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Original Paper

Isolated Extracellular Matrix-based Three-dimensional *In Vitro* Models to Study Orthotopically Cancer Cell Infiltration and Invasion

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An initial event in colon cancer progression is the migration of epithelial cells through the basement membrane (BM) and the invasion of the colon submucosa, where tumour cells enter blood and lymph vessels to spread throughout the body. To interrupt this process would mean the prevention of metastasis. In order to investigate tumour cell invasion orthotopically in the human system, we established novel *in vitro* models which mimic normal human colon tissue (colon reproductions, CoRes) and primary colon carcinomas (artificial tumours, ArTs). These models are based on the isolated extracellular matrix (iECM) of the respective human tissues. Two isolation methods were established, the Digestion Method and the Lysis Method neither of which destroyed the characteristic architecture of the ECM found in the original tissues. BM components, i.e. laminin, fibronectin and collagen IV, were detectable in the iECM isolated with the Lysis Method but not those isolated with the Digestion Method. Scanning electron microscopic analyses of the normal colon iECM demonstrated that even if the BM was missing, the luminal surface consisted of densely packed ECM filaments which do not allow cell infiltration without degradation of the iECM. Furthermore, we demonstrated that iECM can be separately supplemented with different cell types, i.e. colorectal carcinoma cells, normal fibroblasts and immune cells at any desired concentration, combination and localisation. Therefore, these models could be used to determine the role of the BM and of the tumour cell/normal cell crosstalk in the infiltration process of human colorectal carcinoma cells. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: organotypic culture, tumour invasion, extracellular matrix, human colorectal carcinoma
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INTRODUCTION

THE INVASIVE and metastatic potential of tumour cells depends largely on their ability to rearrange and penetrate their connective tissue envelope [1]. The intact extracellular matrix (ECM) of this envelope consists of an impenetrable three-dimensional meshwork of collagen fibres, proteoglycans and glycoproteins. Consequently, the infiltration of this barrier by carcinoma cells can be achieved only by partial degradation of basement membranes (BMs) and the underlying ECM [2]. Therefore, protease production in the tumour area is partly responsible for tumour spread.

Many types of malignant cells, including colorectal carcinoma cells, produce proteases or induce their production by stromal cells within the tumour [3]. Interestingly, the expression of proteases, i.e. matrix metalloproteases (MMPs), is partially regulated by integrin-related signals [4], indicating that the interaction between cancer cells and the connective ECM is involved in the regulation of their infiltrative capacity. In normal tissues, the interaction of integrins with organ-specific matrix receptors seems to be well balanced, controlling both the fixation of cells within an organ and the migration of cells. Carcinoma cells disturb this balance, allowing invasion of the underlining tissue [5]. Apart from interaction with the ECM, the crosstalk with stromal cells regulates the degradation of the ECM in at least two

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ways: direct contact with stromal cells leads to an enhanced expression of hydrolases on tumour cells [6]; and stromal cells, especially at the tumour/stroma interface, express hydrolase receptors, i.e. the urokinase plasminogen activator receptors (uPAR), which activate and focus hydrolytic activity to the tumour/stroma border [7]. It appears that a complex, yet poorly understood interaction of tumour cells with different stroma components determines the malignant behaviour of tumour cells rather than their inherent properties alone.

The infiltrative capacity of cancer cells depends on the organ or site into which they infiltrate. For instance, it has been shown that colorectal carcinoma cells implanted into the caecum of nude mice show a high infiltrative capacity and metastatic potential, whilst the same cells implanted into the subcutis neither metastasise nor show local infiltration [8]. For this reason, an *in vitro* culture system which exactly simulates the structure of the tissue which is infiltrated *in vivo* is needed to improve the relevance of *in vitro* invasion studies. Reconstituted ECMs, such as collagen gels, matrigels and amgels, were established 10 years ago and they are still used in most studies of tumour cell invasion [9–11]. Although great progress was made in the discovery of the molecular basis of tumour cell invasion using these models, they only partially resemble the complex organ-specific ECM and totally ignore the architecture of ECM in human tissue. To overcome these problems, some investigators have used the complete human amniotic membrane or the embryonic chick heart [12, 13], but, tumour cells normally do not infiltrate such tissues *in vivo*.

Although animal models represent the complexity of social cell interactions, they have numerous other disadvantages, the most important one being that the setting is not human. Therefore, the aim of the present study was to establish a link between oversimplified *in vitro* models and animal models in the case of colorectal carcinomas, by establishing a new complex three-dimensional *in vitro* model of the human colon mucosa, the tissue which is the first to be infiltrated by colorectal carcinoma cells.

MATERIALS AND METHODS

Culture conditions and cell lines

All cell types, except lymphocytes, were cultured in Dulbecco's MEM Nut Mix/F12 (1:1) supplemented with 10% heat inactivated fetal calf serum (FCS), 2 mM L-glutamine, 10 mM Hepes buffer, 100 U/ml penicillin and 100 µ/ml streptomycin (Gibco, Paisley, U.K.). Lymphocytes were cultured in RPMI-1640 medium completed with the same supplements. Incubations and cultures were performed at 37°C in a 5% CO_2 humidified incubator (Heraeus, Hanau, Germany).

