Paper-based Diagnostic Devices for Estimating Human Sperm Motility

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Abstract: Low-cost paper-based diagnostic device for human sperm motility has developed to know sperm motility without consulting fertility clinics. We compared the signal pattern of the paper-based sperm motility assay using 4-channel pattern (cross) and 2-channel pattern (line) papers, and investigated difference in signal patterns using some tetrazorium salts for the assay. Using these two paper patterns, we can examine whether human sperm motility in semen is more than 50% or 0%. XTT and WST-8 reagents treated paper-based device can be used. We concluded that MTT treated line pattern paper is suitable for the purpose to reduce the cost of the device.

Keywords: Sperm motility, Paper-based device, Tetrazorium salts

1. Introduction

Male fertility becomes world-wide problems not only in developed countries but also in developing countries (1). To check human sperm motility, usually sperm motion is observed under microscope at fertility clinics. However, some men are reluctant to submit semen samples for examination at fertility clinics (2). There are needs for sperm motility analyses without microscope.

To satisfy the demand with a low cost sperm motility analyzing system, we have developed paper-based device evaluating sperm motility in semen (3). Yellowish 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium salt (MTT) was treated on our paper-based device for a substrate of mitochondrial dehydrogenase enzyme in human sperm. The substrate converts to insoluble purple MTT formazan on the device (4). The reactivity of the enzyme is higher in motile sperm than in non-motile sperm. We estimate the percentage of motile sperms (motility) based on the colorimetry on the paper. The hydrophilic channels were prepared by wax printing method, and this technology was applied for enzyme-linked immunosorbent assay (ELISA) to quantify HIV-1 envelope antigen gp41 in human serum (5, 6). We have to optimize pattern of the hydrophilic channel and reagents for the sperm motility assay to find the best settings for sperm motility assay.

In this study, we compared the signal pattern of the assay using two paper patterns; 4-channel pattern (cross) and 2-channel pattern (line). We tried to find that other tetrazorium salts can improve the sensitivity of the paper-based sperm motility assay. The reagents used in this study were (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) (XTT) and (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium) (WST-8), which are used for cell viability assay as shown in Figure 1 (7).

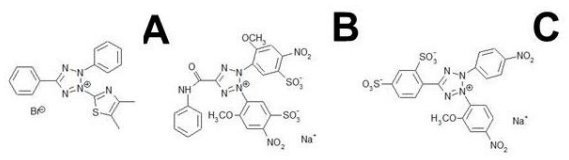

Figure 1. Molecular structures of tetrazorium salts used in this study. (A) MTT, (B) XTT, and (C) WST-8.

2. Methods

~Paper based devices for sperm motility analysis~

The paper-based devices were prepared using wax printer and Whatman filter papers (Figure 2). MTT, XTT, and WST-8 solutions (1 mg/mL) were applied to each circle with 5 μL on every pattern. Volunteer's human semen samples were first checked sperm motility by Sperm Motility Analysis System (SMAS), and 5 μL of the sample was placed onto our reagent-treated paper-based device using a micropipette.

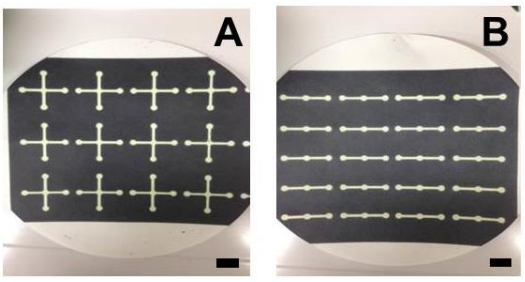

Figure 2. Paper-based devices used in this study. (A) Line and (B) cross pattern. Bars are 1 cm.

~1. Comparison between cross and line patterns~

For MTT treated paper-based device, we divided the initial sample into glass bottles and these semen samples in the glasses were incubated in the heater at 50 °C for 90 min. The rest of initial sample was placed at room temperature without any operations. After every 10 min of incubation, we took out one bottle of semen, checked the sperm motility by SMAS, and applied samples on the paper-based devices until 90 min from the incubation. Finally we checked the semen sample incubating at room temperature for 90 min as well. Pictures of the papers were taken after 30 min of semen applying using digital camera. The sperm motility parameter (MOT) was calculated using the Image J software, and data analysis was conducted by Microsoft Excel software (3). Student's t-test with standard Bonferroni correction was used to determine differences in MOT among three different groups classified with sperm motility. $P < 0.016$ was considered as statistically significant.

