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A New Disaggregation Device for Cytology Specimens[1]

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A new means of disaggregating cytology specimens in suspension using an immersible rotor device is described. The new rotor is compared to an automated syringing apparatus using cervical samples. Similar results using both devices are obtained for both normal and abnormal specimens.

Key terms: Cytology, specimen preparation, automation

A prerequisite for the (pre)screening of cytology specimens by an automated image analysis system is a reproducible and practical method for preparing smears of disaggregated cell suspensions. Many procedures have been described (1,4,10,14,16,17), but most are limited in that they are labor-intensive or very time-consuming. To make automated cytology an attractive addition to routine manual cytology, the specimen should be handled as little as possible, and the processing time should certainly not be much longer than the time needed to prepare a cervical smear with conventional manual methods.

The Biological Precision Encoding and Pattern Recognition (BioPEPR) project (19) is concerned with full automation of cervical smear prescreening, including the preparation phase. In a previous paper (10), we described a semiautomatic procedure by which about 100 cervical smears a day could be prepared. Because it proved difficult to fully automate this entire process as it was, certain steps in the process were reevaluated in order to make a fully automated system feasible.

All of the automated disaggregation systems developed to date have been automated versions of the syringing procedure (1,4,7,9,10,16). With syringing it is difficult to fully automate the changing of the needles or the syringes, and to provide adequate rinsing of the device between samples to prevent specimen contamination.

The present paper describes a new method of disaggregating cell suspensions. Cell disaggregation with this device, a specially designed rotor, is easy to automate and has proved to be efficient. In this paper the rotor is described, and results obtained with this rotor when disaggregating cervical specimens are presented.

MATERIALS AND METHODS

Cervical cells were collected as second or third scrapes using a plastic spatula that was rinsed in a preservative phosphate-buffered saline solution, containing 20% ethanol. Disaggregation was performed either by a syringing technique using a peristaltic pump device (10) for 15 min with 19-gauge needles and a speed of 55 ml/min, or by a rotor immersed in the sample vial containing 9 ml of cell suspension (Fig. 1A). For comparative studies the original sample was divided before disaggregation into two to four sample containers, and the fluid volume was adjusted back to 9 ml.

After disaggregation the cells were deposited onto glass microscope slides, as described elsewhere (10).

The rotor device used consists of a 24-V DC Maxon motor (Sachseln, Switzerland) with a vertical axle on which different cylindrical rotor heads can be mounted. The distance from the outer edge of the rotor head to the vessel wall is about 2 mm. Figure 1B shows two examples of hollow rotor heads, both having an external diameter of 17 mm and an internal diameter of 10 mm. In one of the rotors (right in the figure), 1-mm-diameter holes were drilled at an angle of 30° with respect to the tangent of the outside surface (in the direction of rotation). The holes were thought to provide an action similar to that of syringing. Experiments were performed to compare the two rotor heads. During use, the rotor was driven at a speed of 6,000 rpm. Usually, samples were disaggregated for 30 s. Shorter (15 s) as well as longer (45 s) times were also investigated. After disaggregation, the rotor was spun for a few seconds above the cell suspension to eliminate possible remaining fluid and cells. Thereafter it was rinsed in tap water by spinning

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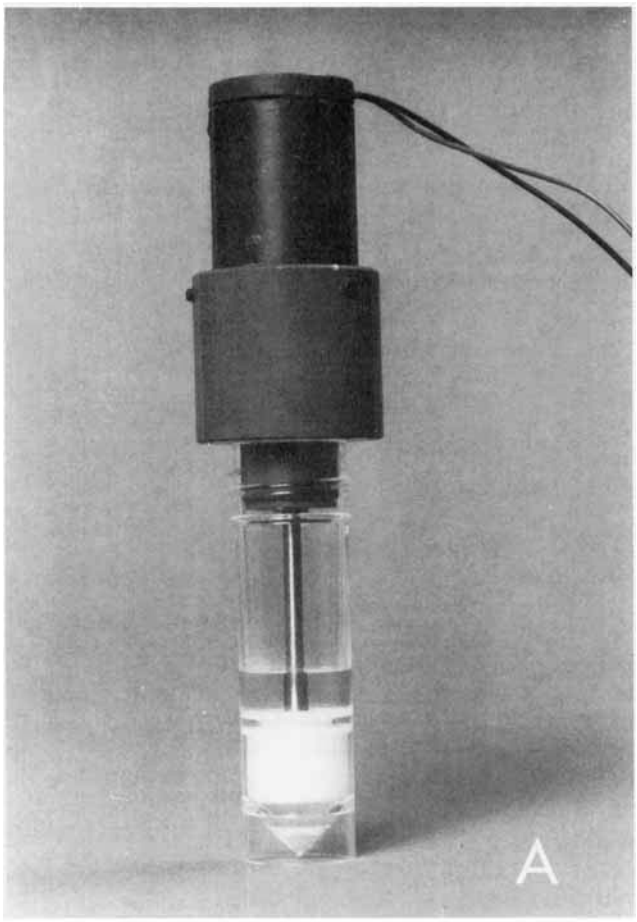

