SOME OBSERVATIONS ON DEHYDRATION AND DRYING OF ERYTHROCYTES FOR SCANNING ELECTRON MICROSCOPY

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SUMMARY

A comparative study on air drying of human erythrocytes for scanning electron microscopy (SEM), between acetone and ethanol for dehydration and drying was carried out, using a quick method. No morphological deformity or artifacts of erythrocytes was observed. More rapid air drying in acetone (3-5 seconds) was noticed as compared to ethanol (20-30 seconds). Acetone was recommended to be used for air drying, in the absence of critical point drying.
INTRODUCTION

The most important step of SEM specimen preparation is to dehydrate and dry the tissue without damaging the morphological structure of the cells. Acetone dehydration and drying was recommended for SEM in general \(^{(1,2,3)}\). Critical point drying method was first developed by Anderson in 1951\(^{(4)}\). This technique has been applied successfully to SEM by Boyde and Wood in 1969\(^{(5)}\). But, no remarkable difference in the erythrocyte morphology between the air drying method and critical point drying was reported\(^{(6)}\).

The aim of this paper is to report some observations on air drying of human erythrocytes for SEM, using acetone and ethanol only for both dehydration and drying.

MATERIALS AND METHODS

0.5 ml of normal human venous blood was aspirated in a syringe containing 20 ml of physiological saline and proceeded following a modified and a quick method of preparation\(^{(6,7,8)}\). Samples of blood without anticoagulants, were washed with physiological saline, fixed in 0.75% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, postfixed in 1% osmium tetroxide, dehydrated with graded ethanol at 25, 50, 65, 75, 85, 95, 99, 100% and three changes of 100% ethanol or with graded acetone at 25, 50, 65, 75, 85, 95, 99% and 4 changes of 99% acetone. Suspensions were centrifuged at 1000 rpm for 5 minutes each except with 75% acetone and above which needed to be centrifuged for 2 minutes only.

One drop of suspension of cells in either absolute ethanol or acetone was placed on a glass slide and aluminium foil, turned obliquely on a filter paper for absorption of the excess and quick air drying. Specimens were coated with gold and observed by Philips 515 SEM.

RESULTS

Erythrocytes were found to be well preserved and maintained their biconcave discocytosis without cellular surface changes or any other deformities such as spherocytosis, echinocytosis, elliptocytosis and knizocytosis in both acetone and ethanol when used (Fig.1). They were more
Fig. 1: Well preserved biconcave erythrocytes dehydrated by acetone. X 5,500.
rapidly air dried in acetone, 3-5 seconds, as compared to ethanol, 20-30 seconds.

Gentle centrifugation at 1000 rpm and for not more than 2 minutes was found to be necessary to avoid stickiness of the precipitated erythrocytes and to achieve easy resuspension when samples were dehydrated with graded acetone at 75% and above.

DISCUSSION

As in well known, specimens for study in high vacuum of EM must be free of water or other volatile matter. Conventionally water was been substituted by acetone prior to drying in air\(^\text{(1,2)}\). Later, acetone was used for dehydration and critical point drying\(^\text{(9)}\) by using F13 as solvent with critical pressure of 38.2 atmosphere at 28.9°C. While for ethanol, F23 is used as solvent with critical pressure of 47.7 atmosphere at 25.9°C or F116 with critical pressure of 29.4 atmosphere at 19.7°C. When Amyl acetate is used as an intermediate reagent between ethanol and liquid CO2, a critical pressure of 72.8 atmosphere at 31°C is required\(^\text{(10)}\).

No remarkable difference in the erythrocyte morphology was found between air drying method and critical point drying\(^\text{(6)}\). This may be due to the fact that the erythrocyte has more stable structure than the ordinary cells. As mentioned above, ethanol needs Amyl acetate and liquid CO2 as intermediate reagents for critical point drying while acetone does not need that. Because of shortage of chemicals at the present time, due to economical sanctions, it is preferrable to use air drying over critical point drying and acetone is recomended for use. Moreover, we believe that rapid air drying with acetone might minimize cellular surface tension.

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REFERENCES

بعض الملاحظات حول تجفاف ونشر كريات الدم الحمراء هويةً للقحص المجهري الإلكتروني الماسح

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الخلاصة

اجريت دراسة مقارنة للفحص الهوائي لكريات الدم الحمراء البشرية للأستجاع
الإلكتروني الماسح ، بين مادتي الاستيكتون والكحول الإيثيل للتجفاف والنشر باستخدام
طريقة تحضير سريعة. لم تلاحظ تشهوهات شكليّة أو شوائب لكريات الدم الحمراء. كما
لم يلاحظ نشف هوائي أسرع في مادة الاستيكتون (3-5 ثوان) مقارنة مع مادة الكحول الإيثيل
(20-30 ثانية). وعليه نوصي باستخدام مادة الاستيكتون للفحص الهوائي في حالة عدم توفر
امكانية النشف عند الدرجة المشرقة.