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Promotion of monolayer formation in cultured whole pancreatic islets by 3-isobutyl-1-methylxanthine

(β cells/cyclic AMP/cell migration)

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ABSTRACT **Normal adult rat islets usually remained intact and encapsulated, even after many days in culture. In contrast, islets cultured in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (0.1 mM) attached more readily to the surface of plastic culture dishes and almost uniformly formed monolayers of endocrine cells. The mechanism of this effect is not known but presumably involves increases in cellular cyclic AMP content. Fibroblast growth did not appear to be stimulated by the inhibitor. These adult pancreatic endocrine monolayer cultures can be produced readily and provide useful preparations for further morphological and biochemical studies of factors affecting the differentiation, growth, and regenerative capacity of islet cells.**

METHODS

Adult rat islets were prepared under sterile conditions by a modified collagenase technique (1). Isolated islets were explanted into plastic petri dishes (35 × 10 mm) containing TCM 199 supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 5 mM glucose, with or without 0.1 mM 3-isobutyl-1-methylxanthine (IBMX). Cultures were maintained at 37° in a humidified atmosphere of 5% CO_2/air. Cultures were viewed by phase-contrast microscopy and were photographed at various intervals with a Polaroid camera attached to a Nikon MS inverted microscope. For examination by scanning electron microscopy, cultured cells were attached to plastic tissue culture discs. Attached cells were subsequently fixed for 1 hr in 2% glutaraldehyde in phosphate-buffered saline, dehydrated, and critical point dried. After coating with gold/palladium, islets were observed with a Coates and Welter model 50 scanning electron microscope.

RESULTS

When examined by phase-contrast microscopy, freshly isolated islets were seen to consist of irregular, large, rounded cell clusters, usually lacking a well-defined capsule (Fig. 1). After 24 hr, islets cultured in the control medium began to develop a more sharply delineated capsule. By the third day after explantation, the few exocrine cells initially present had disappeared and some attached fibroblastoid cells appeared in clusters on the plastic surface near islets that remained intact, encapsulated, and free-floating (Fig. 2*a*). By day 5 fibroblast proliferation was extensive (Fig. 2*c*), and by day 10 many of the islets were surrounded by fibroblasts (Fig. 2*e*). By day 10 most of the islets had become attached to the plastic surface but nevertheless remained rounded and intact, and only rarely did endocrine cells appear to be migrating from them to form a monolayer.

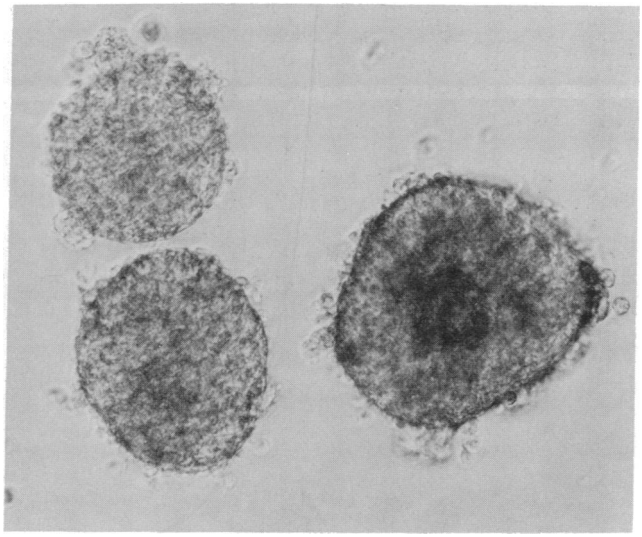

FIG. 1. Adult rat pancreatic islets prior to culturing. Isolated islets consisted of rounded, irregular cell clusters with some adherent fibroblasts and exocrine cells. (×130.)