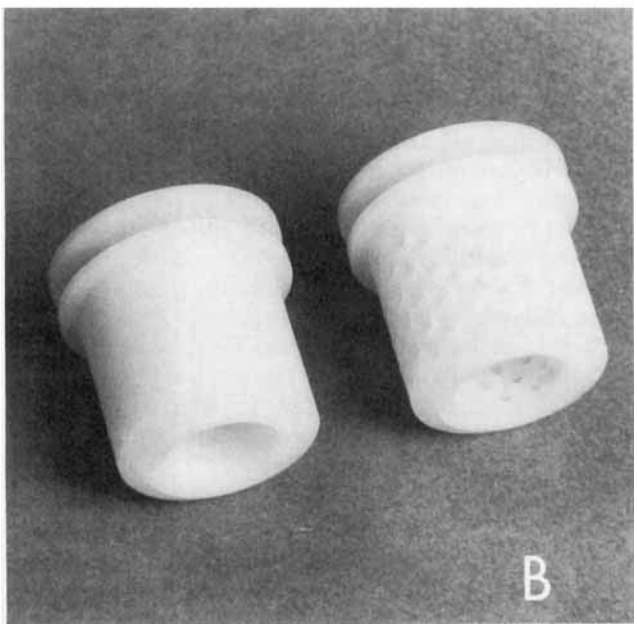

FIG. 1. A) Immersible rotor system for the disaggregation of cytology specimens. B) Two different rotors, which can be mounted on the device in A. The left rotor without holes; the right rotor contains 1-mm holes. For further details see the text.

for about 5 s and spun above the fluid level to remove excess water.

The effectiveness of cell disaggregation achieved by the different techniques was evaluated by counting the number of singly lying epithelial cells, the total number of epithelial cells, including all cells in aggregates such as sheets and clusters, and the total number of leukocytes in the central part of the smear. Singly lying epithelial cells were defined as cells that did not touch or overlap other epithelial cells. Since leukocytes are rather fragile cells, they were counted to see if the disaggregation resulted in a severe destruction of this cell type. Together with the counting procedure the quality of the disaggregated cells was assessed. Although the cell deposition procedure resulted in a certain number of overlapping cells, no attempts were made to discriminate these overlaps from original aggregates, because criteria to distinguish these were difficult to formulate. Counting was done manually under a microscope in ten areas of $1mm^2$ (16× objective field in the microscope) each that were sampled in a regular pattern. The central area was chosen because there is an increase in cell overlap at the beginning and end of the slide owing to the cell deposition procedure (analogous to the "wedge technique," see reference 10).

The reproducibility of the counting procedure was tested in two ways, by repeating the count on different fields of the same slide and by making counts on two different slides made from the same cervical sample.

Using a number of normal samples, a study was made to compare the effectiveness of the syringing technique to that of the rotor device, and to compare the results of the rotor for times of 15 s, 30 s, and 45 s.

For a number of abnormal samples, the number of singly lying cells and total numbers of abnormal cells were counted in a 2 × 2-cm area in the central part of the slide. Abnormal cells were defined as cells consistent with a slight dysplasia or a more severe epithelial abnormality. Singly lying abnormal cells were defined as abnormal cells that did not touch or overlap other normal or abnormal epithelial cells. A comparison of the syringing technique to the rotor device was made, again using three different rotor running periods. In another test a series of 40 "abnormal slides" was evaluated to determine the percentage of singly lying abnormal cells as a function of the degree of abnormality. In this last test all samples were disaggregated using the rotor device for 30 s.

The results of statistical analysis of the data are shown in the Tables 1–4. A regression analysis (15) was performed to compare the results obtained by repeated counting (Table 1), to compare the results of the syringing technique to the results of the rotor (Tables 2,3), and to compare the results of one rotor type to the results of the second rotor type (Table 4).

RESULTS

Counts from the reproducibility study are presented in Table 1. Both the repeat counts from same sample

and the counts from duplicate slides show good agreement.