The cell line CO115 and the fibroblast cell line 902 were kindly donated by A. Pèlegrin (Montpellier) and the Institute of Human Genetics and Anthropology, Freiburg, Germany, respectively. The cell lines SW403, HT29, and CaCo2 were purchased from the ATCC (Rockville, Maryland, U.S.A.). The establishment of tumour-derived fibroblast and lymphocyte cultures and the cell lines w25 and t80 have been previously described [14, 15].

Strategy for the improvement of in vitro *models*

To rebuild a 'colon tissue' *in vitro*, the ECM from a normal colon tissue was isolated and refilled with different cell types, which are most likely involved in tumour cell filtration, such as the tumour cells themselves, stromal cells (fibroblasts) and immune cells (lymphocytes). All these cellular components could be obtained from one tumour tissue specimen, as described previously [16].

Isolation of the ECM

In contrast to previously described methods of *in vitro* ECM reconstitution (gels), the method described in the present report was based on the preparation of the intact ECM of a well-defined human tissue by elimination of non-ECM components. One isolation method made use of a rigorous digestion of the cellular components (Digestion Method) whilst the other was based on the lysis of the stromal cell (Lysis Method).

Normal human colon tissue or colorectal carcinoma tissue samples were washed extensively in phosphate buffered saline (PBS; Seromed, Berlin, Germany) supplemented with 200 µg/ml gentamicin and 2.5 µg/ml amphotericin B (Gibco), and aseptically cut into pieces of approximately 4 cm (normal colon) or 1 cm colorectal carcinoma in diameter.

Digestion method. The pieces were incubated in a trypsin ethylendiamine tetra acetic acid (EDTA) (0.05/0.02%) solution (Seromed) for 3 days. Every day the pieces were rinsed in PBS and the trypsin/EDTA solution was renewed. Thereafter the pieces were rinsed in PBS and incubated in medium containing 100 U/ml deoxyribonuclease I (Serva, Heidelberg, Germany) for 2 days.

Lysis method. Epithelial cells were eliminated by incubation of the tissue with EDTA solution. Stromal cells inside the ECM were then lysed using a lysis buffer containing 1% NP40, 0.5% sodium deoxycholate, 0.1% sodium dodecylsulphate (SDS), 5 mM EDTA, 0.4 M NaCl, 50 mM Tris–HCl (pH 8) and protease inhibitors (2 mM phenylmethylsulphonylfluoride (PMSF), 10 µg/ml each of leupeptin, chymostatin, antipain and pepstatin A; Sigma, St. Louis, Missouri, U.S.A.). Finally the tissue was extensively washed in PBS without Ca^{++}, Mg^{++}. The isolated ECM was checked histologically for the presence of residual cells on slides of paraffin-embedded representative samples.

Both isolation methods of the ECM are simple and minimal technical equipment is needed. The simplest method is the Digestion Method because once the cellular elements are totally digested, further digestion did not change the structure of the ECM. In contrast, to obtain an optimal ECM using the Lysis Method, the isolation time has to be strictly controlled, i.e. the progress of each treatment should be observed using an inverted microscope and stopped either once the epithelial cells started to detach or all stromal cells were lysed. The most critical step in the Lysis Method is the washing procedure, during which cellular debris still present within the ECM after the different treatments has to be eliminated. To avoid damage of the relatively sensitive ECM, it should only be carefully shaken in the buffer until no particles are visible within the ECM. Microbial decontamination of the tissue samples was efficient since cytotoxic concentrations of antibiotics and antimycotics could be used.

Production of artificial tumours (ArTs)

The isolated iECM, isolated from colorectal carcinoma samples, was treated with 500 U/ml collagenase for approximately 30 min, before tumour cells and fibroblasts were plated on the iECM by centrifugation at 50***g*** in a long test

tube. Cells were allowed to grow on the iECM in this tube for 4 h, thereafter the resulting aggregate was transferred into a 25 ml culture flask, gently shaken occasionally to avoid attachment of the aggregate to the bottom of the flask.

Production of colon reconstructions (CoRes)

A wire mesh table with a central hole of 1 cm in diameter was placed into a normal Petri dish. Thereafter, the central hole was covered with the iECM, which was further fixed with a glass cylinder. This results in a two-compartment system separated by the semi-permeable iECM. Epithelial tumour cells were plated on to the surface of the iECM (lumenal side). When the tumour cells had attached to the iECM, the iECM was turned upside down and the fibroblasts and, thereafter, also other cells of interest, i.e. lymphocytes, were added to the other side of the iECM. The equipment and handling of the cell culture system is schematically shown in Figure 1.

