ASSESSMENT OF CELL-SUBSTRATE ADHESION
BY A CENTRIFUGAL METHOD

W. HERTL,1 W. S. RAMSEY AND E. D. NOWLAN

Research and Development Laboratories, Corning Glass Works, Sullivan
Science Park, Corning, New York 14831

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SUMMARY

A centrifugal method has been evaluated for measuring the strength of Vero Green
Monkey kidney cell adhesion to growth surfaces. The centrifugal force necessary to
remove cells gave a quantitative measure of cell adhesion and hence the quality of the
growth surface. After being subjected to high gravity forces, both the remaining attached
cells and the detached cells were viable, indicating the detachment process did not
simply rupture the cell. Electron microscope examination of growth surfaces after cell
detachment suggested that remnants related to filopodia remained.

Key words: centrifugal adhesion; surfaces; Vero; fibroblasts.

INTRODUCTION

In many studies of tissue culture surfaces a
given cell line or strain has been grown on a
variety of different materials. After a number of
days, the cells have been examined for morphol-
ogy and surface coverage. These studies were
necessarily subjective and provide little quanti-
tative information of the adhesive properties of
the cell-surface combination. A method for quan-
titating the "secondary" adhesion to an agar-
based overlay of cells attached to treated poly-
styrene has been described (4,5). This method
does not measure the attachment of cells to a
solid substrate, although it could if an overlay
material of higher adhesiveness to the cells than
treated polystyrene were available.

Another method measures the attachment of
cell monolayer sheets to surfaces through dis-
placement by air pressure (2). Measurement of
the hydrodynamic shear necessary to detach cells
has been used to quantify cell adhesion to a
solid surface (13,15). In this method cells are
allowed to attach to a circular cover slip, which
is then spun rapidly in a fluid. The primary force
applied to the cells is hydrodynamic shear, and
the theory describing these forces has been
developed (6,12,13). The initial adhesion of
leukocytes and platelets to solid surfaces could
be predicted from knowledge of the surface
tensions of the solid surface and the liquid phase
(8,9). Applied centrifugal force has been used to
measure the quality of a tissue growth surface
(1,3). In these experiments slides with attached
cells were mounted perpendicularly to the applied
centrifugal force. The slides were spun at fixed
centrifugal forces and the proportion of cells
detached was used as a measure of adhesiveness.
In an extension of this method, centrifugal force
applied perpendicularly to the growth surface
was used to access the intercellular adhesion
between chick embryo neural retina cells. Adhe-
sion between cells and a growth surface was not
measured in this study (7).

Our interest is in characterizing surfaces for
tissue culture growth. Hydrodynamic shear was
inadequate to remove Vero cells from growth
surfaces after incubation times greater than 30
min. The use of a modified centrifugal method
gave a quantitative measurement of cell adhesion
in the study of the parameters affecting adhesion.
This report deals with the methodology and
precision of the method and an accompanying
report describes the results obtained on a variety
of chemically characterized surfaces (10).

MATERIALS AND METHODS

Cells. Green Monkey Kidney cells, Vero (ATCC
no. CCl. 81, Lot 1323), from American Type
Culture Collection, Rockville, Md. were used
throughout. They were grown in Medium 199

1 To whom requests for reprints should be addressed.
with Earle's salts, 5% fetal bovine serum (not heat inactivated), gentamicin from Schering Corp. Kenilworth, N.J. at 100 μg/ml, and Fungizone at 0.25 μg/ml. No mycoplasmal contamination tests were done.

**Inoculation.** Unless otherwise noted, inocula were equivalent to a monolayer (10⁵ cells/cm²). Cells were suspended in an appropriate volume of growth medium and poured over 2.54 × 7.62-cm sample slides contained in rectangular polypropylene trays (9 × 17 or 12 × 23 cm). The containers were agitated to disperse the cells and were allowed to stand 5 min before transfer to a 36° C incubator gassed with 5% CO₂ 95% air with 95% relative humidity. Incubation was for 24 h unless noted otherwise.

**Sample slides.** The 2.54 × 7.62-cm rectangular slides consisted of polystyrene cut from Falcon bacteriological petri dishes no. 1001 (Falcon Plastics, Los Angeles, CA) or polymethyl pentene cut from Sybron Nalge petri plates no. 5500-0010 from Fisher Scientific Pittsburgh, Pa. The plastic plates were either used after washing with ethanol, treated in an oxygen plasma that is similar to the method used for treating commercial tissue culture plates, or given various chemical treatments (11) to obtain a wide range of cell adhesion strengths. Details of the various surface treatments are given in the accompanying paper (10). Replicate samples were then run as described below to obtain a measure of the precision of the method.

**Centrifugation.** A Sorval centrifuge with swinging bucket head HS-4 was used. Slotted holders were constructed to support the slides in the swinging buckets so that the long axis of the slide was parallel to the centrifuge head radius. Several millilitres of growth medium was placed in the bottom of the buckets before capping to ensure that a humid atmosphere was maintained during centrifugation. The centrifuge buckets were balanced to ensure that the slides were in a horizontal position during centrifugation and that the applied gravity force was parallel to the long axis of the slides. This configuration gave a gradient of gravity forces along the length of each slide. Unless noted otherwise, the duration of centrifugation was 5 min. For each sample at least four slides were used, an uncentrifuged control and three slides that were centrifuged at several discrete speeds to provide a complete spectrum of gravity forces from about 400 to 7500 ×g. After changing the speed control with this type of centrifuge, it is not possible to preset a desired rotor speed precisely. The actual rotor speed attained was measured during each run with a photoelectric tachometer.

**Staining.** Each sample was rinsed with cold Dulbecco's phosphate buffered saline, fixed 1 min in absolute methanol, and stained 2 min in a solution of 0.3% methylene blue in 30% absolute ethanol. The samples were rinsed with water to remove excess dye and air dried. After staining, the position on the slide where complete cell removal occurred (see Figs. 1 and 2) was measured, and this distance from the axis of the rotor was determined. The rotor speed attained was constant during any one centrifugation but varied between centrifugations. The centrifugal force (RCF) for each run was calculated using the measured rotational speed and the point of complete cell removal was measured from the rotor axis.

Scanning electron micrographs were made of slides from which cells had been removed by the centrifugal process using the following fixation and coating procedure. The slides were fixed 1 h in 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3. The slides were rinsed 1 min in 2.0 M cacodylate buffer, pH 7.3, followed by 1 min rinse in 1% OsO₄ in 1.0 M cacodylate buffer, pH 7.3, and a final rinse in 0.2 M FIG. 1. Fused quartz slide with multilayer cell inoculation after centrifugation and staining. Scale zero is 9.5 cm from rotation axis. Slide shows gradient from region of no cell removal (less than 2 cm on scale or 11.5 cm from rotation axis) to dispersed layer and also the region of complete cell removal (greater than 5.5 to 6.0 cm on scale or 15 to 15.5 cm from rotation axis). The strips along the edges of the slide are from the slotted slide holder used in the swinging bucket. See Fig. 2 for the cell micrographs. Cell detachment from this particular surface was measured from the point of nearly complete cell removal near 6 cm. Photograph ×0.628.