Virchows Arch (2001) 439:571–578
DOI 10.1007/s004280000380

ORIGINAL ARTICLE

Paulo Tambasco de Oliveira
Márcia Martins Marques Jaeger
Sueli Patrícia Harumi Miyagi · Ruy Gastaldoni Jaeger

The effect of a reconstituted basement membrane (Matrigel) on a human salivary gland myoepithelioma cell line

Received: 15 September 2000 / Accepted: 6 December 2000 / Published online: 24 February 2001

Abstract We have already demonstrated that a reconstituted basement membrane (Matrigel) is a key modulator of morphogenetic changes and cytodifferentiation of pleomorphic adenoma cells in culture. Myoepithelioma is considered to be a neoplasm closely related to pleomorphic adenoma and should experience similar induction processes. Thus, the aim of this study was to investigate whether Matrigel would influence myoepithelioma cells. We used a cell line derived from a human salivary gland plasmacytoid myoepithelioma (M1 cells) grown in a three-dimensional preparation of Matrigel. Phenotype differences were assessed using conventional light microscopy technique (haematoxylin and eosin) and phase and differential interference contrast (Nomarski). Immunofluorescence was carried out to detect smooth-muscle actin, laminin and type-IV collagen. M1 cells exhibited all proteins studied, showing a myoepithelial differentiation. M1 cells grown inside Matrigel presented morphological changes and changes in smooth-muscle actin status. By growing M1 cells inside Matrigel, it was possible to reproduce the tumour architecture with no duct-like structures. Based on our findings, we suggest that myoepithelioma would be derived from a cell with a commitment to myoepithelial differentiation. We also suggest that the mechanical properties of the matrix environment will likely regulate smooth-muscle actin expression in myoepithelioma.

Keywords Salivary gland neoplasms · Myoepithelioma · Extracellular matrix · Smooth-muscle actin · Cell culture

P.T. de Oliveira · M.M.M. Jaeger (✉) · S.P.H. Miyagi · R.G. Jaeger
Department of Oral Pathology, School of Dentistry,
University of São Paulo, Av. Prof. Lineu Prestes 2227,
São Paulo SP 05508-900, Brazil
e-mail: rgjaeger@fo.usp.br
Tel.: +55-11-8187902, Fax: +55-11-2104409

P.T. de Oliveira
Department of Oral Histology, School of Dentistry,
University of São Paulo at Ribeirão Preto, Brazil

Introduction

Myoepithelioma is defined as a rare tumour of myoepithelial cell differentiation [9, 25]. Histopathological growth patterns may be solid, myxoid and reticular [7, 8, 9]. Two main cytological subtypes have been described for this tumour: spindle-shaped and plasmacytoid [23, 24, 25]. Myoepithelioma differs from pleomorphic adenoma in that it has little or no ductal component [24]. For some authors, myoepithelioma is part of the spectrum of pleomorphic adenoma [4, 9, 26].

It has been suggested that the extracellular matrix plays an important role as a regulatory factor of phenotypic differences among salivary gland neoplasms [13, 14, 16]. We have already demonstrated that a three-dimensional preparation of a reconstituted basement membrane (Matrigel), a supramolecular array of extracellular matrix proteins, is a key modulator of morphogenetic changes and cytodifferentiation of pleomorphic adenoma cells in culture [14]. Myoepithelioma is considered to be a neoplasm closely related to pleomorphic adenoma [4, 23, 24, 26] and should experience similar induction processes. Therefore, it would be interesting to study whether basement membrane molecules could influence myoepithelioma cells.

We have previously established a neoplastic myoepithelial cell line (M1 cells) derived from human salivary gland plasmacytoid myoepithelioma [16, 20]. M1 cells were grown in contact with Matrigel in a three-dimensional preparation. Phenotype differences were assessed using conventional light microscopy technique [haematoxylin and eosin (HE)], phase and differential interference contrast (Nomarski) and fluorescence microscopy.

Materials and methods

Cell culture

M1 cells were derived from a human plasmacytoid myoepithelioma [16, 20]. These cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM, Sigma Chemical Co., St.

Louis, Mo.) supplemented with 10% fetal bovine serum (Cultilab, Campinas, SP, Brazil) and 1% antibiotic–antimycotic solution (Sigma). The cells were maintained in 25-cm^2 flasks in a humidified atmosphere of 5% CO_2 at 37°C. To study phenotype differentiation, M1 cells were plated on no. 1 round glass coverslips (Ted Pella Inc., Redding, Calif.). Morphology of M1 cells was analysed using phase contrast.

Immunofluorescence

Detection of smooth-muscle actin was carried out to confirm the myogenous differentiation of M1 cells. Labelling of smooth-muscle actin was performed as described previously [16]. For laminin and type-IV collagen immunostaining, M1 cells grown on coverslips were fixed in 1% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min and rinsed in PBS. Cells were then subjected to an immunofluorescence protocol [15] using mouse monoclonal antibodies against laminin (Biogenex Laboratory, San Ramon, Calif.), diluted 1:50 in PBS and type-IV collagen (Biogenex), diluted 1:20 in PBS. An anti-mouse fluorescein (FITC) conjugate (Amersham Co., Arlington Heights, Ill.) was used as a secondary antibody. All incubations were done for 60 min at room temperature. The mounting medium was Pro Long (Molecular Probes, Eugene, Ore.). Replacement of the primary antibody with PBS was used as a negative control.