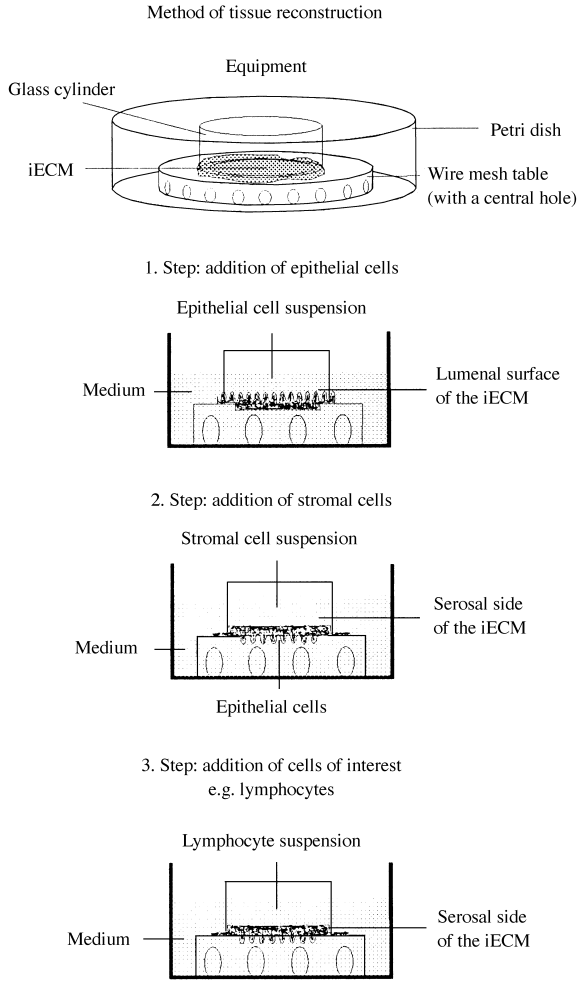

Figure 1. The figure illustrates the equipment and the method of isolated extracellular matrix (iECM) supplementation with different cell types. The central hole of a wire mesh table within a Petri dish was covered with the iECM, with the lumenal surface up. Thereafter, the iECM was fixed with a glass cylinder, leading to a two-compartment system separated by the iECM. The lower compartment was filled with culture medium and the upper with an epithelial cell suspension. Attachment and confluence of epithelial cells could be easily controlled with an inverted microscope. Once the epithelial cells had formed a complete monolayer on the lumenal surface, the iECM was turned upside down. Thereafter the stromal cell suspension was added to the upper compartment. To favour stromal cell sedimentation, the cell suspension was cooled to 0°C. When the stromal cells had attached to the iECM, the whole structure was once again turned upside down to optimise medium supply from the large lower compartment.

Histological investigations

Immunoperoxidase staining for the determination of the presence of BM components was performed on slides of formalin-fixed, paraffin-embedded three-dimensional cell cultures using antilaminin, antifibronectin and anticollagen IV monoclonal antibodies (Sigma) and the standard avidin–biotin technique.

Electron microscopy

For scanning electron microscopy, the tissues were prefixed with 2.5% glutaraldehyde and postfixed with 1% OsO_4. After dehydration in an acetone series, the specimens were dried by the critical point method (CO_2), sputtered with gold–palladium and examined in a scanning electron microscope (S-520 Hitachi).

RESULTS

Structure and architecture of the iECM

After elimination of the cellular components of either the colon mucosa or the primary colon carcinoma specimens, the maintained iECM was examined by light and scanning electron microscopy. No difference in respect to its architecture was observed between the ECM isolated by the two methods, i.e. Digestion Method and Lysis Method. Interestingly, histological investigation of the iECM derived from the normal colon tissue samples showed that the mucosal and the submucosal part could be easily identified within the iECM (Figure 2a). The iECM of the mucosa was characterised by densely packed fibres and crypt-like structures. The submucosa was made up of a loose meshwork containing multiple blood and lymph vessel-like structures. As shown in Figure 2(b) and (c), the lumenal surface showed multiple crypts of 50 μm in diameter, equal to the diameter of crypts in the normal colon. Scanning electron microscopic analyses demonstrated that the lumenal surface of the whole iECM, including the crypts, was built of densely packed filaments (Figure 2d and e). No remaining cellular components were found in the iECM. The specimen to specimen variation was minimal, if they were obtained from identical locations of the large intestine. The iECM from primary human colorectal carcinomas was an unstructured network of ECM fibres without any visible tissue architecture and the specimen to specimen variation was high (data not shown).

iECM components

An important area of intestinal ECM with respect to carcinoma cell invasion is the BM. When we analysed the Digestion Method iECM by immunohistochemistry, we could not detect BM components either on the lumenal surface nor in the vessel-like structures. All stainings using antifibronectin, antilaminin and anticollagen type IV antibodies were negative (Figure 3a). However, iECM isolated by the Lysis Method contained clearly detectable BM components at their physiological location (Figure 3b and c), i.e. at the top of the lumenal surface, around blood vessels and along the lamina muscularis mucosae. Immunohistologically, the

most intensive staining was seen with the anticollagen type IV antibodies, but laminin and fibronectin were also clearly detectable (data not shown).