Table 2 shows the comparison between two different disaggregation procedures for the normal samples: syringing for a period of 15 min, and treatment with the rotor with 1-mm holes for three different periods of time. Extensive studies elsewhere (1,4,10,16) had proven that the syringing method yielded a considerable increase of single cells, compared to nonsyringed samples, without additional significant cell loss or morphological cell damage. In previous studies in our laboratory syringing was shown to increase the percentage of single cells counted on the slide from an average of 23% to an average of 50% (10). Because the effectiveness of syringing had already been shown, and because the samples used in this comparative study were typical of those used in our previous study, "nonsyringed" data are not presented here.

As can be seen in Table 2, no significant difference is noted between the number of single cells, the total number of cells, and the number of leukocytes, whether treated with the rotor or the syringing device. Also no significant difference is noted between the different durations of rotor treatment. A minimal time of 15 s resulted in optimally disaggregated cell samples.

The same experiment was also performed on a variety

Table 1
Reproducibility of the Cell Counting[a]

	Same slide, different fields				Same sample, different slides			
	Epithelial cells/mm^2		% single epithelial cells		Epithelial cells/mm^2		% single epithelial cells	
Sample No.	First count	Second count	First count	Second count	Slide A	Slide B	Slide A	Slide B
1	116	114	39	33	63	66	41	40
2	108	114	36	37	95	98	32	37
3	117	108	41	34	61	62	53	46
4	50	79	42	28	78	79	31	37
5	95	81	58	60	69	67	35	38
6	52	60	64	64	117	113	38	37
7	67	72	67	64	114	121	25	21
	r = 0.893		r = 0.948		r = 0.989		r = 0.845	
	P<0.01		P<0.01		P<0.001		P<0.05	

[a]r, coefficient of correlation;
P, level of significance.

Table 2
Comparison of an Automated Syringing Apparatus With the Rotor on Normal Cervical Samples[a]

	% single epithelial cells				Epithelial cells/mm^2				Leukocytes/mm^2			
		Rotor treatment				Rotor treatment				Rotor treatment		
Sample No.	Syringing	15 s	30 s	45 s	Syringing	15 s	30 s	45 s	Syringing	15 s	30 s	45 s
1	67	61	89	77	48	59	38	44	8	13	15	11
2	44	52	54	46	33	33	42	48	10	12	12	10
3	74	78	66	64	21	18	40	56	9	5	13	10
4	59	40	34	51	22	26	22	27	1	0	0	0
5	81	66	68	71	36	49	45	44	3	4	3	3
6	66	42	38	48	30	43	50	46	12	13	11	11
7	45	44	38	53	54	48	65	48	34	18	32	22
8	58	81	78	76	49	44	37	29	16	19	39	15
9	81	92	80	79	47	44	47	44	3	2	4	3
10	62	74	62	68	47	56	47	40	11	23	16	16
11	76	71	73	75	58	50	39	40	12	14	12	15
12	65	73	76	72	44	41	37	36	15	23	15	15
13	73	64	58	68	42	49	51	37	5	11	14	8
14	83	76	73	76	26	27	42	35	28	39	46	36
15	54	64	67	60	96	77	63	86	17	37	46	42
16	57	45	62	54	32	24	26	27	5	12	9	10

[a]% single epithelial cells, syringing versus 15-s rotor treatment: r = 0.588, P < 0.05; % single epithelial cells, 15-s versus 30-s rotor treatment: r = 0.514, P < 0.05; % single epithelial cells, 15-s versus 45-s rotor treatment: r = 0.842, P < 0.001; epithelial cells/mm^2, syringing versus 15-s rotor treatment: r = 0.872, P < 0.001; epithelial cells/mm^2, 15-s versus 30-s rotor treatment: r = 0.629, P < 0.01; epithelial cells/mm^2, 15-s rotor treatment: r = 0.703, P < 0.01; leukocytes/mm^2, 15-s versus 30-s rotor treatment: r = 0.868, P < 0.001; leukocytes/mm^2, 15-s versus 45-s rotor treatment: r = 0.941, P < 0.001. r, coefficient of correlation; P, level of significance.

