Tr. J. of Medical Sciences
28 (1998) 383-387

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The Role of Serum on the Adhesion of Cultured Chinese Hamster Lung (CHL) Cells

Received: September 20, 1996

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Abstract: The roles of serum on the initial attachment and adhesion strength of CHL cells, which is gained following initial attachment, spreading and growth, on tissue culture polystyrene dish were determined by using a convergent Microflow chamber. In the presence of 10% fetal calf serum 98%±2% of cells were attaching within 2 hours at 37°C. While only 74%±3% of cells were attaching during the same period of incubation in serum free medium. The critical shear stresses of detachment of CHL cells were 3.01±0.25 Nm^{-2}, 5.38±0.55 Nm^{-2}, 7.22±0.54 Nm^{-2}, 9.47±0.43 Nm^{-2}, and 9.54±1.15 Nm^{-2} in the presence of 0%, 0.05%, 0.1%, 1%, and 5% fetal calf serum respectively. In addition the effect of serum origins on the adhesion strength of this cell was determined. The the critical shear stresses of detachment of CHL cells were1.73±0.80 Nm^{-2}, 11.6±0.66 Nm^{-2}, and 10.96±0.73 Nm^{-2} in the presence of 10% of horse serum, new born calf serum, and fetal calf serum respectively. These results suggest that 1% serum concentration will be sufficient for cells to gain the maximum possible adhesion strength.

Key Words: CHL Cells; Adhesion strength; serum proteins.

Introduction

Cell substrate adhesion is a multistep process including initial cell contact to the substratum, attachment, spreading and growth (1-3). An understanding of animal cell adhesion may be important in controlling practical problems such as the control of cell growth on a biocompatible substrate, the proper anchoring of connective tissue on to metal bone prostheses and the prevention of attachment of blood cells to vascular prostheses. The investigation of all these areas requires the measurement of cell adhesion (4).

It is generally believed that the growth of almost all types of mammalian cells in culture require the presence of added serum in the culture medium (5,6). Serum is an extremely complex mixture of many molecules. There might be components of serum which are yet undefined. However, the major functions of serum can be broadly defined: these arettchment and spreading, nutrition, stimulation, and protection (7). Recently, rapid progress has been made in the identification and characterization of the serum proteins involved in cell adhesion such as fibronectin, vitronectin, laminin, thrompospondin (8,9). There are also many poorly studied proteins (adhesive proteins) found to mediate cell adhesion (10.11). In conrast, some serum proteins interfere with cell attachment, anti adhesive proteins, (12,13). For example, it has been shown that both α-1-antitrypin and albumin reduce adhesiveness of BHK cells (14) while immunoglobulin G (lgG) inhibits hepatocyte adhesion (15).

However, most of above serum-cell adhesion studies deal qualitatively with only initial cell attachment which is the first step in cell adhesion. Whereas, in this study the role of serum in cell adhesion, that is gained following initial attachment, spreading, and growh (3,6), was determined by using the Microflow chamber which has been developed recently (16). Hence, the relationships between the strength of cell binding on a tissue culture dish and origins of serum or concentrations of fetal calf serum in culture medium have been studied.

Materials and Methods

Cell Culture: Cultured Chinese Hamster Lung (CHL) cells were obtained from Flow Laboratories UK. CHL cells were maintained in minimum essential Eagle Medium (EMEM) with Earls salts supplemented with 20mM N-2-hydroxyethyl piperazine-N-ethanesulphonic acid (HEPES) buffer, 10% (v/v) fetal calf serum, 200 IU penicillin/ml, 200mg streptomycin/ml, 2mM glutamine and 2% non essential amino acids. Cultures were incubated in an atmosphere of 5% Co_2/air (17).