Supplementation with different cell types

To evaluate the growth characteristics of colorectal carcinoma cells on the lumenal surface of the iECM cells from five different colorectal carcinoma cell lines, i.e. HT29, CO115, SW403, CaCo2 and w25, were plated on to the iECM. The undifferentiated CO115 cells contracted the iECM, resulting in a 2–3-fold decrease of the iECM in diameter. In addition, this contraction was associated with total destruction of the colon tissue-like architecture. Consequently, some cell aggregates growing on the base of the crypts were totally enveloped by the iECM (Figure 4a). Cells from all other cell fines did not significantly contract the iECM. SW403 cells grew as a multilayer of undifferentiated cells on the iECM, and cell contact was not well established (Figure 4b). In contrast, the moderate differentiated HT29 cells were arranged in a single cell layer with close cell/cell contacts. HT29 cells showed the greatest colonisation of the crypts in the iECM. It was very rarely observed that these cells had

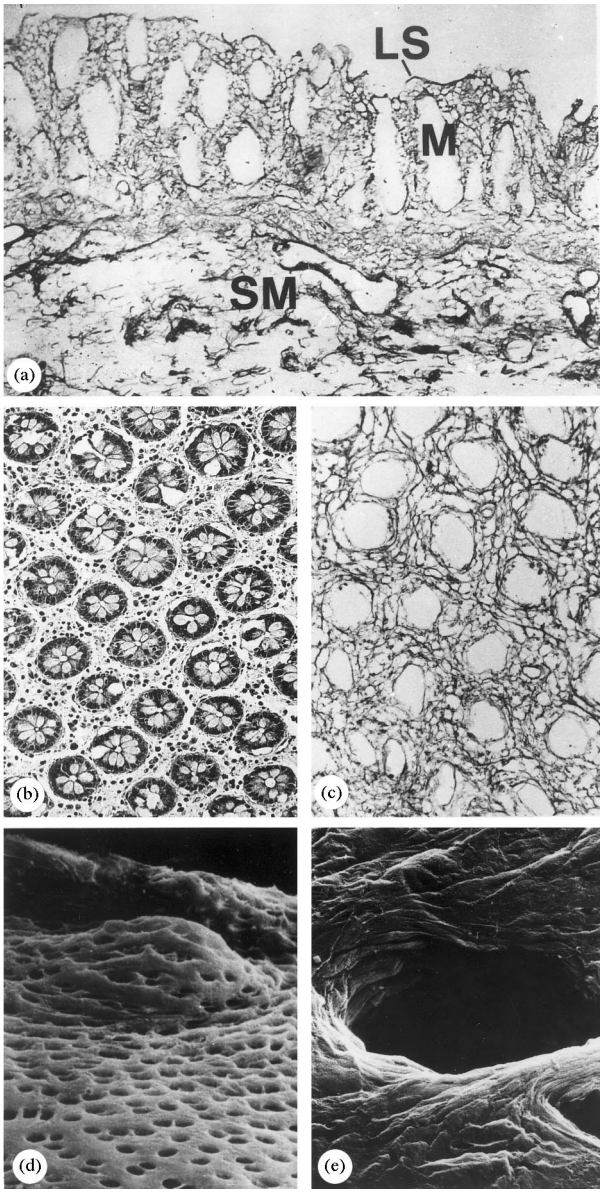

Figure 2. Morphology of the isolated extracellular matrix (iECM) from human colon. (a) Haemalum stained section (vertical to the lumenal surface (LS)) of the iECM showing the typical structure of the colon, i.e. mucosa (M) and submucosa (SM). (b) Haemalum stained section (parallel to the lumenal surface) of human colon mucosa and (c) of the iECM showing the maintenance of the typical colon mucosa architecture with numerous crypts. (d) Scanning electron micrograph of the lumenal surface of the iECM. (e) Higher magnification of (d) showing the entrance of a crypt (original magnification: (a)–(c)×140; (d) ×40 and (e)×700).

