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Letter to the Editor

GROWTH OF MDCK CELLS ON NON-TRANSPARENT SUPPORTS

Dear Editor:

Primary cell cultures or continuous cell lines are frequently used model systems in biomedical research (1,2). Most in vitro experiments are performed in polystyrene culture dishes, which allow the excellent and immediate evaluation of growth characteristics by simple phase contrast microscopy. Besides the conventional, fully-transparent cell culture laboratory ware, semi-transparent filter insets and various non-transparent biomedical materials are frequently used to test cell growth under in vitro conditions. Such tests are performed on surgical implantation grafts and are useful to evaluate the biocompatibility of solid or fibrous polymers for artifical articulations, blood vessels or dental prostheses (1,2,7). The identification of cultured cells by phase contrast microscopy on these materials is impossible. For that reason we developed a simple technique which would allow the estimation of cell growth on non-transparent surfaces within a reasonable time and without major technical equipment.

For the present experiments the established renal Madin Darby canine kidney (MDCK) cell line was cultured according to the literature (3,4,6). The cells were kept in 24 well tissue culture plates (Becton Dickinson, Heidelberg, FRG) containing minimal essential medium (MEM) with Earle's salts. The medium was supplemented with 10% heat inactivated calf serum. Media and additives were obtained from GIBCO-BRL Life Technologies, Eggenstein, FRG. Culture was carried out in a CO_2-incubator (Heraeus, Hanau, FRG) at 37° C with 5% CO_2 and 95% air under humid atmosphere. A complete medium change was performed daily.

To test transparent and non-transparent supports for cell growth commercially available materials in 13 mm diameter size were used (Thermanox - Nunc Wiesbaden; Cyclopore - Dunn, Asbach; Nucleopore - Reichelt, Heidelberg; Polycarbonate black/white - Poretics-Biotech Trade, St. Leon-Rot; Cellophane - Müller, Neutraubling; all FRG). Capsula fibrosa from pig kidney was sized to the standard diameter of 13 mm with a punching tool. The supports were then placed into special setup rings (Fig. 1; Minucells and Minutissue, Starenstr. 2, D-93077 Bad Abbach); (5). After autoclaving, the sterile setup rings were transferred into a 24-well tissue culture plate and 1 ml of culture medium with suspended cells (2×10^4/ml) was pipetted onto each. After seeding, the cultures were maintained for 7 days.

Determination of growth characteristics on fully transparent supports can easily be obtained by conventional phase contrast microscopy. However, with semi- and non-transparent supports the conventional optical methods fail to determine the appropriate parameters. The ring-method (Fig. 1) (5) offers a dependable tool to overcome most of the inherent difficulties. The cell-mounted support, including the setup rings, was transferred with a fine forceps into ice-cold 70% ethanol, where it remained for 10 minutes. It was then incubated in propidium iodide (4 μg/ml) (Sigma Chemie, Dei-

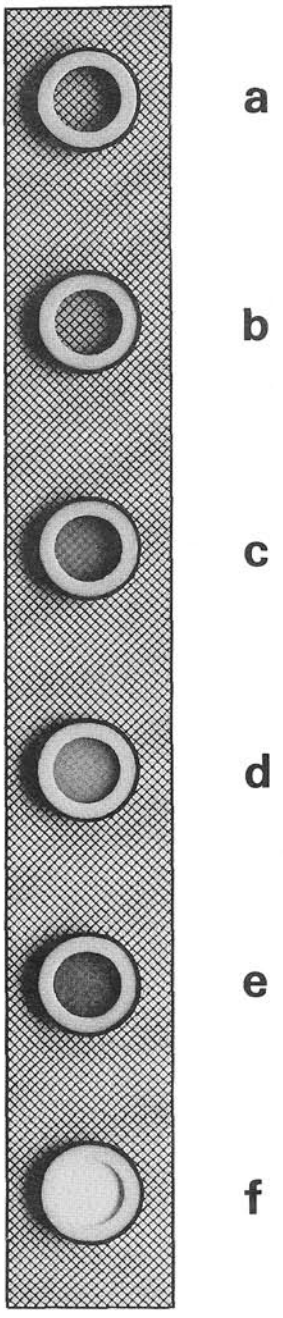

FIG. 1. The micrograph shows the setup rings in original size with transparent, semi-transparent and non-transparent supports used in the cell culture experiments: glass (*a*), Cyclopore (*b*), capsula fibrosa (*c*), Nuclepore (*d*), Polycarbonate black (*e*), and nylon (*f*).

