Contact behaviour during the reassociation of dissociated epithelial cells in primary culture

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Summary

The behaviour in culture of dissociated epithelial cells from chick embryo pigmented retina epithelium (PRE), corneal epithelium (CE) and epidermis has been studied using time-lapse cinematomography. The analysis concentrated on the contact behaviour of 60 previously isolated cells of each type during a 24h period starting 3-5h after the cells were plated out.

During the period analysed the number of isolated cells in cultures of all three types gradually decreased as they became incorporated into islands and sheets of cells. However, there were significant differences in behaviour between the cell types during the establishment of these sheets and islands. In PRE cell cultures, islands of cells developed because, throughout the period of analysis, collisions involving previously isolated cells almost invariably resulted in the development of a stable contact. Once having established contact with another cell these cells rarely broke away again to become reisolated. In contrast the contacts formed between colliding CE and epidermal cells were, at least initially, much less stable and cells of both these types were frequently seen to break away and become reisolated after colliding with other cells. Sheets and islands of cells eventually developed in these cultures because the frequency with which isolated cells become reisolated decreased with increasing time in culture.

The possible reasons underlying the different behaviour of PRE cells, when compared with that of CE and epidermal cells, are discussed. It is suggested that the decreasing tendency of isolated CE and epidermal cells to become reisolated may be related to the formation of desmosomes.

Key words: cell behaviour, cell culture, cell contacts, epithelial cells.

Introduction

The behaviour of epithelial cells in culture has not yet been so extensively characterized and quantified as that of fibroblasts. However, a detailed knowledge of the behaviour of this cell type is required for a fuller understanding of such processes as morphogenesis, wound healing and malignant neoplastic invasion.

The most significant behavioural difference between epithelial cells and fibroblasts in culture is that, in general, epithelial cells tend to form long lasting contacts with one another while fibroblasts do not (Abercrombie, 1970; Middleton, 1973). Thus, when migrating from an explant, epithelial cells usually form a sheet of tightly coherent cells (Vaughan & Trinkaus, 1966; Abercrombie & Middleton, 1968; Dipasquale, 1975) while fibroblasts form a meshwork of loosely associated cells. Similarly, in subconfluent cultures of dissociated cells, contacts between colliding epithelial cells, unlike those between colliding fibroblasts (Abercrombie, 1970) are long lasting and, as a result, the cells tend to reassociate as sheets or islands of cells (Middleton, 1973; Garrod & Steinberg, 1975; Brown & Middleton, 1982; Nicol & Garrod, 1982).

It is clear that the formation of long lasting lateral contacts between epithelial cells is of fundamental importance in determining their behaviour. To study this in more detail we have observed cultures of three types of chick embryo epithelial cells - pigmented retinal epithelium (PRE), corneal epithelium (CE) and...
epidermal cells — to establish how islands of coherent cells develop from populations of previously dissociated cells.

Materials and methods

Cell culture

The medium throughout consisted of Ham's F-10 tissue culture medium (Flow Ltd) supplemented with 10% foetal calf serum (Grand Island Biological Co.) and containing 50 units ml\(^{-1}\) penicillin and 50 \(\mu\)g ml\(^{-1}\) streptomycin.

Single-cell suspensions of PRE cells from 10-day-old chick embryos were prepared as described by Middleton (1977), single-cell suspensions of CE cells from 12-day-old chick embryos were prepared as described by Brown & Middleton (1981) and single-cell suspensions of epidermal cells from 8-day-old chick embryos were prepared as described by Brown & Middleton (1985).

Samples (0.2 ml) of suspensions of PRE, CE and epidermal cells, containing 2.4 \times 10^5 cells ml\(^{-1}\), 6.4 \times 10^5 cells ml\(^{-1}\) and 4.0 \times 10^5 cells ml\(^{-1}\), respectively, were plated out into 9 mm diameter glass rings attached to collagen-coated coverslips (see Middleton & Pegrum, 1976) with silicone grease. Cultures were maintained at 37°C in an atmosphere of 5% CO\(_2\) in air.