When islets were cultured for 2 days in medium containing 0.1 mM IBMX, 70–80% of the islets became attached, and by 3 days after explantation all of the islets were adherent. Concomitant with attachment, the capsules of the islets appeared to be disrupted (or their formation was inhibited), and endocrine cells from the islets began to migrate out to form monolayers. By 3 days all of the islets maintained in medium containing 0.1 mM IBMX had attached to the petri dish and in most cases their capsules were beginning to break down to allow monolayers of epithelioid cells to form (Fig. 2*b*). A few islets remained intact and did not begin to form monolayers. By day 5, attached fibroblasts were evident in both control and experimental culture dishes (Fig. 2 *c* and *d*). After 10 days in culture, the islets in the control medium remained intact and were surrounded by fibroblasts (Fig. 2*e*) whereas monolayers had progressively developed by extension from the islets cultured in IBMX (Fig. 2*f*). Light microscopic examination of aldehyde-fuchsin stained epithelioid monolayers after 6 days of culture revealed the presence mainly of β cells. Biosynthetic experiments performed with islet cultures maintained in IBMX-containing medium for 6 days revealed active incorporation of [^{3}H]leucine into proinsulin and insulin and a stimulation of this process by glucose (data not shown).

In separate experiments we assessed the effect of high glucose

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Abbreviations: IBMX, 3-isobutyl-1-methylxanthine; cAMP, adenosine 3′,5′-cyclic monophosphate.

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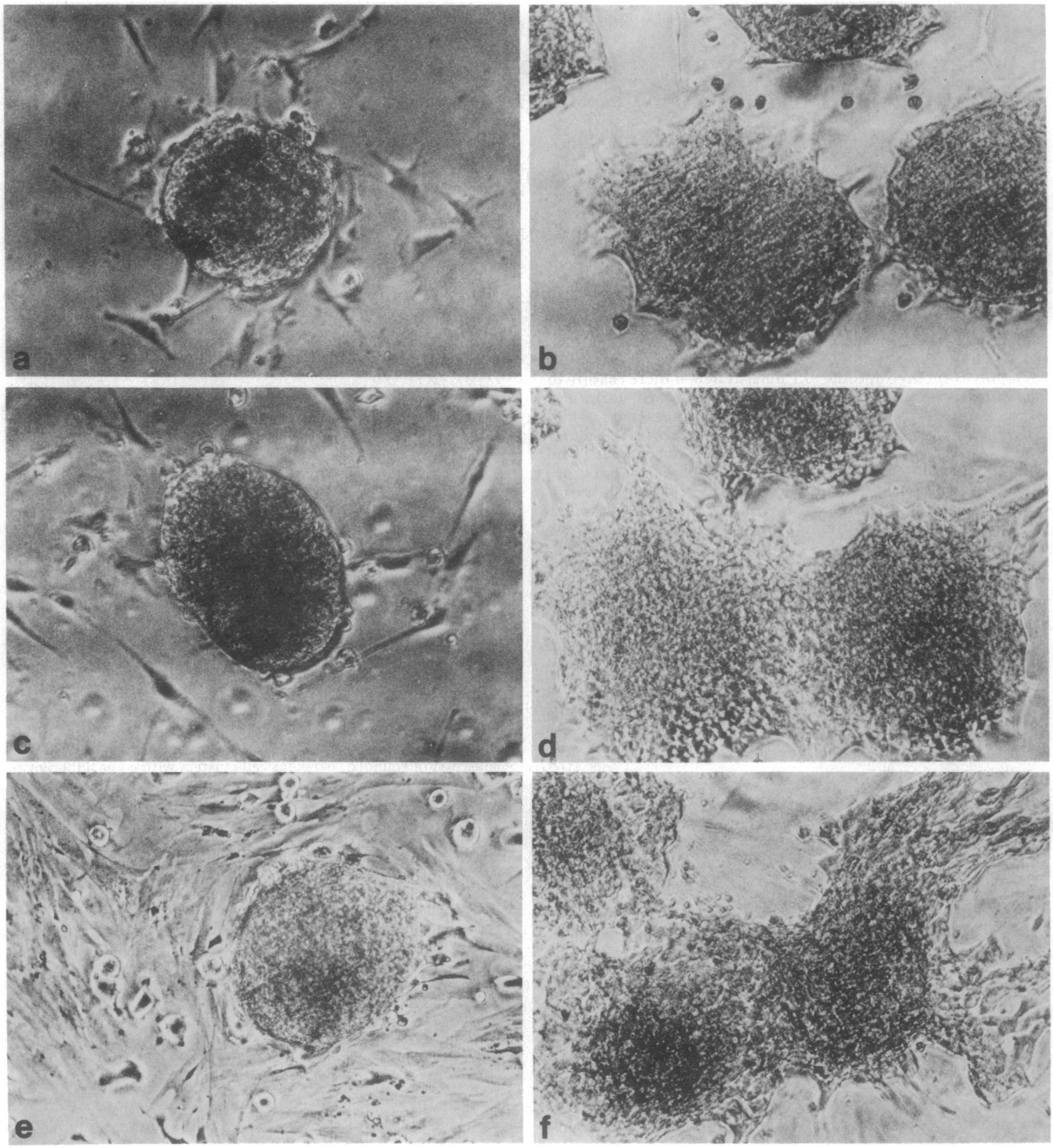