Immunofluorescence labelling of M1 cells was carried out at least five times, and a minimum of 100 cells was examined each time. The observations and photographic recording were carried out under a Zeiss Axiophot 2 fluorescence microscope (Carl Zeiss Inc, Oberköchen, Germany), using the objectives 40× Plan Neofluar, 1.4 NA and 63× Plan Apochromatic, 1.4 NA.

Three-dimensional preparation of Matrigel

M1 cells were plated on no. 1 round glass coverslips coated with a reconstituted basement membrane (Matrigel, kindly provided by Dr. Matthew Hoffman NIDCR, NIH, Bethesda, Md.). The coating procedure was as follows [14]: we prepared a thick gel for growing cells within a three-dimensional matrix, simulating an intact basement membrane. Matrigel was thawed, homogenised and diluted in cold serum-free DMEM to reach a final concentration of 6 mg/ml (stock solution 13 mg/ml). M1 cells were then harvested from the culture flasks and resuspended inside Matrigel preparation. After that, this Matrigel containing M1 cells was placed (100 µl/cm^2) on cold coverslips. Cells were then incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. M1 cells were grown on this three-dimensional preparation of Matrigel for 1 week. Two controls were used: (1) M1 cells plated on plain glass coverslips and (2) M1 cells plated on coverslips with a thin coating of Matrigel (3 mg/ml; 50 µl/cm^2).

Light microscopy

Coverslips with M1 cells growing within Matrigel were fixed in 10% formalin for 24 h. Then, Matrigel was carefully removed from the coverslips, paraffin-embedded, and stained with HE. Cells growing inside Matrigel were also studied using phase contrast, Nomarski and fluorescence microscopy. For fluorescence microscopy, we used samples immunostained with smooth-muscle actin, in order to access overall morphology of M1 cells inside Matrigel. For comparative analysis between in vitro and in vivo situations, we obtained new HE-stained sections from the tumour that originated the M1-cell line [16, 20].

Results

Phase contrast microscopy showed that M1 cells, when grown on a glass surface, presented a polyedrical morphology with abundant cytoplasm and visible stress fibres (Fig. 1).

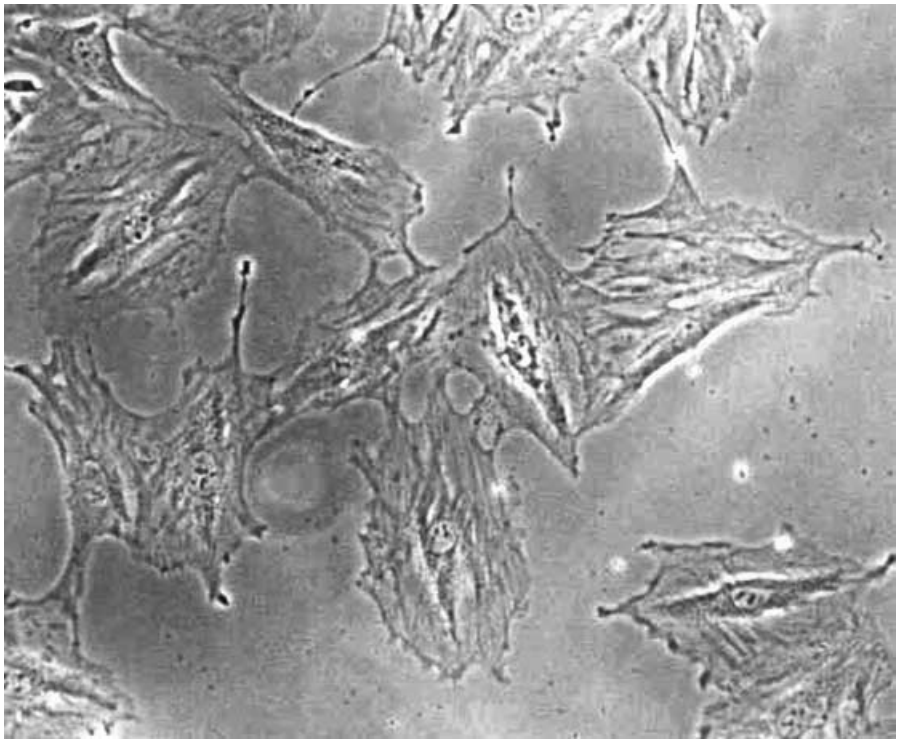

Fig. 1 Phase-contrast microscopy of M1 cells (derived from a human salivary gland plasmacytoid myoepithelioma) grown on a glass surface. A flat monolayer of polyedrical cells is observed. The nuclei are round, with two or three nucleoli. The cytoplasms are abundant, with stress fibres. Magnification 400×