Filming and film analysis

Cultures were incubated for 1–3 h and then transferred to filming slides. To maintain the pH of the cultures during filming the medium was supplemented with 20 mM-Hepes. Cine films (16 mm) were obtained using Bolex-Wild time-lapse filming equipment in conjunction with a Reichert Biovert inverted microscope equipped with phase-contrast optics and a heated stage. The films were exposed at 1 frame/25 s.

Analysis of the films started 3–5 h after the cells were plated out and continued for the subsequent 24 h. 60 cells of each type which were isolated at the start of the analysis were selected and their behaviour was monitored continually throughout this period. To obtain this number of cells three films each of CE and epidermal cultures and 6 films of PRE cultures were analysed and the data pooled appropriately. The films were projected onto paper so as to give a total magnification (microscope plus projector) of \(\times 1600\). The frame number in which any of the selected cells made contact with another cell or island of cells was recorded and the analysis was continued to establish whether the cell subsequently became reisolated or remained in contact with other cells. For the purposes of this analysis we made no distinction between the contacts that the selected cells made with other isolated cells and those that they made with islands of cells.

Although previously isolated cells which had become incorporated in an island of cells sometimes broke some of their intercellular contacts while exchanging neighbours, they were still considered to have made a stable contact unless they broke all their intercellular contacts and became reisolated. When a cell did become reisolated the film frame number in which this occurred was recorded and analysis of the cell continued. For the purpose of analysis the frame numbers were converted to real time, i.e. hours since the start of the culture.

Results

At 3.5 h after plating out, cultures of all three types contained numerous isolated cells (Fig. 1A, B, C) but in the PRE cultures in particular some small islands of cells were also present (Fig. 1A). 24 h later, cultures of all three cell types appeared essentially similar; few isolated cells of any type remained because the majority had become organized into sheets and islands of cells (Fig. 1D, E, F). However, preliminary observations of the films suggested that there were significant differences in the behaviour of the three cell types during the establishment of these sheets and islands and this has been investigated in more detail.

Incorporation of isolated cells into sheets and islands

Analysis of the films started 3–5 h after the cells had been plated out. 60 cells of each type that were isolated at that time were selected and their interactions with other cells were continuously monitored for the next 24 h (see Materials and methods).

At 4 h intervals throughout this period the percentage of the selected cells that remained isolated was recorded. This figure included cells that had not yet collided with other cells as well as cells that had become reisolated after such collisions. It is clear from Fig. 2 that the percentage of isolated cells of all types gradually declined with time until, at the end of the period of analysis, none of the CE or epidermal cells and only one of the PRE cells remained isolated. It should be stressed that all the filmed cultures were subconfluent and that even at the end of the analysis large areas of the substratum remained unoccupied (see Fig. 1D, E, F).

Stability of cell contacts

Table 1 records the total number of collisions involving the selected cells of all three types and the number of these that were followed by the cells becoming reisolated. It is clear that the behaviour of PRE cells differed from that of the other types for in only one case did a cell of this kind become reisolated after colliding

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Total no. of collisions</th>
<th>Total no. of reisolations</th>
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<tbody>
<tr>
<td>PRE cells</td>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>CE cells</td>
<td>153</td>
<td>93</td>
</tr>
<tr>
<td>Epidermal cells</td>
<td>155</td>
<td>95</td>
</tr>
</tbody>
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\(n = 60\) for each cell type.
Fig. 1. Appearance of the cultures 3-5 h (A,B,C) and 27-5 h (D,E,F) after plating out. A,D, PRE cells; B,E, CE cells; C,F, epidermal cells. Phase-contrast. Bar, 100 μm.
with other cells. All the other observed collisions between PRE cells resulted in contacts that remained stable throughout the subsequent period of analysis. As a result of this behaviour the islands and sheets of cells that developed in these cultures tended to increase gradually in size as more previously isolated cells became attached to them.