FIG. 2. Effect of phosphodiesterase inhibitor on rat islet culture. Control cultures (*a*) in the absence of IBMX and experimental cultures (*b*) with 0.01 mM IBMX on day 3. Day 5 control (*c*) and IBMX-experimental cultures (*d*). Day 10 control (*e*) and IBMX-experimental cultures (*f*). Note that islets in control medium remained intact, whereas islets cultured in 0.1 mM IBMX exhibited capsular breakdown by day 3, followed by progressive islet cell monolayer formation. (×130.)

concentration, alone or in the presence of IBMX, on monolayer formation. The results are illustrated in Fig. 3 and summarized in Fig. 4. After 14 days in culture with low glucose alone, about 60% of the islets had become adherent to the dishes, but only 19% of these had developed monolayers. High glucose alone for this period definitely enhanced monolayer formation. Under low glucose conditions, IBMX slightly increased islet attachment but had a marked effect on the extent of monolayer formation. In the presence of high glucose and IBMX, the effect was more pronounced and all the attached islets (80% of total) developed extensive monolayers.

Observation by scanning electron microscopy revealed attached cell monolayers characteristically exhibiting microvilli on the cell surfaces (Fig. 5).

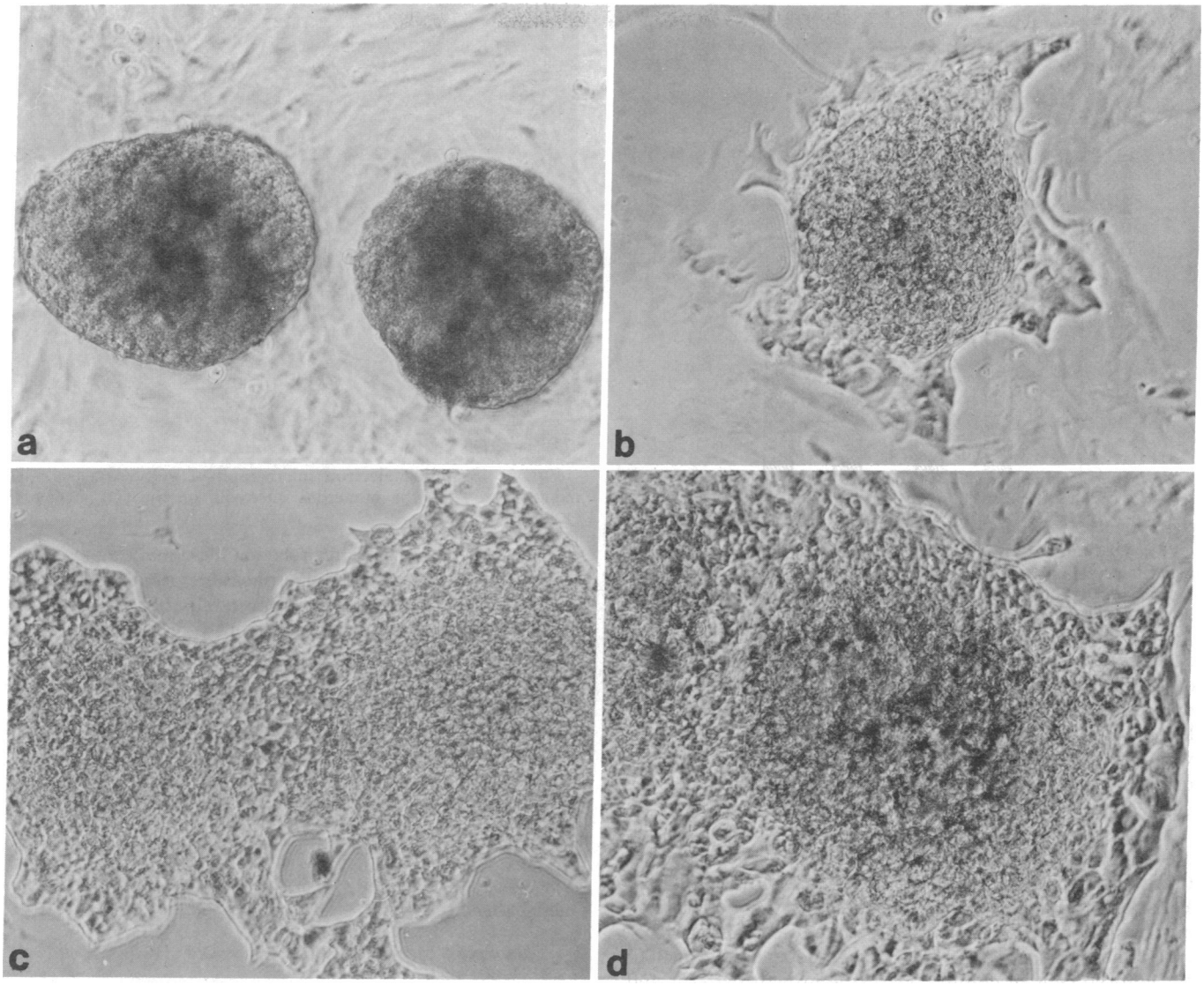

FIG. 3. Modulation of islet cell monolayer formation by glucose-containing medium and by medium containing both glucose and IBMX. Medium additions for cultures consisted of: (*a*) 5.5 mM glucose; (*b*) 16.7 mM glucose; (*c*) 5.5 mM glucose and 0.1 mM IBMX; and (*d*) 16.7 mM glucose and 0.1 mM IBMX. The presence of IBMX greatly enhanced monolayer formation in either high- or low-glucose medium. (×130.)

DISCUSSION

Several laboratories have reported observations on the maintenance of β cells in organ cultures of isolated islets or in fetal or neonatal pancreatic monolayer cultures (see ref. 2). Andersson and Hellerström (2) maintained isolated mouse islets for several weeks in a culture system similar to that used in the present studies. They noted that the islets gradually became attached