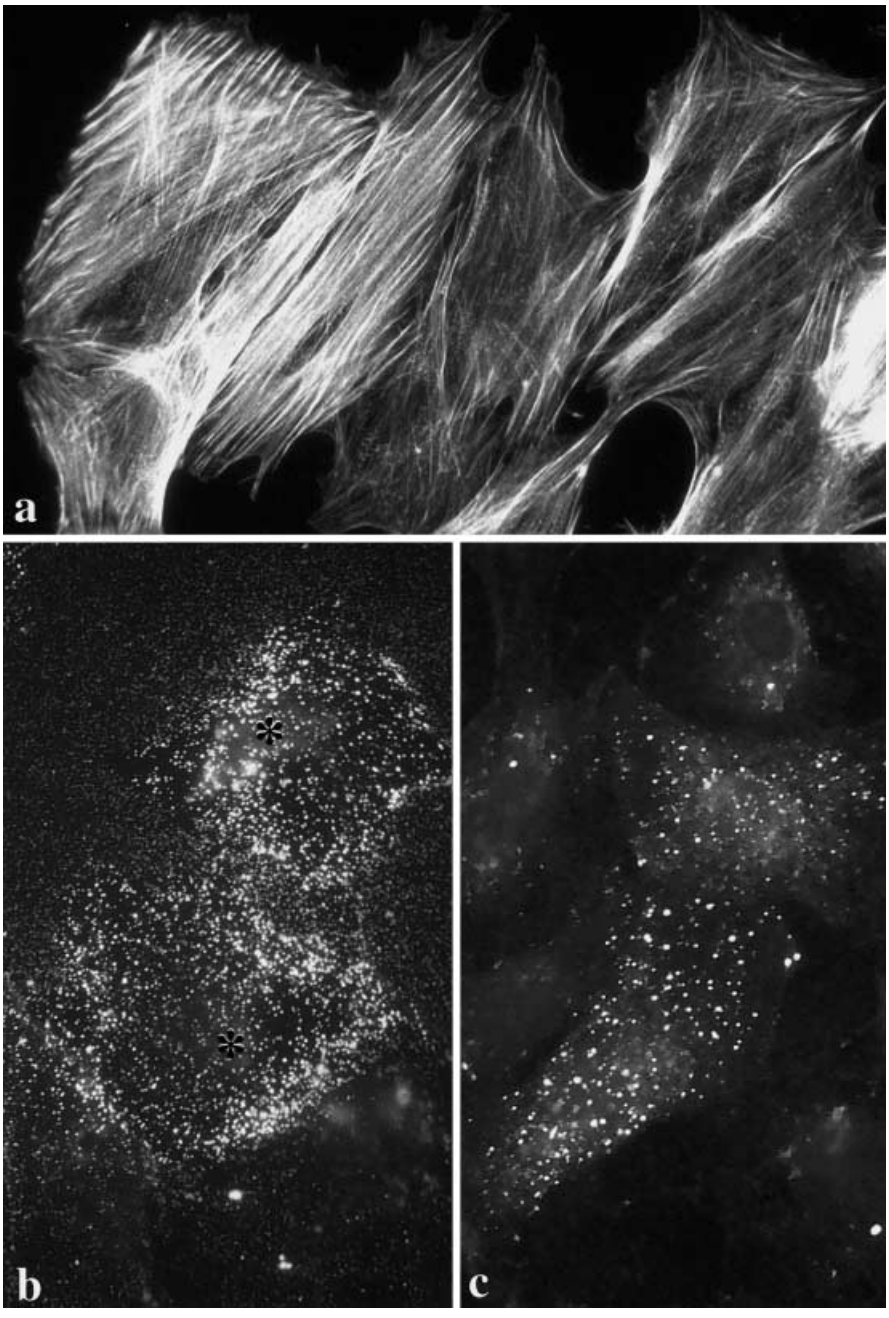

Fig. 2 Characterisation, by means of immunofluorescence, of the M1-cell line (a cell line derived from a human salivary gland plasmacytoid myoepithelioma). These cells express smooth-muscle actin (**a**), mostly as stress fibres. Laminin (**b**) and type-IV collagen (**c**) are also observed, forming multiple dots throughout the cell surface. Laminin label is distributed on the entire surface of M1 cells, making their nuclei (**b**, *) hardly visible. Type-IV collagen expression appears to be weaker when compared with laminin immunostaining. Magnifications 400×

Immunofluorescence

Smooth-muscle actin appeared as parallel bundles of filaments dispersed throughout the cytoplasm (Fig. 2a). Laminin (Fig. 2b) and type-IV collagen (Fig. 2c) exhibited a patchy pattern of distribution throughout the cell surface. Laminin label was more abundant than that of type-IV collagen.