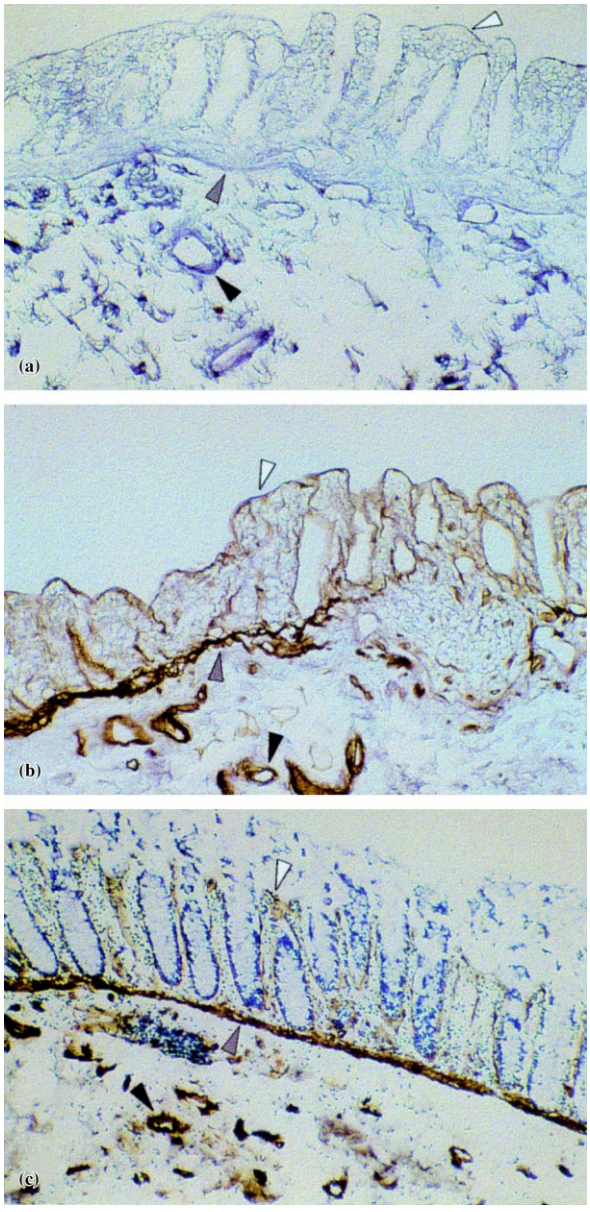

Figure 3. Demonstration of basement membrane (BM) components: (a) Isolated extracellular matrix (iECM) isolated by the Digestion Method, containing no BM components, (b) iECM isolated using the Lysis Method; the staining demonstrates that the BM was maintained, (c) normal colon. The BM was detected by anticollagen IV immunohistochemistry. Similar results were obtained using antilaminin and antifibronectin Monoclonal Antibodies. The typical distribution of BMs in colon tissues is indicated by arrowheads (original magnification: (a), (b)×100; (c)×60).

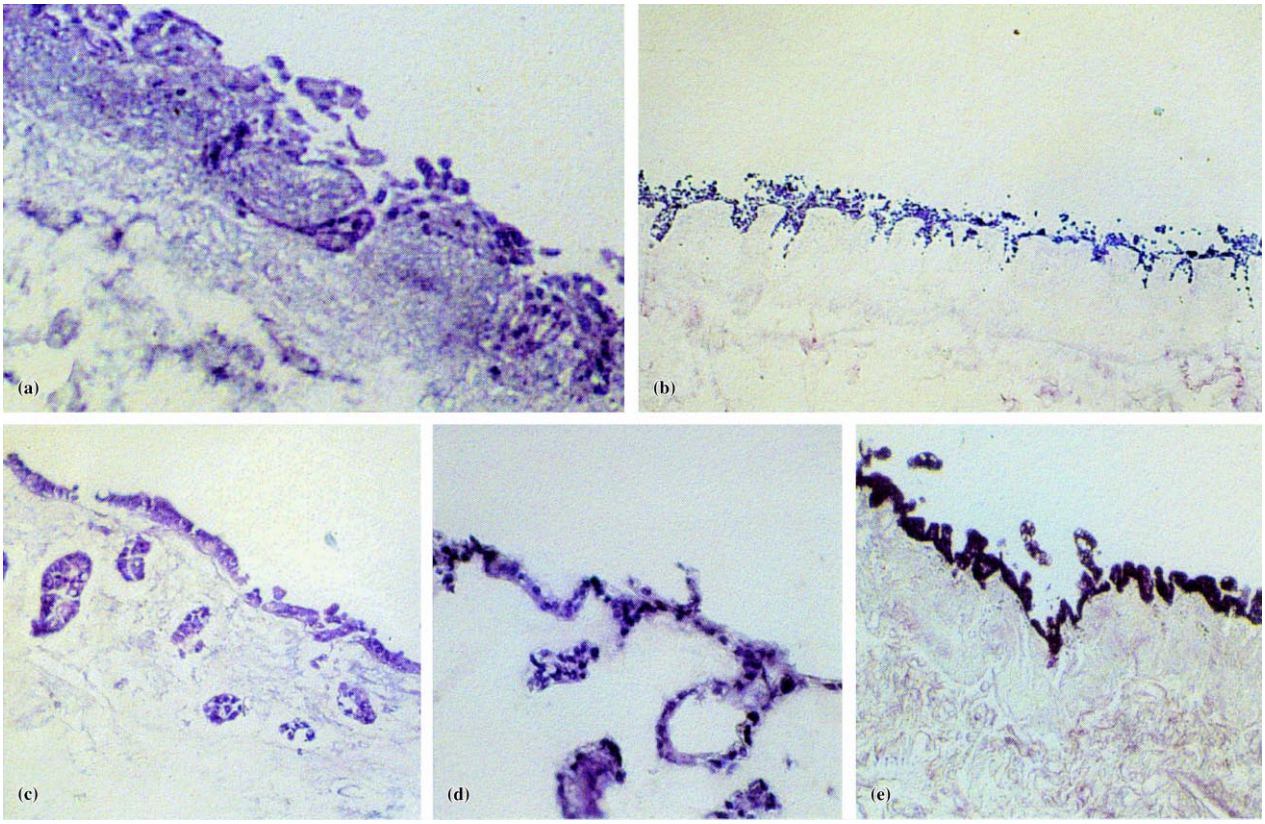

Figure 4. Comparison of the growth characteristics of different Colorectal carcinoma cell lines plated on to the lumenal surface of the isolated extracelluar matrix. (a) Growth of the undifferentiated cell line Co115 which forms a multilayer, resulting in contraction of the ECM. (b) Growth of the cell line SW403 also growing as a multilayer, but without contracting the iECM. (c) The more differentiated cell lines HT29 (c) and CaCo2 (d) formed a pseudo epithelium (monolayer consisting of polarised epithelial cells) on the iECM. Both cell lines colonised the crypts. (e) Growth of the newly established cell line w25 (original magnification: (A) ×200; (B), (E) ×60; (C) ×100; (D) ×140).