Table 3
Comparison of an Automated Syringing Apparatus With the Rotor on Abnormal Cervical Samples[a]

	% single abnormal cells				Total No. of abnormal cells				Epithelial cells/mm^2			
		Rotor treatment				Rotor treatment				Rotor treatment		
Cytology	Syringing	15 s	30 s	45 s	Syringing	15 s	30 s	45 s	Syringing	15 s	30 s	45 s
Slight dysplasia	35.0	28.5	–	–	20	35	–	–	73.2	62.2	–	–
Slight dysplasia	3.8	9.7	5.4	15.0	159	93	56	60	103.3	76.5	91.3	98.8
Slight dysplasia	22.2	18.1	14.3	8.2	27	33	28	49	64.0	47.5	54.3	63.2
Slight dysplasia	33.3	27.0	12.9	–	48	37	62	–	40.3	17.5	35.5	–
Slight dysplasia	5.0	4.9	5.7	4.5	60	61	88	67	62.8	84.8	88.3	79.2
Slight to moderate dysplasia	34.6	39.1	30.0	37.5	26	23	40	24	45.3	43.3	42.8	51.8
Moderate dysplasia	14.2	19.4	21.4	10.2	14	31	28	49	15.2	21.8	11.0	23.7
Moderate dysplasia	50.6	31.4	–	–	77	102	–	–	45.8	69.2	–	–
Moderate dysplasia	34.0	25.0	–	37.0	55	146	–	119	40.0	65.1	–	55.8
Moderate dysplasia	9.0	24.0	35.0	21.0	207	251	311	324	23.1	27.2	37.9	43.8
Severe dysplasia	17.3	17.1	17.6	15.5	369	404	427	245	44.0	64.2	56.3	44.7
Severe dysplasia	4.8	6.0	7.0	8.1	272	386	345	321	56.2	63.8	66.0	72.3
Severe dysplasia	37.0	32.0	–	33.0	696	815	–	675	23.2	20.2	–	20.8
Severe dysplasia/CIS	28.0	25.0	–	–	64	71	–	–	70.8	71.9	–	–
CIS	25.1	23.0	22.6	–	351	357	381	–	72.3	62.5	50.2	–

[a]% single abnormal cells, syringing versus 15-s rotor treatment: $r = 0.850$, $P < 0.001$; % single abnormal cells, 15-s versus 30-s rotor treatment: $r = 0.731$, $P < 0.05$; % single abnormal cells, 15-s versus 45-s rotor treatment: $r = 0.859$, $P < 0.0l$; total No. of abnormal cells, syringing versus 15-s rotor treatment: $r = 0.982$, $P < 0.001$; total No. of abnormal cells, 15-s versus 30-s rotor treatment: $r = 0.982$, $P < 0.001$; total No. of abnormal cells, 15-s versus 45-s rotor treatment: $r = 0.971$, $P < 0.001$; total No. of epithelial cells, syringing versus 15-s rotor treatment: $r = 0.730$, $P < 0.01$; total No. of epithelial cells, 15-s versus 30-s rotor treatment: $r = 0.903$, $P < 0.001$; total No. of epithelial cells, 15-s versus 45-s rotor treatment: $r = 0.851$, $P < 0.01$. r, coefficient of correlation.
P, level of significance.
CIS, carcinoma in situ.

Table 4
Comparison of Two Different Types of Rotors Used on Normal Cervical Samples[a]

	% single epithelial cells		Epithelial cells/mm^2		Leukocytes/mm^2	
Sample No.	Rotor with 1-mm holes	Solid rotor	Rotor with 1-mm holes	Solid rotor	Rotor with 1-mm holes	Solid rotor
1	50	55	120	187	6	4
2	28	28	62	124	0	0
3	68	78	89	52	22	23
4	52	40	69	79	20	13
5	42	48	70	63	2	7
6	47	45	20	23	0	0
7	52	59	100	86	20	14
8	40	40	129	140	175	138
9	57	56	64	63	12	11
10	52	48	46	49	1	1
	$r = 0.886$, $P < 0.001$		$r = 0.769$, $P < 0.01$		$r = 0.998$, $P < 0.001$	

[a]r, coefficient of correlation.
P, level of significance.

of abnormal cervical specimens (Table 3). Special attention was given to both disaggregation procedures with regard to their influence on abnormal cells. Again no significant differences concerning the number of singly lying abnormal cells as well as the total number of abnormal cells were observed between the syringing and the rotor procedure. Also for abnormal cells a minimal time of 15 s resulted in optimal disaggregation.

In Table 4, the results of a comparison of two different types of rotors are given, one rotor with 1-mm holes, and one without holes. No significant differences were observed between the results obtained with these two different rotors.