Light microscopy

The M1-cell line, when cultured on a glass surface, formed a flat-cell monolayer composed of juxtaposed cells (Fig. 1). Individually, these cells exhibited a polyedrical shape and abundant cytoplasm. The M1-cell line, when cultured within Matrigel, assumed a new overall morphology, represented by cellular cords in a branching configuration (Fig. 3a–d). No duct-like structures were observed. Individually, M1 cells grown inside Matrigel were mostly spindle-shaped, with a hypercromatic nucleus with a barely discernible nucleolus (Fig. 3e). The plasmacytoid-like phenotype of M1 cells was occasionally observed in this Matrigel preparation (Fig. 3f).

The histopathology of the original tumour was similar to that observed in the three-dimensional preparation of M1 cells in Matrigel. Islands and cords of non-cohesive cells lying in either hyaline or myxoid matrixes

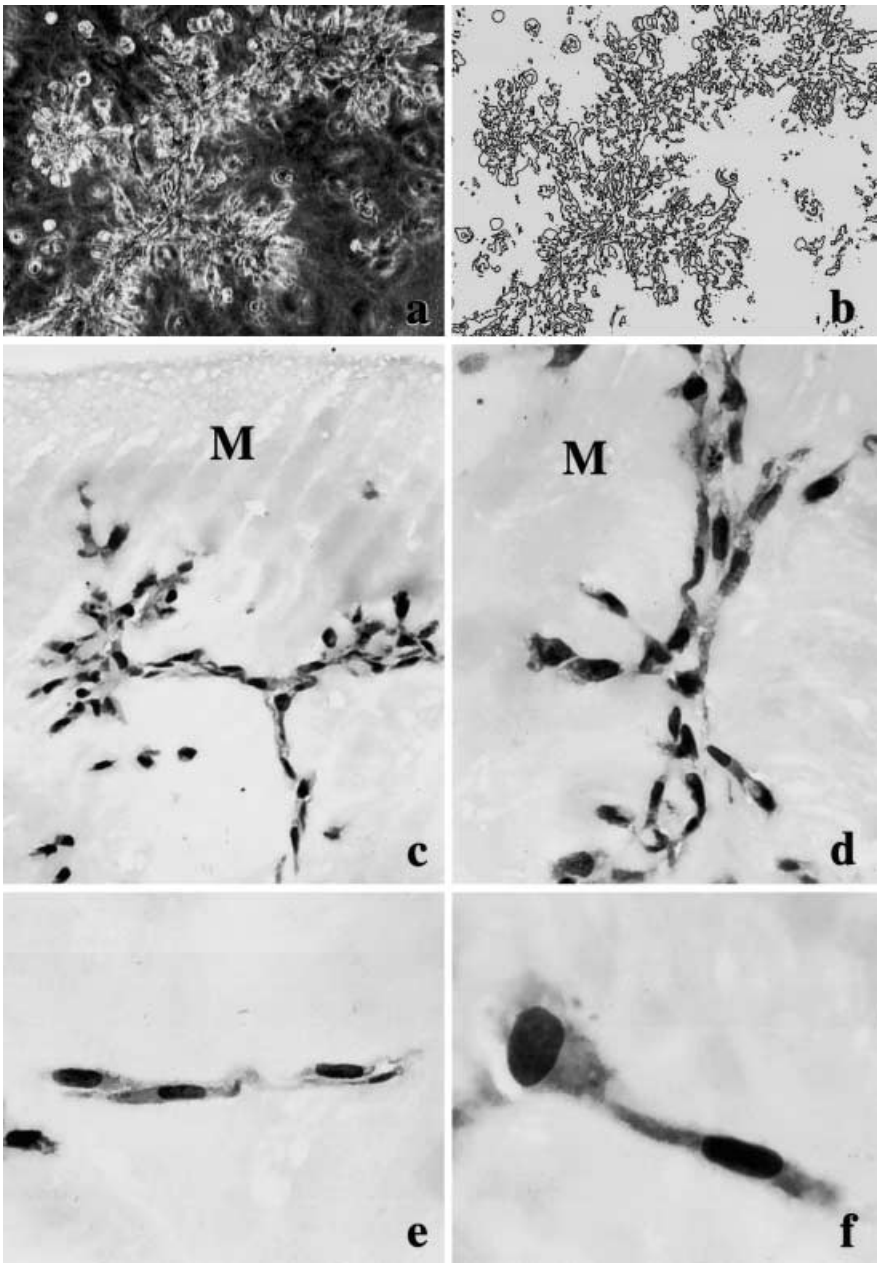

Fig. 3 Morphology of M1 cells (derived from a human salivary gland plasmacytoid myoepithelioma) when grown inside a three-dimensional preparation of Matrigel. Phase contrast shows M1 cells in a branching configuration (**a**). This configuration is clearly depicted when the image shown in (**a**) is processed using the Adobe Photoshop "trace contour" filter (**b**). **c**, **d** Haematoxylin and eosin preparations of M1 cells grown inside Matrigel (*M*). M1 cells are mostly elongated, forming anastomosing cords. Close-up view shows cells with hyperchromatic nucleus with barely discernible nucleoli (**e**). Plasmacytoid-like phenotype was occasionally observed in this Matrigel preparation (**f**). Magnifications **a**, **b** 200×; **c**, **d** 400× and **e**, **f** 800×

(Fig. 4a, b) represented neoplasm architecture. Cells showed glassy and hyaline cytoplasm, and most of them were plasmacytoid in shape. However, the majority of cells facing the extracellular matrix presented an elongated morphology (Fig. 4a, *arrows*).