In contrast both CE and epidermal cells frequently became reisolated after colliding with other cells (Table 1). On average individual isolated cells of both these types each became reisolated approximately 1·5 times during the period of analysis. As a result, the composition of the sheets and islands which formed was initially much more labile than that of those formed in PRE cell cultures because cells frequently made and subsequently broke contact with them. It was, however, clear from our films that the sheets and islands of cells formed by CE and epidermal cells later became more stable because the frequency with which cells broke away from them decreased. This observation suggested that collisions involving these cell types that occurred later in the period of analysis were more likely to result in stable contacts than were collisions which occurred earlier.

To investigate this further we scored the total number of collisions made by the selected CE and epidermal cells in consecutive 4 h periods during the first 16 h of analysis. We then monitored the cell involved in each collision to determine whether it had formed a stable contact or whether it subsequently became reisolated (see Materials and methods). We ceased scoring new collisions after 16 h, so that cells which came into contact late in this period could be observed for at least an additional 8 h (i.e. until the end of the period of analysis at 24 h) to determine whether the contact that they had made was stable or not. The number of collisions in each 4 h period that resulted in the cells forming a stable contact is shown in Table 2. For both cell types the percentage of cells developing stable contacts in each period shows a significant linear increase over the 16 h of observation (Fig. 3).

**Discussion**

During the period of analysis previously isolated epithelial cells of all three types formed stable contacts with other cells and, as a result, their numbers gradually decreased as islands and sheets of cells developed. However, despite this overall similarity between the

<table>
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<th>Time interval (hours since plated out)</th>
<th>No. of collisions</th>
<th>No. of collisions resulting in stable contact</th>
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<tbody>
<tr>
<td></td>
<td>CE cells</td>
<td>Epidermal cells</td>
</tr>
<tr>
<td>3·5–7·5</td>
<td>43</td>
<td>68</td>
</tr>
<tr>
<td>7·5–11·5</td>
<td>61</td>
<td>42</td>
</tr>
<tr>
<td>11·5–15·5</td>
<td>36</td>
<td>21</td>
</tr>
<tr>
<td>15·5–19·5</td>
<td>9</td>
<td>13</td>
</tr>
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</table>
cell types, our results show that two different patterns of behaviour were involved.

In PRE cultures, islands and sheets of cells developed because, throughout the period of analysis, collisions between cells of this type nearly always resulted in the formation of stable contacts, in fact in only one case did a collision involving these cells result in a cell subsequently becoming reisolated (see Table 1). In contrast, collisions involving either CE or epidermal cells often failed to result in the formation of a stable contact and cells of both types frequently became reisolated as they moved away from contacts they had developed. Islands and sheets of cells eventually developed in these cultures because there was, with time, a decrease in the frequency with which cells became reisolated after collisions.

Whether or not stable contacts develop between cells which have collided in culture must depend to a great extent on the balance between the strength of any adhesion that develops between them and the locomotory force that the cells can exert against this adhesion. Normal fibroblasts colliding in subconfluent cultures usually undergo contact inhibition and, after a short delay, move away from each other over an unoccupied area of the substratum (e.g. see Abercrombie, 1970). The locomotory forces that these cells can exert are clearly normally sufficient to break any adhesion that may have developed between them.

Our results, and those from a previous investigation (Middleton & Pegrum, 1976), suggest that the opposite is true for PRE cells. Even from the earliest stages that we have analysed, collisions between these cells nearly always result in the formation of stable contacts. In this study our analysis only started after the cells had been in culture for 3-5 h, but previously (Middleton & Pegrum, 1976) stable contacts were observed to develop between colliding PRE cells after they had been in culture for as little as 1-25 h. These results must suggest that the adhesions developed between these cells are normally of sufficient strength to resist any locomotory forces that the cells may exert against them. The reasons that may underlie this behaviour have previously been considered (Middleton & Pegrum, 1976; Middleton, 1982). Briefly, two factors seem likely to be important. First, although isolated PRE cells may spread extensively they do not normally adopt a polarized morphology and they have only a very limited capacity for directed migration (Brown & Middleton, 1985). This may suggest that they can exert only relatively weak locomotory forces against any adhesion that they develop. Second, after collisions, ultrastructurally well differentiated junctional complexes develop between the cells (Middleton & Pegrum, 1976; Nicol & Garrod, 1982). Although these complexes lack desmosomes (Nicol & Garrod, 1982; Docherty et al. 1984) they seem likely to be the sites of strong adhesion between the cells (Middleton & Pegrum, 1976; Middleton, 1982). Whatever the relative importance of these factors, it is clear that in this cell type the balance between the strength of their adhesions and the locomotory forces that they can exert favours the development of stable contacts.