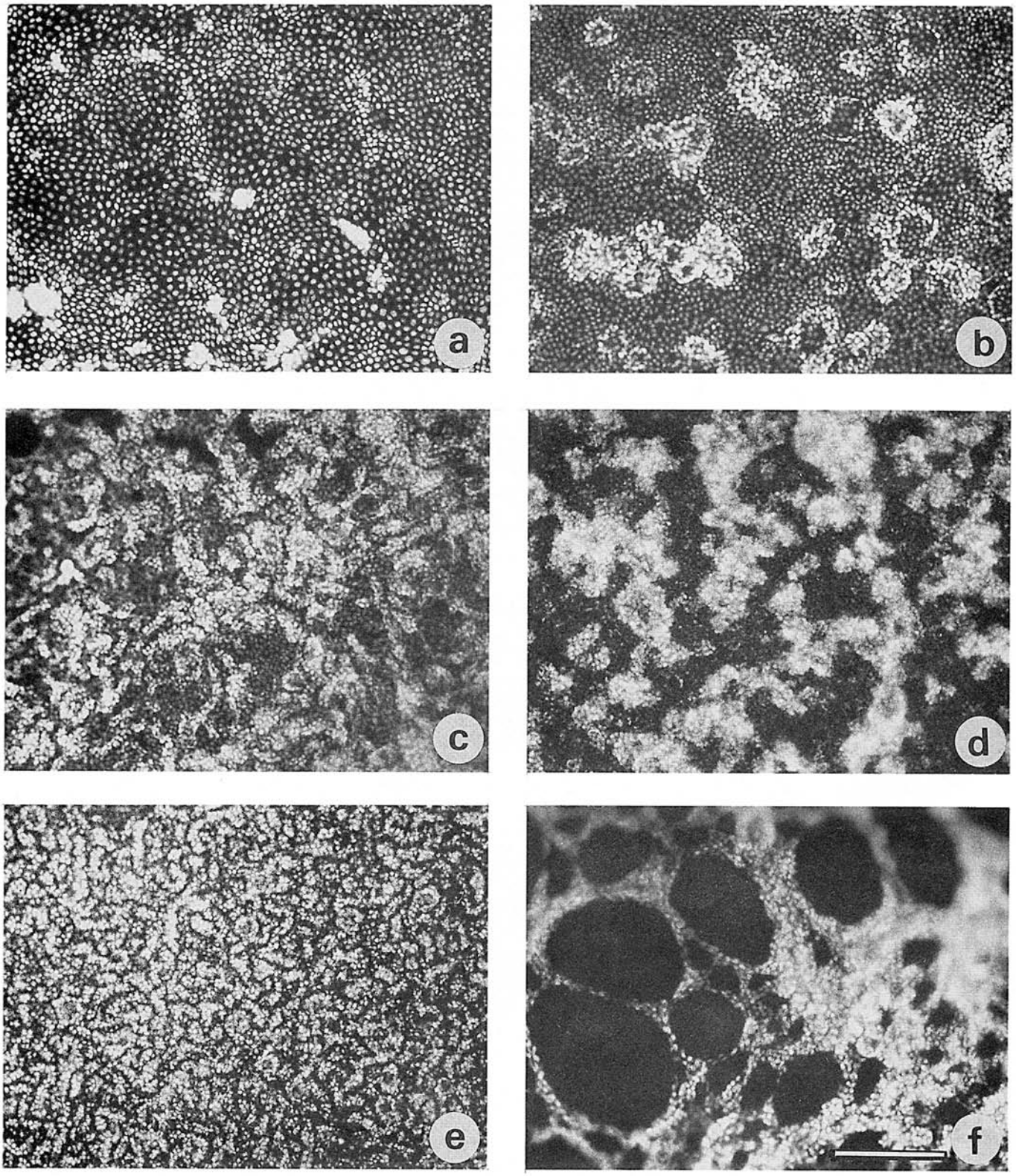

FIG. 2. Identification of MDCK cells grown on different supports: glass (*a*), Thermanox (*b*), Nuclepore (*c*), Polycarbonate black (*d*), nylon (*e*), and Cellophane (*f*) by fluorescence microscopy after propidium iodide labeling. The cells grown on glass (*a*) show a confluent monolayer with few clusters, while Thermanox (*b*) reveals multiple clusters and domes. Nuclepore (*c*) and Polycarbonate black (*d*) membranes show extensive tubular cluster formation. Nylon (*e*) reveals irregular tubular aggregations, while cellophane shows non-adherent tubular aggregations within wide areas of the eyefields. *Bar* = 200 μm.

senhofen, FRG) in phosphate buffered saline (pH 7.5) without Ca^{2+} and Mg^{2+} for 30 min at room temperature, finally the cells were rinsed three times with PBS. Microscopy of the cells could be performed on the same culture plates which were used for culture. It is important for the further procedure that the setup rings had to be turned upside down with a fine forceps before microscopy. An inverse epifluorescence microscope (Axiovert 35, Zeiss, Oberkochen, FRG) was used to record cell growth. The fluorescent nuclei were photographed at a final 32× magnification. Tripan X 400 films (Kodak) were used for documentation.

Cultured MDCK cells on different supports were investigated for individual growth characteristics (Fig. 2). Extreme differences in phenotypical appearance could be observed between cells grown on transparent and non-transparent supports. Although the cells were cultured within the same medium, and under the same conditions, the individual supports influenced the appearance of the cells remarkably. When glass (Fig. 2 *a*) was used as a support, only a few clusters were found within the confluent MDCK monolayer. The number of domes and clusters increased, however, when Thermanox (Fig. 2 *b*) was used as support. The use of Nuclepore (Fig. 2 *c*) and Polycarbonate black (Fig. 2 *d*) induced further cluster formations. The tubular clusters on the Nuclepore membranes (Fig. 2 *c*) were frequent but smaller than the extended clusters on the Polycarbonate black membranes (Fig. 2 *d*). On the Nylon support (Fig. 2 *e*), clusters showed a distinct pattern resembling small folds or waves on the "whale filter surface". Attempts to culture MDCK cells on Cellophane supports failed (Fig. 2 *f*). Wide areas of the cellophane were free of cells.