M1 cells grown within Matrigel revealed a stellate phenotype with long and thin cytoplasmic processes branching out from the cell body (Fig. 5a–c). These elongated multipolar processes established cell–cell contacts (Fig. 5a). The smooth-muscle actin network showed some degree of rearrangement when M1 cells were cultured inside Matrigel. Cells plated on both plain glass coverslips (Fig. 2a) and coverslips with a thin coating of Matrigel (data not shown) presented smooth-muscle actin mostly distributed as bundles of filaments (stress fibres). However, when grown inside Matrigel, M1 cells exhibited mostly globular actin (Fig. 5a). By comparison, the amount of actin in M1 cells grown inside Matrigel (Fig. 5a) was apparently smaller than that of cells grown on plain coverslips (Fig. 2a).

Discussion

A cell line (M1) derived from human plasmacytoid myoepithelioma expressed markers for myoepithelial differentiation, such as smooth-muscle actin, laminin and type-IV collagen. The M1-cell line was cultured inside Matrigel. The culture grew in a branching pattern without formation of duct-like structures. There were confor-

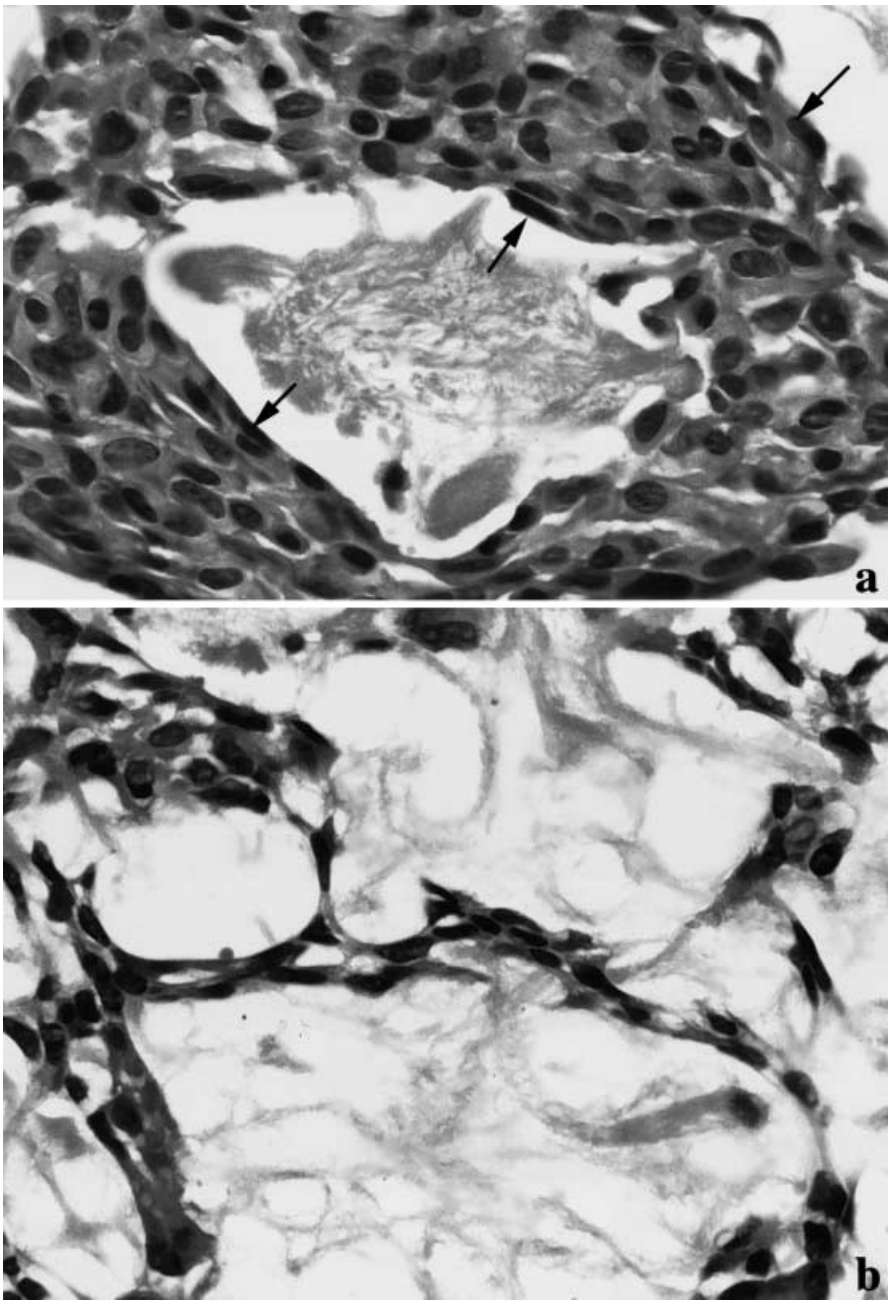

Fig. 4 Morphology (haematoxylin and eosin) of the tumour that originated the M1-cell line (a cell line derived from a human salivary gland plasmacytoid myoepithelioma). The overall picture is similar to the M1-cell line grown inside Matrigel. Islands (**a**) and cords (**b**) of non-cohesive cells lying in either hyaline or myxoid matrix is observed. Most cells are plasmacytoid, with a glassy and hyaline cytoplasm. However, the majority of cells facing the extracellular matrix present an elongated morphology (**a**, *arrows*). Magnifications 400×