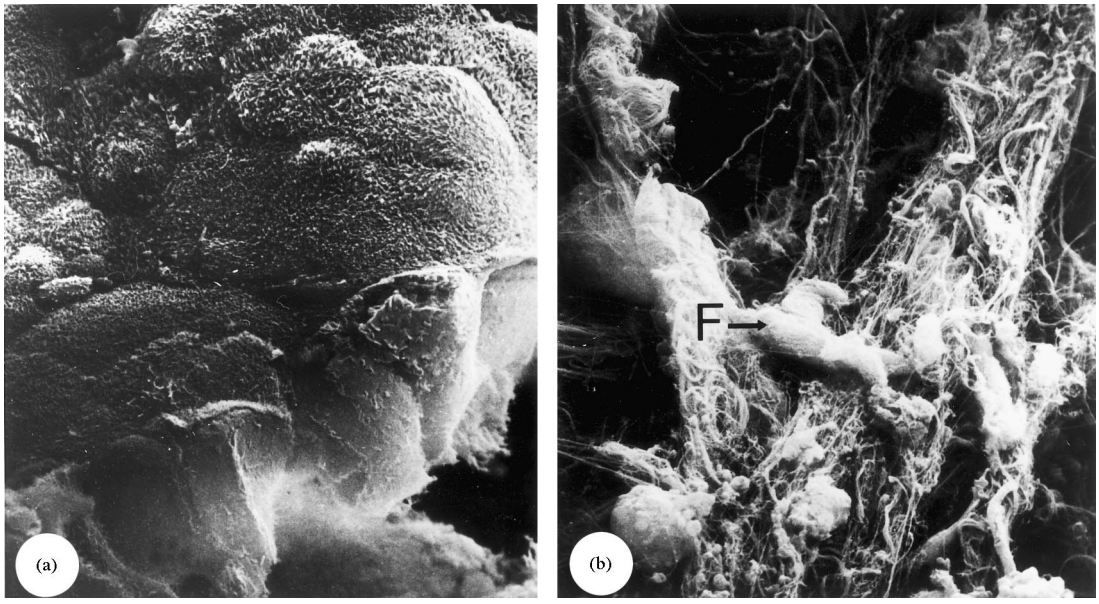

Figure 5. (a) Scanning electron micrograph of the lumenal surface of a w25 colon reproduction (CoRe). Note the continuity of the apical cell membrane surfaces characterised by the presence of numerous microvilli. (b) Scanning electron micrograph of the mesenchymal surface. Note the fibroblast (F) growing on the filaments and thereby connecting them (original magnification: (A)×1000; (B)×600).

bridged the crypts (Figure 4c). A very similar growth pattern was observed when well-differentiated CaCo2 cells grew on the iECM. Polarised CaCo2 cells also covered the crypts and formed glandular structures (Figure 4d). The newly isolated cell line w25 could not be separated into a single cell suspension without killing the cells. Therefore, they were placed on to the iECM as small cell aggregates. After 1 week of culture they had formed a solid pseudo epithelium (Figure 4e). However, cells which had colonised the crypts were only rarely observed. Between their lateral cell membrane domains they formed tight intercellular contacts, including tight junctions (data not shown). Scanning electron microscopy showed that the close contact between these cells resulted in a surface of the CoRes built only by apical membranes, characterised by the presence of multiple microvilli (Figure 5a), a structure which is very similar to the lumenal surface of normal colon. No cell of any cell line moved into deeper areas of the iECM within a 4-day culture period, indicating that the lumenal surface had no pores large enough for cell infiltration without partial degradation of the iECM.