to the dishes and tended to flatten out to form a thin peripheral monolayer. They noted no morphological differences in islets cultured, with glucose at 1.2 or 5.0 mg/ml. Thus, although they used a higher serum concentration (20% calf serum) in their experiments, their results appear to be comparable to our observations on the morphology of rat islets cultured in high- or low-glucose medium alone. It is not clear as yet whether the rather dramatic effect of IBMX in promoting monolayer extension from attached islets is due to the breakdown of an islet capsule that apparently forms during the early culture period or to a more direct effect of IBMX on the β cells which constitute the major cell population of these islets.

IBMX is a potent phosphodiesterase inhibitor and it has been shown to elevate cyclic AMP (cAMP) levels in islets (3, 4), especially in the presence of glucose, and to enhance the secretion (4) and biosynthesis of insulin (5). The optimal concentration for maximal effects on insulin secretion has been shown previously (4) to be 0.1 mM, the concentration chosen for these studies. However, this concentration does not produce a maximal increase of cAMP. We have also recently found that this concentration of IBMX stimulates DNA synthesis in cultured islets under certain conditions (unpublished data). Glucose nonetheless appears to be the preeminent stimulus to insulin biosynthesis and secretion in β cells, whereas cAMP may serve mainly as a modulator or promoter of the glucose response. Such a role for cAMP is quite different from its effects in some other endocrine cells, where it appears to function as a true second messenger to regulate secretion (6).

In addition to its well-known metabolic effects, cAMP also has been reported to affect the morphology and growth characteristics of both normal and transformed fibroblasts (6) and other cell types (7, 8) in culture. It has been suggested that the morphological changes may result from alterations in the distribution and state of aggregation of intracellular microfilamentous and microtubular structures (9), which in turn influ-