mational and molecular changes in these cells, represented by a stellate phenotype and a depolymerisation of smooth-muscle actin, respectively.

We previously described that M1 cells, in addition to smooth-muscle actin, laminin and type-IV collagen, also express vimentin and pan-keratin [16]. Our findings are in agreement with Dundas et al. [10]. These authors demonstrated that clones of cultured myoepithelial cells derived from rat normal mammary gland express variable amounts of cytokeratin 8 and 14, smooth-muscle actin, laminin, type-IV collagen and vimentin.

Morphogenetic studies of normal and neoplastic salivary glands have been carried out through culturing cells in a three-dimensional matrix environment [3, 6, 11, 13, 14, 17, 31, 32, 33]. Most of the studies on neoplastic salivary glands have cultured cells from pleomorphic adenoma [13, 14, 31]. We have used a unique cell line (M1) derived from a rare human salivary gland plasmacytoid myoepithelioma [16, 20]. Moreover, we believe that culturing M1 cells inside Matrigel will provide important information on myoepithelioma biology. This reconstituted basement membrane has been used in many laboratories as a means of preserving, enhancing or inducing phenotypes of a variety of epithelial cells [17, 18, 19, 22, 30]. Matrigel has in its composition important morphoregulatory molecules, such as laminin and type-IV collagen, playing important roles either in cell proliferation or in cell differentiation [19, 30].