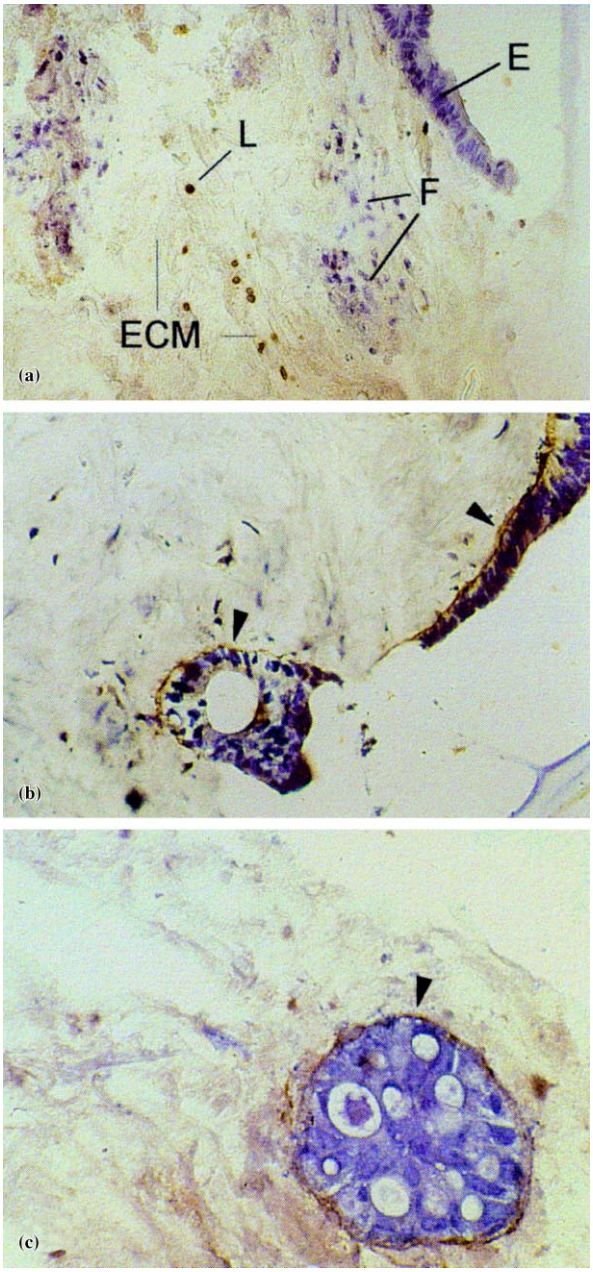

Figure 6. Demonstration of the deposition of basement membrane (BM) components in artificial tumours (ArTs). (a) Composition of a complete ArT (tumour derived extracellular matrix (ECM), epithelial cells (E), fibroblasts (F), and lymphocytes (L)). (b), (c) Immunohistochemical detection of rebuilt BM at the tumour cell/stroma interface (original magnification: (a), (b) ×200; (c) ×300).

Fibroblasts were added to the serosal side of the iECM. Since the iECM there has large, free spaces, cells could easily migrate into deeper areas. However, while fibroblasts were found all over the submucosal part of the iECM, only a very limited number was detectable in the mucosal area after culture for 4 days. In contrast, lymphocytes added in the same way were able to infiltrate the whole iECM. Since one fibroblast can adhere to and thereby connect different ECM fibres, a strong contraction of the iECM occurred, which correlated with the amount of added fibroblasts (Figure 5b). The number of added fibroblasts was also crucial to the lifespan of the cultures, since the time until a large amount of dead cells were observed mainly depended on the medium supply within and through the ECM. For example, if no fibroblasts were added, tumour cells could be cultured several weeks on top of, or within the ECM. However, once enough fibroblasts were added to get a strong contraction of the ECM, culture for longer than 4 days leads to large necrotic areas in the centre of the aggregates (data not shown).

Deposition of BMs

Usually, in colorectal carcinomas, tumour cells are at least partially separated from the tumour stroma by rebuilt BMs, which is thought to be the result of a co-operative production of BM components by malignant epithelial and stromal cells. Since in iECM-based *in vitro* models malignant and stromal cells can co-operate, we tested whether deposition of BMs occurs. To favour close contact between epithelial and stromal cells, iECM derived from colon carcinomas was used (Figure 6a). As shown in Figure 6(b), strong staining with anticollagen type IV monoclonal antibodies was observed around the pseudo glands of malignant cells. Since we used the iECM isolated with the Digestion Method the negativity of vessel-like structures demonstrated that the detected BMs were deposited by tumour/stromal cells. Interestingly, deposition of BM components could not be observed in homotypic spheroids of the same tumour cells (data not shown), demonstrating that it was a result of tumour cell/stroma interaction.

DISCUSSION

The relatively new, but rapidly growing field of so-called tissue engineering shows that there is an increasing interest and need for improved complex, i.e. three-dimensional, *in vitro* models in many fields of the life sciences. Therefore, great efforts have been made to develop different three-dimensional cell culture systems, e.g. spheroids [17], heterospheroids [18], hollow fibre culture systems [19], encapsulation techniques [20], collagen gels, matrigels and amgels [9–11] and microcarrier-based bioreactors [21]. In this report, we describe a culture system which is based on isolated ECM of the parental tissue to generate a homologue of the human large intestine and of primary colorectal carcinomas. These models are nearly identical in structure and architecture to the original tissues and most likely share many functional

properties with them, indicating that this culture system may have relevance for cancer research, as well as for pharmacological and toxicological *in vitro* assays.

For the usefulness of a new *in vitro* model, it is decisive how it compares with the models which already exist and whether it improves these models. There are several issues where *in vitro* models of the large intestine or colorectal carcinomas are useful. For example colorectral carcinoma cells have the ability to survive and grow in different parts of the human body, and in each part they have to tackle a special micro-environment composed of specialised cells, a special ECM and special humoral factors. Furthermore, we have previously shown that differentiated colorectal carcinoma cells have at least two different organisation forms which may influence their interactions with the immune system of the host [16]. Therefore, it seems to be important to have the proper model for colorectal carcinoma cells for each localisation and stage of cancer development, i.e. directly after the transformation of epithelial cells, the primary tumour, in the circulation, and the sites of metastasis, if a relevant investigation of the effects of different treatment modalities, i.e. chemo-, radio- or immunotherapy, is envisaged. Most, if not all, previously described *in vitro* tumour models simulate tumour cells in the circulation, micrometastases or already established tumours i.e. single cells, spheroids, heterospheroids, encapsulated tumour cells. ArTs improve these models in the case of primary tumours, since the tumour-specific ECM is included and the CoRes go one step back in the process of tumour progression and simulate the normal tissue, where epithelial cells are already transformed, but where the separation of cancer cells from the connective tissue envelope still exists.

