Technical note

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Treated glass coverslips for in situ culture of amniotic cells: better cell adhesion and growth than on plastic

In culturing amniotic cells for antenatal diagnosis, particular attention has been paid to the reduction of culture time to minimise the interval between amniocentesis and termination when results justify the latter.[1–3] The in situ culture method[4] is now used in many laboratories as an alternative to mass culture techniques, since it is more error-free.[5] The in situ procedure involves the seeding and harvesting of amniotic cells on glass coverslips. Rappaport *et al*[6] have shown that the charge density on glass surface is critical for adhesion and growth of mammalian cells. Furthermore, the glass charge density can be increased by a simple procedure.[7]

We recently experienced poor growth of some amniotic cell cultures set up according to the in situ technique. The difficulty could be definitely attributed to the coverslips, since concomitant cultures of the same amniotic fluid samples on plastic containers exhibited satisfactory growth. Because of this, we reviewed the procedure of Rappaport and Bishop.[7] In this note we report the results of 120 consecutive amniotic fluid cell cultures on glass coverslips treated according to their procedure.

The modified glass coverslip treatment was as follows. They were autoclaved in 0·1 mol/l EDTA in dilute NaOH (0·025N) at 125°C for 30 minutes, then rinsed, and autoclaved again in Na_2CO_3 (0·01 mol/l) at 125°C for a further 30 minutes. Finally, they were rinsed in distilled water, dried, and sterilised by dry heat.

The amniotic samples (average 15 ml), obtained by amniocentesis at the 17th week of gestation, were centrifuged and the pellets seeded in Petri dishes (35 mm, Corning) with treated coverslips on the bottom. The coverslips are cut to cover over 90% of the area of the Petri dish. The cultures were incubated in 5% CO_2 at 37°C. The culture medium (RPMI-1640+16% FCS, Flow Laboratories) was changed twice a week. Colcemid was added for the last 4 hours of incubation. The results are summarised in tables 1, 2, and 3. Data given in table 1 are for 20 consecutive amniocenteses. Sixty percent of the cell pellet was used for cytogenetic studies. The remaining 40% was divided equally between four Petri dishes, two of which did not contain a coverslip, and harvested after 10 days. This was done in order to compare the growth supporting abilities of plastic dishes and treated coverslips. Glass coverslips were compared to plastic because plastic is an excellent reference material. Moreover, in parallel cultures of 10 amniotic fluids, plastic yielded better results than non-treated glass coverslips.

The ratio

$$\frac{291 \text{ (total number of colonies on coverslips)}}{205 \text{ (total number of colonies on plastic dishes)}} \text{ is } 1{\cdot}42$$

($p = 0{\cdot}00001$). This quantifies the advantage of treated coverslips over plastic, as regards the number

TABLE 1 *Data for 20 consecutive amniocenteses.*

Colonies/20 amniotic fluids*			
Coverslips		*Plastic*	
291		205	
E	AF+F	E	AF+F
160	131	123	82

*Colonies were classified morphologically (E=epithelial cells; AF=amniotic fluid cells; F=fibroblasts) according to Hoehn *et al*.[8] Only colonies larger than 2·5 mm diameter were scored.

TABLE 2 *Number of days between initiation of culture and harvest for 100 consecutive amniocenteses (*includes one with 47,XX,+21 karyotype).*

Days elapsed						
4	5	6	7	8	9	10
2	5	17	44*	20	6	6

No of cultures (100)
$\bar{x} = 7{\cdot}17 \pm 1{\cdot}22$

TABLE 3 *Number of colonies/ml seeded amniotic fluid calculated after 10 days. Only colonies larger than 2·5 mm diameter were scored (*includes one with 47,XX,+21 karyotype).*

Colonies/ml						
0·6–1	1–2	2–3	3–4	4–5	5–6	6–7·78
9	28	31*	18	7	5	2

No of cultures (100)
$\bar{x} = 2{\cdot}68 \pm 1{\cdot}36$
Range: 0·60–7·78

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of colonies. If epithelial cells and AF cells+fibroblasts are considered separately, the ratios become:

$$\frac{160 \text{ (epithelial cells growing on coverslips)}}{123 \text{ (epithelial cells growing on plastic dishes)}} = 1 \cdot 30,$$

and

$$\frac{131 \text{ (AF cells+fibroblasts growing on coverslips)}}{82 \text{ (AF cells+fibroblasts growing on plastic dishes)}} = 1 \cdot 60.$$

This means that AF cells and fibroblasts are more sensitive to the improved culture conditions than epithelial cells. This can be regarded as a positive achievement, particularly when cultures are established for biochemical purposes, since AF cells and fibroblasts are more suitable for subculturing than epithelial cells. In addition, in our experience, AF cells and fibroblasts yield better metaphase spreads than epithelial cell colonies.

The second series of experiments (tables 2 and 3) refer to 100 consecutive amniotic cell cultures. The cell pellet was seeded into five Petri dishes containing treated coverslips. The two dishes showing most rapid growth were used for cytogenetic studies (table 2). The three remaining dishes were harvested after 10 days, in order to evaluate the number of colonies/ml of seeded amniotic fluid (table 3). No culture failed. The high number of colonies obtained and their rapid growth allowed us to harvest over 80% of the cultures within 8 days of seeding, as shown in table 2, and no culture lasted more than 10 days. At least six clones per culture were analysed. In our hands not less than three or four well spread metaphases can be easily obtained from small colonies (1·5 to 2 mm diameter). The 'islands' colonies[8] alone are unsuitable for cytogenetics, but they account for a very small percentage of the observed colonies.

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