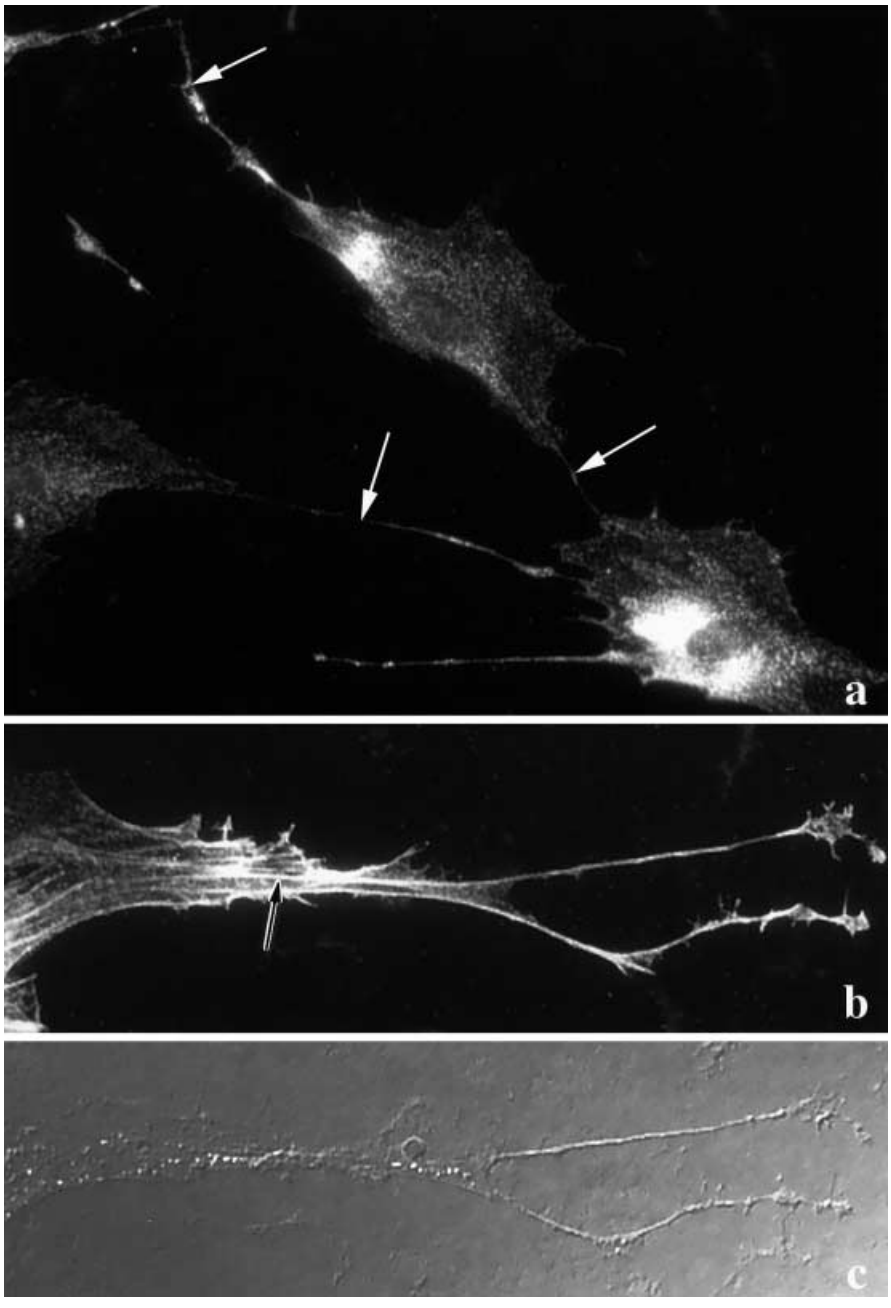

Fig. 5 Cytoplasmic distribution of smooth muscle actin in M1 cells (derived from a human salivary gland plasmacytoid myoepithelioma) grown within Matrigel. Fluorescence microscopy (**a**, **b**) and differential interference contrast (Nomarski, **c**) show cells with stellate phenotype with long and thin cytoplasmic processes branching out from the cell body (**a**–**c**). Cell–cell contacts are noteworthy (**a**, *arrows*). Smooth-muscle actin network shows some degree of rearrangement when M1 cells were cultured inside Matrigel. These cells exhibit mostly globular actin (**a**, **b**) and few stress fibres (**b**, *arrow*). Magnifications **a** 400× and **b**, **c** 630×

The arrangement of M1 cells inside Matrigel mimicked the original tumour in both architectural growth pattern and cytologic subtypes. Islands and cords of noncohesive cells lying in either hyaline or myxoid matrix represented the tumour. Myoepithelioma cells showed glassy and hyaline cytoplasm, and most of them were plasmacytoid in shape. However, the majority of cells facing the extracellular matrix presented elongated morphology, as previously described by Bhaskar and Weinmann [5]. Likewise, M1 cells grown inside Matrigel formed island and cords intermingled in the extracellular matrix. Elongated cells were predominant, growing in direct contact with the extracellular matrix. Plasmacytoid cells, although in smaller amounts, were also present. Duct-like structures were absent in both in vivo and in vitro conditions.

The reproduction of the tumour architecture in our in vitro assay could be due to either the physical or biochemical properties of Matrigel. We have previously shown that spatial arrangement of this reconstituted basement membrane is a key modulator of morphogenetic changes and cytodifferentiation of a pleomorphic adenoma cell line [14]. We showed that M1 cells secrete extracellular matrix molecules, such as laminin and type-IV collagen. Matrigel is basically an enriched preparation of laminin and type-IV collagen [19]. Thus, these molecules may act as autocrine factors, determining the morphogenetic changes of M1 cells.

Differently from the cell line derived from pleomorphic adenoma (AP2 cells) [14], M1 cells exhibited no epithelial differentiation inside Matrigel. M1 cells presented a myoepithelial phenotype with no formation of duct-like structures. Indeed, the myoepithelioma generally lacks duct formation in vivo [7, 8, 9]. Jaeger et al. [14] suggested that pleomorphic adenoma would be derived from a "reserve" cell still capable of differentiation into either direction, epithelial or myoepithelial, depending on the extracellular induction. Based on these findings, we suggest that the myoepithelial cell is the "reserve" cell of the salivary gland myoepithelioma. This should be further emphasised, because it provides evidence that myoepithelioma cells are truly committed to myoepithelial differentiation rather than representing an end of a spectrum of differentiation between ductal and myoepithelial phenotype.

The smooth-muscle actin network showed some degree of rearrangement when M1 cells were cultured inside Matrigel. Cells plated on both plain substrates and on a thin coating of Matrigel presented smooth-muscle actin mostly distributed as bundles of filaments (stress fibres). However, when grown inside Matrigel, M1 cells exhibited mostly globular actin (G-actin). Stress fibres form in response to tension generated across a cell [1]. Cells in culture exhibit different levels of intracellular tension, as shown by the variation in the abundance and distribution of actin stress fibres in different systems [2]. Thus, cells in monolayer cultures on a plastic substrate developed high levels of intracellular tension, whereas in anchored gels, they developed a moderate tension [2]. This could explain the changes in actin status in M1 cells, ranging from stress fibres in cells grown on plain coverslips and on thin coating of Matrigel to mostly G-actin in cells grown inside an anchored Matrigel. Moreover, our results suggest that the amount of actin in cells grown inside the Matrigel was smaller than that of cells grown on plain coverslips. It is in accordance with findings of Arora et al. [2], who showed that smooth-muscle actin transcription is regulated in part by intracellular tension.

In addition to its mechanical properties, Matrigel has chemical factors that may have an impact on actin expression in M1 cells. It has been suggested that a mechanism by which a putative specialised extracellular matrix molecule, under the control of transforming growth factor (TGF)-β, is required for outside–inside signalling and possibly for the development of the intracellular tension necessary for smooth-muscle actin induction [2]. It is known that Matrigel has in its composition TGF-β [30]. Hence, the changes in smooth-muscle actin expression could also be due to this chemical factor.