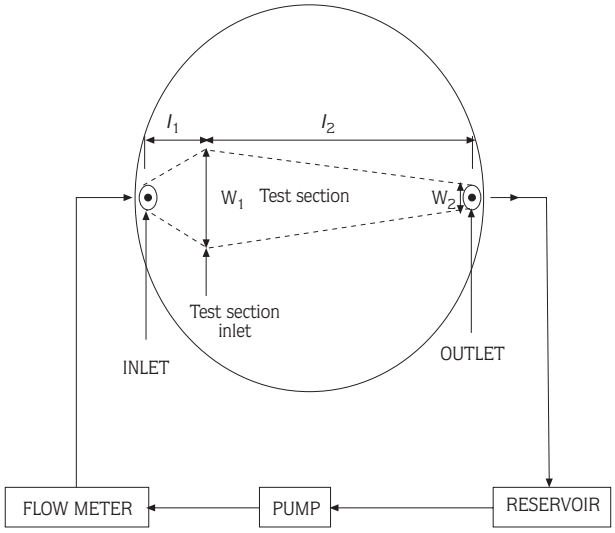

Figure 1. General Arrangement of Microflow Chamber and the Attached Apparatus (l_1:20mm, l_2:47mm, w_1:20mm, w_2:7mm)

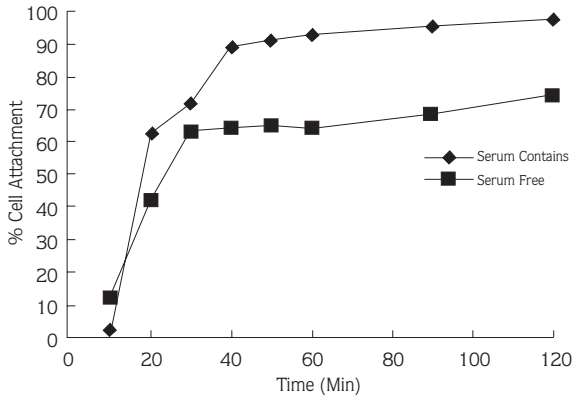

Figure 2. CHL cells attachment in the presence of 10% fetal calf serum or in the absence of serum.
Attachment studies were performed on 35mm tissue culture grade plastic dishes. 2ml of cell suspension contain totally $1x10^6$ cells were added into these dishes. After incubation at 37°C for indicated period the number of attached and non attached cells were determined.

Attachment Assay: A Sub confluent monolayer of cells was trypsinized with 0.05% w/v typsin in ethylenediamintetraacetic acid-phosphate buffered salina (EDTA-PBS) buffer and the action of this proteolytic enzyme wes stopped by using serum containing culture medium. The density of the cells was determined by means of a Neubauer hemocytometer. Then a stock cell suspension that contained $5x10^5$ cells/ml was prepared by

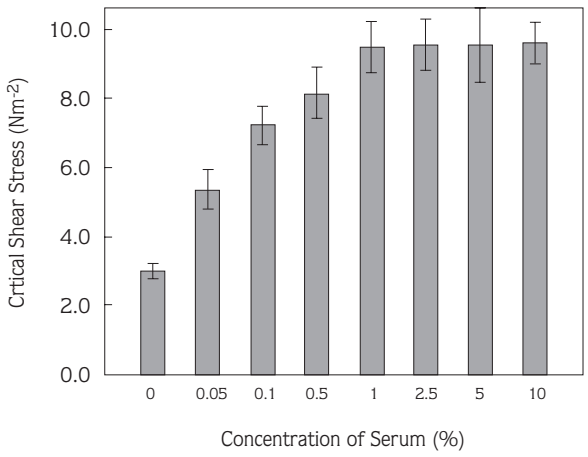

Figure 3. CHL Cells Adhesion Strength at the Various Concentrations of Fetal Calf Serum.
Sub confluent CHL cells weresubcultured and maintained in the culture medium that was supplemented with 0% to 10% (v/v) serum. The adhesion strength of the CHL cells growing in these media is measured in terms of the critical shear stress (c.s.s) of detachment.

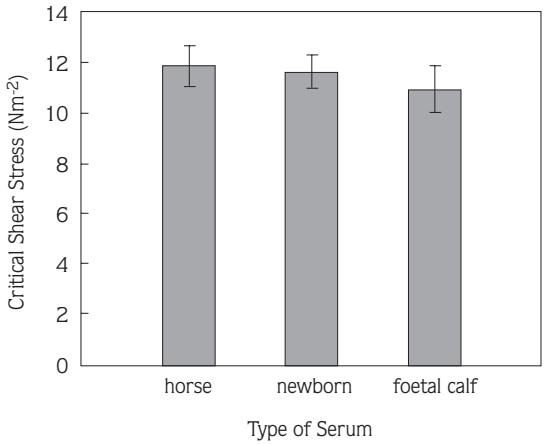

Figure 4. The Effect of Origin of Serum on CHL Cells Adhesion Strength.
CHL cells were seeded in the medium supplemented with 10% horse serum, or new born calf serum, or fetal calf serum on to plastic substratum. The adhesion strength of CHL Cells growing in these mediums is measured in terms of the critical shear stress (c.s.s) of detachment.
(P=0.02 between horse serum and fetal calf serum; P=0.04 between new born calf serum and fetal calf serum).