Initially, at least, the same was not true of collisions involving either CE or epidermal cells for, during the early period of analysis, the majority of the collisions involving them resulted in the cells moving apart again to become reisolated. In fact, during the first 8 h of analysis only approximately 30% of the collisions between cells of both these types resulted in stable contacts being formed (data from Table 2). Sheets and islands of cells eventually developed because later the behaviour of the cells changed and in the subsequent 8 h period approximately 60% of the collisions between these cell types resulted in stable contacts (data from Table 2). More detailed analysis (see Results) confirmed that in both cell types there was, with time, a significant increase in the frequency with which stable contacts were formed. It is clear, therefore, that in these cell types the balance between the strength of their adhesions and the locomotory forces that they can exert initially favours the cells becoming reisolated after collisions but that later this changes to favour the development of stable contacts.

In the initial 8 h of analysis there was a clear difference between the behaviour of PRE cells and that of both CE and epidermal cells. This difference may be due to the fact that isolated CE and epidermal cells show a greater potential for directed migration than do PRE cells. Isolated cells of both these types spend a significant proportion of the time exhibiting a polarized morphology and migrating in an essentially fibroblast-like manner (Brown & Middleton, 1985). The speeds of movement attained by CE and epidermal cells when migrating in this way are substantially slower than those reported for many fibroblastic cell types but they are approximately 3-5 times faster than those attained by isolated PRE cells (Brown & Middleton, 1985). This ability of isolated cells of these types to polarize and undergo directed migration may explain the fact that, during the early periods of analysis, the locomotory forces that they could exert were, in the majority of cases, sufficient to overcome the strength of any adhesions that they developed with other cells. However, later this ceased to be the case and the majority of collisions between both CE and epidermal cells resulted in stable contacts. Such a change in behaviour could result from an alteration in either the locomotory forces exerted by the cells, or the strength of the adhesions between them, or both.

We have no direct evidence to suggest that the locomotory potential of either CE or epidermal cells changed during our period of analysis. Certainly, in a
previous study of the locomotory behaviour of isolated cells of both these types (Brown & Middleton, 1985) we did not find any evidence to suggest that either the proportion of the time for which the cells were polarized, or their speed of locomotion, changed significantly over a period of time that was identical to that used in the present investigation. However, we do have some evidence that the strength of the adhesions between the cells may change with time in culture. We have made an ultrastructural study of the junctions present between both CE and epidermal cells after different times in culture (Brown & Middleton, unpublished data). Our data suggest that desmosomes are initially absent from junctions between the cells, but that they start to develop between cells of both types after they have been in culture for about 12 h. The time that they first appear approximately coincides with the time at which, in this investigation, the behaviour of both cell types changed in favour of the development of stable contacts. It seems possible that the development of desmosomes may be responsible for this change in behaviour since they would be expected to strengthen the adhesions between the cells (e.g. see Nicol & Garrod, 1982; Garrod, 1986), which might then enable them to resist the locomotory forces exerted by the cells and so lead to the development of stable contacts. However, further investigations will be required to establish if this suggestion is correct. In particular it will be interesting to establish whether culturing the cells in medium with a low calcium ion concentration, which inhibits desmosome formation (Hennings et al. 1980; Watt et al. 1984; Jones & Goldman, 1985; Mattey & Garrod, 1986), will prevent CE and epidermal cells developing stable contacts.

This investigation has confirmed that the development of stable lateral contacts between the cells is a major factor in determining the behaviour of epithelial cells in culture, but it has also demonstrated that the way in which such contacts develop varies with cell type and that in some cell types the ability to form these contacts changes with time in culture.

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References


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