In pharmacological and toxicological *in vitro* assays, the intestine barrier is usually simulated *in vitro* by a monolayer of CaCo2 cells grown on a simple culture plate insert. Since we demonstrated that CaCo2 cells grow as a confluent monolayer on the iECM, but also the same as an *in vivo*-like architecture and underlayered by a BM and mucosal stromal components, it seems to be likely that CoRes can improve the relevance of these *in vitro* studies.

In our view, the most important characteristic of our model for three-dimensional cell culture is that the cells grow *in vitro* on an identical substrate (the complete ECM) to that found *in vivo*. This seems to be especially relevant for epithelial cells, since their growth *in vivo* is regulated by tissue-BMs and species-specific ECM [22]. For example, the tissue-specific composition of the BMs influences differential gene expression in mammary epithelial cells, thereby controlling epithelial cell differentiation [23]. Therefore, providing the proper ECM signals, using the iECM as a culture substrate, may improve the survival of normal enterocytes *in vitro* compared with the use of collagen gels, as previously described [24], and we are currently investigating this possibility. Furthermore, the epithelial cell layer in the normal intestine shows a spatial separation of proliferative cells (on the bottom two-thirds of the crypts) and functional cells (on the top of the crypts), resulting, with only few exceptions, in the continuous linear migration of 'new' cells from the lower crypt upwards to the lumenal surface. How exactly this migration is regulated is not known, but there is substantial evidence that the different expression of BM molecules, such as fibronectin, tenascin and different laminin isoforms along the crypt–villus axis play an important role in the regulation of enterocyte adhesion and migration [25]. Interestingly, signalling through the cellular receptors of these BM molecules, the integrins, is crucial for cancer cell invasion. Since the BM existing in CoRes was formed in the colon *in vivo*, this model seems to be the most relevant to study cancer cell infiltration *in vitro*. However, strong invasion was not observed, even when colorectal carcinoma cells were grown on the iECM isolated with the Digestion Method which eliminated the BM. This would argue in favour of the BM not being the main barrier for epithelial cell invasion. Since the ECM could be isolated both with and without the maintenance of the BM using the described methods, it is now possible for us to verify this hypothesis.

In addition, it is noteworthy and important that the iECM not only maintained the ECM components, but also the characteristic architecture of the parental tissue ECM, since the architecture determines both the cell–matrix interactions, and the cell–cell contacts, thereby regulating cell–cell communication. That these social signals strongly influence epithelial cells *in vitro* is indicated by the observation that adenoma, carcinoma and normal epithelial cells survive better in culture when the cell–cell contacts are not disturbed or/and when they grow within an ECM substrate [26]. This and the finding that colorectal carcinoma cells in ArTs rebuild a BM, support the view that the use of iECM as a culture substrate may be optimal for an *in vivo* like behavior of epithelial cells *in vitro*. Furthermore, the importance of the architectural restriction under which tumour cells grow is stressed by the fact that colorectal neoplasms are believed to arise from mutations within the pluripotent stem cells, located at the base of the crypts [27]. Crypts are highly specialised three-dimensional structures, which promote the adhesion and fixation of stem cells at their physiological position, at the bottom of the crypt, which is, therefore, the site of interest for the very early events of cancer development. In our system, this was also the place where the CO115 cells were totally enveloped by the ECM, indicating that the three-dimensional architecture of crypts favours sequestration of undifferentiated cancer cells, thereby enhancing the selection of tumour cells with an infiltrative capacity. Therefore, it seems likely that the invasiveness of tumour cells growing in the three-dimensional iECM differ significantly from cells growing on a plan surface of an ECM gel, the former probably reflecting more truly their invasive potential. Since CoRes are the first *in vitro* models which exactly maintain the architecture of the normal colon, it is now possible to study the role of tissue architecture in colon carcinoma cell invasion. This seems to be important, since there is no *in vivo* model available which allows the investigation of the very early events in human colon cancer progression. Even the orthotopic transplantation of human tumour cells as described by Fu and colleagues started at a stage where tumour cells had already penetrated the BM and the closely underlying tissue [28].

In accordance with previous findings, the growth characteristics of the investigated pure colorectal carcinoma cell lines on the iECM indicated that the invasion capacity of most colorectal carcinoma cells is weak [29, 30], and that the stromal cells of the underlining tissue play a very important role in enhancing the invasive capacity of colorectal carcinoma cells. This view is further supported by the finding that the presence of stromal cells can enhance the expression of proteases by colorectal carcinoma cells and that the uPAR is

mainly expressed by stromal cells [7, 8]. It was further demonstrated that tumour cells induce various processes, which may collectively be called 'cancer cell-directed tissue remodelling'. Most of these processes are based on the interaction of tumour cells with normal stromal cells, i.e. induction of angiogenesis and immune cell invasion. Since the CoRes can be completed by addition of different stromal cells, CoRes may be useful to investigate the importance of the tumour cell/stromal cell interactions for cancer cell invasion.

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