Equation 2 where (ε^*_p) and (ε^*_m) are complex permittivity of the particle and medium. Finally, complex permittivity (ε^*) determine by the dielectric constant(ε), conductivity (σ), field frequency(ω), and imaginary unit (i) with Equation 3.(8)

Equation 1:	Equation 2:	Equation 3:
$F_{DEP} = 2\pi\varepsilon_m R^3 Re\,(F_{CM})\nabla E^2_{RMS}$	$F_{CM} = \frac{(\varepsilon^*_p - \varepsilon^*_m)}{(\varepsilon^*_p + 2\varepsilon^*_m)}$	$\varepsilon^* = \varepsilon + \frac{i\sigma}{\omega}$

With average size, shape and complex permittivity of chromosomes and estimation of F_{DEP} from the length of chromosomes movement, the F_{DEP} applied to chromosome should be adequate to move chromosomes a small deviation between (10_{um} to 100_{um}). A signal generator (Twintex-TFG320) has been adjusted to generate the desired AC ramp voltage. (9)

[2] Time-Dependent Dielectrophoretic

The final AC ramp voltage create by a signal generator (Twintex-TFG320) with six characteristics properties setting:1- The peak-to-peak voltage (V_{PP}) adjusted to 25 volts.2- The shape of the signal should be ramp wave.3- The frequency of this signal adjusted to 20 Mhz.4- Duty-cycle set to 50%.5- The effective voltage (E_{RMS}) adjusted to 25 volts. 6- DC offset voltage adjusted to 0 volts. In the next step, microelectrodes attached to the signal generator and placed on the edge of each droplet cell on the lam. After drying, the slide was stained with Giemsa, and the chromosomes were examined under a microscope. The images obtained from chromosomes, in this case, were compared to the chromosomes prepared by conventional karyotypes. (10, 11)

Results

In the images obtained from the karyotype slides, the chromosomes in the AC voltage treated group (Figure 3C to 3F) have been displaced towards the end of terminals, but chromosomes in the control group were centrally folded (Figure 3A-3B). However, the integrity of chromosomes in slightly compromised compared to chromosomes in the control group. In final karyotype slides of AC voltage treated group, movement of chromosomes measured by ImageJ software. The results indicate that the evolution of chromosomes is about 30_{um} (Table 1).

Label	Angle	Length
Min	26.5	10.61
Max	355.2	51.25
Mean	190.85	30.93

Table 1 results of chromosomes movement

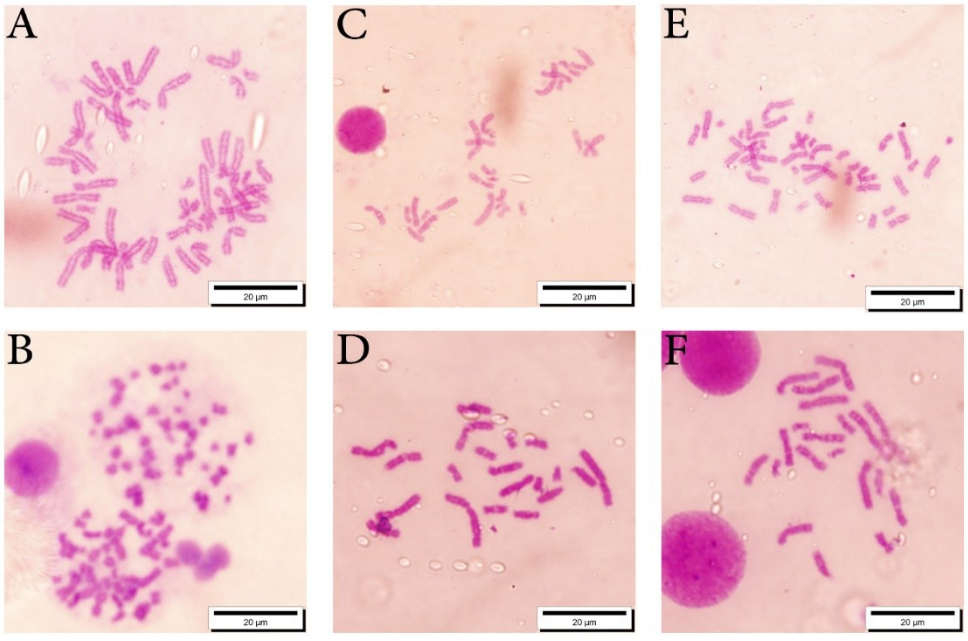

Figure 3 A and B: control group C to F: AC voltage treated group

Discussion

This study, based on the interaction of the AC voltage with macromolecules such as DNA or chromosomes, if DC signal used instead of AC voltage, macromolecule move towards the positive terminal. However, AC voltage polarities change in periodic time so, macromolecule movement will be back and forth between terminals, this phenomenon makes chromosomes to unfold and untwist. Also, the signal should not be destructive to the structure of the chromosome or cause excessive energy and cause the chromosome to break. Further studies are required to determine the rate of chromosomal movement by changing signal frequencies.

Conclusion

Successful standing chromosomes and aligned chromosomes are essential for high-quality karyotype analysis. The force of AC ramps signals interact with chromosomes can be used to move chromosomes to unfold and untwist them. This method based on the sending of AC family signals with ramp shape into karyotype slide to move the chromosomes back and forward in the desired length. A most crucial factor to the successful movement was the setting of characteristics properties of ramp signal made by a signal generator. Several parameters should calculate the proper amount of force applied to chromosome. Further investigation of useful parameters and adjusting the correct setting of characteristics properties of the signal generator is recommended.

Consent

Written informed consent for publication of the patient's details.

Author contributions

All authors were involved in the revision of the draft manuscript and have agreed to the final content.

Competing Interests

No competing interests were disclosed.

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