The rotor with 1-mm holes was used in a large-scale screening study, for the routine preparation of 5,500 cervical slides made from second scrapes. After cytologic evaluation to determine abnormality, 40 slides were chosen from this material containing cells consistent with varying degrees of epithelial abnormalities, and the number of singly lying abnormal cells was determined. As Table 5 shows, the proportion of singly lying abnormal cells remained fairly constant, but the total

Table 5
Performance of the Rotor in Disaggregation of Abnormal Cells[a]

Cytology	No. of samples	% single abnormal cells	Total No. of abnormal cells per slide	Epithelial cells/mm^2
Slight dysplasia	10	31 ± 14	298 ± 164	52 ± 22
Moderate dysplasia	10	22 ± 14	400 ± 292	48 ± 15
Severe dysplasia	10	33 ± 23	526 ± 348	48 ± 22
Carcinoma in situ	10	25 ± 16	790 ± 542	45 ± 35

[a]Numbers shown are mean ± standard deviation.

number of abnormal cells counted in each sample increased with the increasing degree of abnormality of the cell sample.

DISCUSSION

The syringing technique works by exerting a shear force at the tip of the needle that breaks clusters of cells preferentially along the natural boundaries between cells. The syringing method is difficult to fully automate, however, primarily because of inadequate rinsing, which causes specimen contamination, and because of the necessity to frequently change the needles or the syringes. The need for an equally effective, yet more automatically workable disaggregation technique led to the development of the rotor device described here.

The rotor system also makes use of shear forces. Here, a circumferential shear force is formed in the cell suspension layers adjacent to the rotor head, owing to the rotation of the cylinder, the viscosity of the fluid, and the size of the cellular clumps. Larger clusters are exposed to a gradient of fluid velocity extending from the rotor to the inner vessel wall, providing the force to tear the clusters along the path of least resistance at their natural boundaries. The irregular shape and size of the clusters ensures the oscillating movement of the clusters into the higher-level forces close to the rotor head.

The rotor head can be constructed as a simple cylinder that is easy to clean, eliminating cell contamination from one specimen to another. As shown in Table 4, the use of holes drilled through the cylinder (which were thought to induce a syringing effect) did not improve disaggregation. The circumferential shear force seems to be the dominant effect. The rotor also works considerably more rapid than a typical syringing system—15 s proved to be adequate. For the automated syringing system used in this study 15 min were required (10), whereas for other automated versions 2 (16) to 3 (1) min have been reported.

From the presented data it may be concluded that the rotor device gives very similar disaggregation results as the syringing device. This finding was consistent for a wide variety of cervical specimens, both normal and abnormal. Using both methods, no differences in cell morphology of the squamous epithelial cells were seen, whereas the same number of the more fragile leukocytes was found (see Table 2). Other studies (11) in which the rotor device has been used show that the morphology of columnar epithelial cells and other more fragile epithelial cells is well preserved. It should be noted that both disaggregation techniques were designed for use in preparing slides for an image analysis system. Such a system is tolerant of a fair number of touching or slightly overlapping cells. Observations indicate that the size and numbers of the remaining clusters might be an additional parameter in the further classification of atypical or abnormal specimens. It is possible that a greater degree of disaggregation could be obtained through higher-speed rotation or modification of the rotor head, or perhaps through the use of (bio)chemical methods (8). The rotor has not been tested at higher speeds. Increasing the shear force might result in cell damage, whereas the gain in rotating time is not relevant. A very high rotational speed of the rotor head should provide results similar to those of the more forceful syringing technique now used in such cases (7,9). No further attempts to disaggregate cell clumps by (bio)chemical means have been made, since these procedures generally fail or result in severe aspecific cell damage (5,6,8,10,18).

It is important, of course, to determine how any disaggregation device will work on abnormal cells. In our study using abnormal samples, the rotor device again performed very similarly to the syringing apparatus. For abnormal cells, a lower percentage of singly lying cells was found than for normal cells. In general only 22–33% of the abnormal cells were disaggregated, compared to 60% of the normal ones. Again, an automated image analysis system should be capable of recognizing many of these abnormal cells, even when clustered, since good nuclear information is generally still available, and simple algorithms are capable of identifying cells with overlapping cytoplasms (20). It is of interest to note that the fraction of abnormal cells increased with samples of increasing abnormality (see Table 5); this same tendency has been reported elsewhere for conventional smears made from first scrapes (2,3,12,13). For slight dysplasia, abnormal cells constituted 0.75% of the total number of epithelial cells, whereas for carcinoma in situ a fraction of 2.2% was found.

In the study described here a new rotor disaggregation device was used in the preparation of cervical cytology specimens. The use of this method with other cytological

material such as sputum, ascitic, and pleural fluids is clearly possible. Preliminary results with sputum indicate results similar to those achieved with cervical scrapes (data not reported here). The rotor disaggregation technique seems, therefore, well suited to a variety of clinical and research applications in which disaggregated cell suspensions are required.

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