As has been shown in earlier investigations, the growth and differentiation of cells in culture is highly dependent upon the individual supports on which the cells attach (3,4,8,9). This was shown by coating culture dishes or filter insets with molecules such as poly-L-lysine, collagens, laminin, or fibronectin in order to mimic the natural extracellular matrix. Depending on the coatings selected, the cells changed their growth characteristics and morphological appearance (9). In the present experiments, MDCK cells were cultured without coating on three different groups of supports (Fig. 1): transparent, semi-transparent and non-transparent. It was possible to study the growth characteristics of MDCK cells on all chosen supports (Fig. 2). The growth characteristics of MDCK cells on the individual supports depended largely on the materials used. The general conclusion is that a prediction of cell growth on an individual support is not possible. Our experiments further demonstrated that poorly assessable physical surface properties influence the growth characteristics of MDCK cells (Fig. 2). Since the surface properties of the supports can hardly be defined in terms of physicochemical parameters such as molecular sizes, electric charges, hydrophobic - hydrophilic bondings or adhesion forces, it is speculative to speak in favor of these parameters' specific influence. Up to now it was generally believed that only pH, specific sequences of extracellular matrix proteins, hyperosmolality or the administration of hormones significantly influence the quality of growth and differentiation of cells in culture (3,4,6,8,9). However, the present experiments clearly show that dome, cluster and tubular formations of MDCK cells can be provoked by surface factors of the individual supports.

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