diluting the above cell suspension with growth medium. Subsequently, 2mls of the latter cell suspension were distributed onto 35 mm round tissue culture dishes which then were incubated at 37°C. Finally, after the incubation period, the culturemedium was transferred into a tube and all unattached cells were removed by washing dish with serum free medium. Any cell not removed by series of these gentle washes were considered to be attached. The numbers of attached and non- attached cells were counted in an haemocytometer. Experiments 5 times repeated in each of which 2 determinations were made. Without serum, attachment assay was performed as above except that trypsin was inhibited by soybean trypsin inhibitor (Sigma) and cells were cultured in serum free medium.

Detachment Assay: The 20 ml cell suspension, that was prepared from subconfluent cells as described above, was poured into a 100 mm tissue culture grade plastic dish. The cells were allowed to grow for 24 hours in 5% (V/V) CO_2/air atmophere at 37°C. Finally, the adhesion strength of these cells were measured by inserting this cell growing substratum on the Microflow chamber and after passing the running medium (serum free medium) over test substratum at defined flow rates. After 10 minutes of running; the distance from the beginning of test section to a point at which cells start to detach (critical distance) was measured by a ruler. By inserting this value and flow rate in equation:

$$\tau = \frac{13.15 \times V}{73 - L}$$

where

τ= Shear stress (N/m^2),

V= Flow rate (ml/s),

L= Critical distance (mm),

the shear stress of detachment of cell was determined (16). Experiments were repeated 4 times in each of which 10 determinations were made.

The Microflow chamber, basically, is a round shaped aluminum casting 100 mm diameter and coated with nylon.. A convergent channel is incorporated into this chamber. The width of this convergence at the beginning of this channel is 20mm tapering to 7mm at the end of the channel (figure 1). The depth of channel is constant (1mm). The cell culture dish forms the lid of the chamber. The dish and chamber is clamped tight and running medium at a convenient flow rate (determined by several trials) is pumped into chamber. Hence channel is convergent, the velocity of fluid passing through channel increase from inlet to outlet and hydrodynamic shear stress on cell growing substratum increase as well. At a certain critical point the surface shear stress becomes sufficiently large to cause the detachment of the cells. The distance from test section inlet to this detachment point called critical distance and is used in equation above to determined call adhesion strength (16).

Results

The amount of atached cells in 10 minute of incubation was higher in the absence of serum then those in 10% serum containing medium. However, after 60 minutes of incubation 93%±3% cells were attaching in the presence of serum while 64%±4% of cells were attaching during the same period in the absence of serum (figure 2). Over 60 minutes of incubation, there were slight increases in the amount of attached cells. Nevertheless, these were not significant in both cases. Hence after 2 hours of incubation 97%±2% and 74%±3% of cells were attching in the presence of 10% serum and in the absence of serum respectively.

Cell adhesion strength increased with increasing serum concentrations up to 1% of serum. While above 1% increasing the serum concentration did not have any noticeable effect upon the strength of CHL cells. In the absence of serum the adhesion strength of CHL cells was very low; the c.s.s of detachment being 3.01±0.25 Nm^{-2}. When the serum concentration was increased, the critical shear force required to detach cells from tissue culture dish increased as well. That is the values for the critical shear stress of detachment were 7.22±0.54 Nm^{-2}, 8.14±0.74 Nm^{-2}, and 9.47±0.43 Nm^{-2} in the presence of 0.1%, 0.5%, and 1% fetal calf serum in the culture medium respectively. Thus it is clear that upto a concentration of 1% serum, cell adhesion strength was serum-dosage dependent. Increasing serum concentration above 1% did not affect cell adhesion significantly. Hence the c.s.s of detachments were 9.54±1.15 Nm^{-2} and 9.60±0.75 Nm^{-2} in the presence of 5%, and 10% serum respectively. Although there were slight differences between these values, these were not statistically significant. For instance there was no significant difference in the c.s.s of detachments even between 1% and 10% of serum (P=0.67). (Statistical calculations were done by using Minitab software version 8.2)