~2. Effects of different reagents on the MOT analysis~

To evaluate availability of XTT and WST-8 treated paper

devices, we incubated the semen sample at 50 °C for 120 min, and compared MOTs of the applied samples without incubation and incubated at room temperature for the same time. Sperm motility was checked under microscope and applied as the methods for MTT assay.

3. Results and discussion

~1. Comparison between cross and line patterns~

We found that the MOT for sperm motility more than 50% was significantly higher than MOT for sperm motility less than 10% in both line and cross patterns (Figure 3). The sperm sample with motility between 10%-30% was hard to prepare because sperm motility sharply decreased from more than 50% to less than 10% after incubation for 20-30 minutes. Sample number for the group of motility between 10%-30% was less than other two groups. This little sample number induced the difference in MOT between the two papers. To clarify availability to investigate the sperm motility between 10%-30% using these devices, we need to increase the sample number. Similar results were observed between group less than 10% and more than 50% using these two paper patterns. We recommend to use line pattern in order to decrease amount of reagent for preparation of the paper-based device.

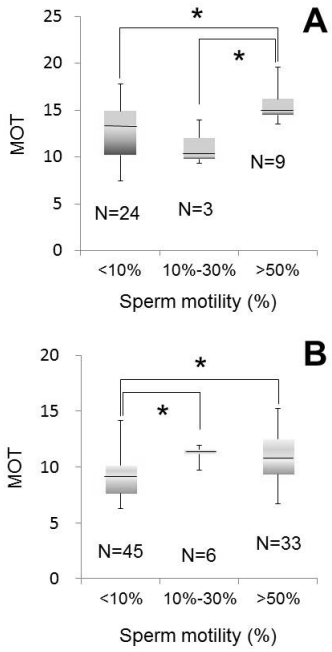

Figure 3. MOT of human sperm by MTT assay using (A) cross and (B) line pattern papers. The thin line in the boxes, lower and upper edges of the boxes, and lower and upper bars outside the boxes represent median values, 25th and 75th percentiles of all data, minimum and maximum, respectively. *p < 0.016 (Student's t-test), indicating statistically significant differences compared with two groups. The value N shows the sample number.

~2. Effects of different reagents on the MOT analysis~

Figure 4 shows relationship between MOT and sperm motility using XTT and WST-8 treated paper-based devices. MOT parameters were higher in motile sperm than non-motile sperm for XTT and WST-8 using human sperm, suggesting these three reagents can reflect the sperm motility by paper-based device. We suggest to use MTT reagent for sperm paper-based assay, because MTT would lower the experiment costs comparing to other two reagents.

4. Conclusion

We could find similar difference in MOT parameter using line and cross patterns, when we compare the samples between sperm motility more than 50% and less than 10% because of large sample number. However, more sample number of the motility 10-30% is required to conclude the accuracy of MOT parameter. We can evaluate motility more than 50% using MTT, XTT, and WST-8 applied papers. Because of higher cost of XTT and WST-8 compared to MTT, MTT treated line pattern paper is the best choice to use this paper-based assay system for routine use.

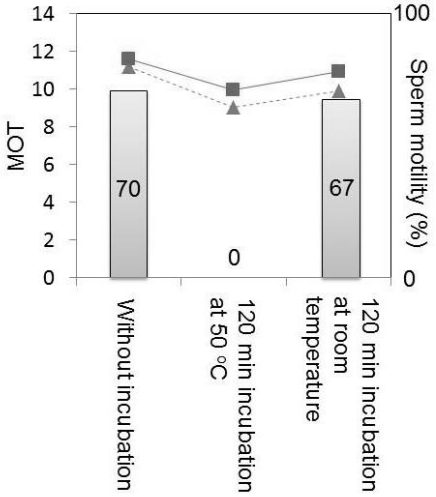

Figure 4. Relationship between MOT and sperm motility using XTT and WST-8 treated paper-based devices. Squares and triangles show results of XTT and WST-8 treated papers, respectively.

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