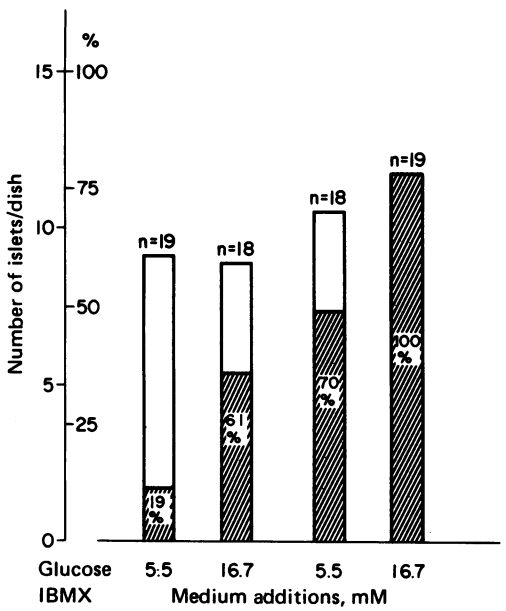

FIG. 4. Summary of the effect of glucose concentration alone or in the presence of IBMX on islet attachment and monolayer formation. □, Islets adherent; ▨, islets forming monolayers. n = number of dishes sampled; 15 islets cultured per dish.

ence the formation of microvilli and the distribution or properties of cell membrane receptors (10). This effect of the cyclic nucleotide is unmasked in transformed cells which have lower levels of cAMP than do normal fibroblasts (6). However, whether these effects of cAMP are direct or are mediated by changes in cell nutrition and metabolic activity remains unclear.

There is also an extensive literature on the influence of secreted proteolytic enzymes, such as plasminogen activators, on the morphological phenotype of cultured transformed cells (11). Pollack and Rifkin (12) have recently shown that both trypsin and plasmin can disperse intracellular actin-containing structures that are associated with anchorage-dependent growth of rat embryo cells in culture. Thus, the growth patterns and morphology of cultured cells may be modified by various factors that govern protease secretion including, of course, cAMP (13). We have previously noted that IBMX and high glucose stimulated the *in vitro* secretion, from islets, of small amounts of a serine protease into the medium along with insulin (14). In this connection, it recently has been observed that islets release plasminogen activator under similar conditions (M. A. Virjy, J.-D. Vassali, R. D. Estensen, and E. Reich, personal communication). Thus, it is conceivable that both capsular breakdown and β cell migration may be related to the increased release of proteases, especially in the presence of IBMX.

Further experiments obviously will be required to determine whether the observed effects on islet morphology stem directly from cAMP elevation in response to phosphodiesterase inhibition by IBMX or are due to extrinsic (serum) proteases evoked by secretory products (activators) from the islet cells. Finally, increased cellular motility in response to enhanced substrate utilization and metabolic activity due, in turn, to increased glucose and cAMP levels must also be considered as a possible indirect cause of all the above phenomena.

cAMP has recently been shown to serve as a chemotactic signal for aggregation of cells in certain systems such as the slime mold (15) and perhaps also in early chick embryos (16). Sensitive cells of *Dictyostelium discoideum* have cell surface receptors for sensing external cAMP levels and respond by changes in cell motility while relaying the signal via increased secretion of both cAMP and phosphodiesterase. Whether differentiated cells of higher organisms retain such chemotactic mechanisms remains unclear at present.

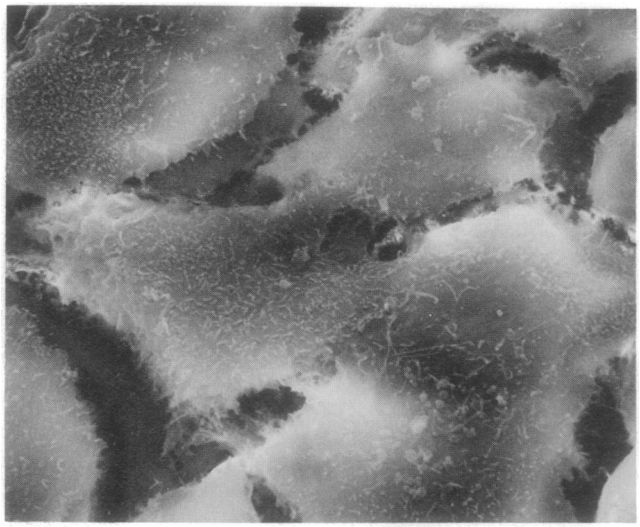

FIG. 5. Scanning electron micrograph of cultured islet cell monolayers. Note the numerous microvilli on the cell surfaces. (×2590.)

Among other factors that may possibly contribute to islet cell monolayer formation in response to IBMX should be included inhibition of fibroblast growth as well as local surface conditioning through the release of proteins (i.e., collagen, lectins, etc.) that may render the plastic surface more suitable for cell migration and attachment. Further study of this interesting problem may provide new insight into factors regulating β cell growth and islet structure.

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