The CHL cell adhesion strength was highest in 10%horse serum and lowest in the fetal calf serum. The actual values for the c.s.s. of detachment were 11.73±0.80 Nm^{-2} and 10.96±0.73 Nm^{-2} in horse serum

and fetal calf serum respectively (P=0.02) and it was 11.66±0.66 Nm^{-2} in new born calf serum (P=0.04, between new born calf serum and fetal calf serum respectively), figure 4. These results indicate that there is no significant effect of the origin of serum in cell adhesion strength, as far as above serum origins and CHL cells are concerned.

Discussion

The role of serum on the cell attachment, which is only initial step in cell adhesion, have previously been studied (18). However, there are so far no reports that indicate the role of serum on the cell adhesion process. In the present study, the effect of concentrations and origins of serum on the CHL cells adhesion strength, that is ganied following initial contact, attachment, spreading and growth (1,2,3), were determined quantitatively by Microflow chamber. In addition the attachment rates of CHL cells in the presence of 10% fetal calf serum or in the absence of serum were determined.

There was a lag phase in the CHL cells attachment curve in the serum presence, while in the absence of serum there was no lag phase. This difference could be due to that in the presence of cells do not directly adhere to the surface, instead they adhere via cell surface receptors. Whereas in the absence of serum there are no preadsorbed protenis on the substratum and simply cells adsorb to the surface non specifically (19). Thus in the first case cell attachment involves different subsequent steps such as, receptor-ligand binding, signaling, and reorganizations of other molecules that are involved in cell adhesion (20,21,22). This will of course take time and therefore as soon as the cells touch the surface they adsorb to it (23). In fact the duration of the lag period in the presence of serum increased as the incubation temperature decreased. Whereas in the absence of serum there was no lag phase even at 4°C (data is not shown).

In the absence of serum cell adhesion is performed by direct interaction between cell surface molecules and substratum (23,24). Hence, there is no further process for the cell to strengthen its adhesion. While in the presence of serum, cell adhesion is mediated by surface adsorbed serum proteins. If serum is considered to be the only source of proteins for cell adhesion, it appears that fetal calf serum, even at a concentration of 1%, provided sufficient amounts of adhesive proteins for CHL cells to gain their maximum possible adhesion strength. Fibronectin and vitronectin are two major cell adhesion proteins in serum (10,25). However, some of the proteins in serum affect the adsorptive properties of the adhesive proteins adversely. For example, albumin, and α-2-macroglobulin, serum anti adhesive proteins, reduce fibronectin adsorption (12). It was reported that the adsorption of fibronectin decreases at a serum concentration of 2% or above while the vitronectin adsorption increases (19,25). The above reports, coupled with CHL cell adhesion strength-serum concentration studies make it possible to suggest that at low serum concentrations CHL cells gain their adhesive strength mainly through fibronectin, while at high serum concentrations fibronectin has a limited role and at such concentrations CHL cells will attain their adhesion strength through vitronectin. Consequently, it is possible to suggest that, since fibronectin receptors and vitronectin receptors are integrin receptors (26) both of these receptors might exert the same effect on the cell, as far as the strength of cell adhesion is concerned.

There was no significant effect of origins of serum on cell adhesion strength, as far as the serum origins and CHL cells used in this study are concerned. In fact it has been reported that there is no detectable difference between horse serum fibronectin and calf serum fibronectin, as far as structure and functions are concerned (27). Moreover, it has been previously determined that six different animals vitronectins, which play a major role in the cell adhesion at 10% serum in the culture medium (19), did not significantly vary in their effect on BHK cells adhesion (28) These findings support results obtained in the present study, although there are as yet no reports that indicate the relationship between the origin of the sera and cell adhesion strength. In this work it was possible to show quantitatively that origin of serum has no significant effect on CHL cell adhesion strength.

Perhaps it sholud be mentioned here that the effect of serum in cell adhesion is not limited to the adhesion proteins. There are other serum components that might also have a role in cell adhesion. These components may include molecules such as tranferrin and growth hormones (29,30,31).

Acknowledgments

Author wishes to express his sincere thanks to Dr. WJD Whish of Department Biochemistry at Bath

University for his permission author to perform this work on his laboratory and for very fruitful discussion.

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