An increase in smooth-muscle actin leads to retardation of motility. Any reduction in the pool of filamentous actin would increase the rate of motility [21]. Therefore, in cells grown inside Matrigel, the reduction of stress fibres could facilitate the movement of proliferated cells, resulting in the formation of cellular cords in a branching configuration, a finding similar to that reported by Azuma and Sato [3]. The cells grown inside Matrigel would be in a more labile situation capable of remodeling the extracellular matrix by proteolytic enzymes and their inhibitors [27].

In conclusion, we presented the effects of a three-dimensional preparation of Matrigel on cells derived from a human salivary gland plasmacytoid myoepithelioma. The cells grown inside Matrigel presented morphological changes and changes in the smooth-muscle actin status. It was possible to reproduce the tumour architecture with no duct-like formation. Additionally, we showed positivity to smooth-muscle actin. We previously suggested that smooth-muscle actin expression in M1 cells would be regulated by the extracellular matrix [16]. The present investigation is a step further, showing that the plasticity of Matrigel directly influences smooth-muscle actin cytoplasmic organisation. This finding may explain the well-known variations in smooth-muscle actin expression in human salivary gland myoepitheliomas [12, 26, 28, 29].

Acknowledgements This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil. PTO was supported by Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil. SPHM is currently sponsored by Fundação para o Desenvolvimento Científico e Tecnológico da Odontologia (FUNDECTO), Brazil.

References

1. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (1994) The cytoskeleton. In: Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (eds) Molecular biology of the cell, 3rd edn. Garland Publishing Inc., New York, pp 787–861
2. Arora PD, Narani N, McCulloch CAG (1999) The compliance of collagen gels regulates transforming growth factor-β induction of α-smooth muscle actin in fibroblasts. Am J Pathol 154:871–882
3. Azuma M, Sato M (1994) Morphogenesis of normal human salivary gland cells in vitro. Histol Histopathol 9:781–790
4. Barnes L, Appel BN, Perez H, El-Attar AM (1985) Myoepitheliomas of the head and neck: case report and review. J Surg Oncol 28:21–28
5. Bhaskar SN, Weinmann JP (1955) Tumors of the minor salivary glands. A study of twenty-three cases. Oral Surg 8: 1278–1297
6. Burford-Mason AP, Dardick I, MacKay A (1994) Collagen gel cultures of normal salivary gland: conditions for continued proliferation and maintenance of major cell phenotypes in vitro. Laryngoscope 104:335–340
7. Dardick I (1995) Myoepithelioma: definitions and diagnostic criteria. Ultrastruct Pathol 19:335–345
8. Dardick I, Thomas MJ, Van Nostrand AWP (1989) Myoepithelioma. New concepts of histology and classification: a light and electron microscopic study. Ultrastruct Pathol 13:187–224
9. Dardick I, Cavell S, Boivin M, Hoppe D, Parks WR, Stinson J, Yamada S, Burns BF (1989) Salivary gland myoepithelioma variants. Histological, ultrastructural, and immunocytological features. Virchows Arch 416:25–42
10. Dundas SR, Ormerod MG, Gusterson BA, O'Hare MJ (1991) Characterization of luminal and basal cells flow-sorted from the adult rat mammary parenchyma. J Cell Sci 100:459–471
11. Durban EM (1990) Mouse submandibular salivary epithelial cell growth and differentiation in long-term culture: influence

of the extracellular matrix. In Vitro Cell Dev Biol Anim 26:33–43
12. Franquemont DW, Mills SE (1993) Plasmacytoid monomorphic adenoma of salivary glands. Absence of myogenous differentiation and comparison to spindle cell myoepithelioma. Am J Surg Pathol 17:146–153
13. Fujita Y, Yoshida T, Sakakura Y, Sakakura T (1999) Reconstruction of pleomorphic adenoma of the salivary glands in three-dimensional collagen gel matrix culture. Virchows Arch 434:137–143
14. Jaeger MMM, Araújo VC, Kachar B, Jaeger RG (1997) The effect of spatial arrangement of the basement membrane on cultured pleomorphic adenoma cells. Study by immunocytochemistry, electron and confocal microscopy. Virchows Arch 430:467–477
15. Jaeger RG, Jaeger MMM, Araújo VC, Kachar B (1995) Analysis of the interdependent localization of vimentin and microtubules in neoplastic myoepithelial cells. Cell Motil Cytoskeleton 32:289–298
16. Jaeger RG, de Oliveira PT, Jaeger MMM, de Araújo VC (1997) Expression of smooth-muscle actin in cultured cells from human plasmacytoid myoepithelioma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 84:663–667
17. Kibbey MC, Royce LS, Dym M, Baum BJ, Kleinman HK (1992) Glandular-like morphogenesis of the human submandibular tumor cell line A253 on basement membrane components. Exp Cell Res 198:343–351
18. Kim ND, Oberley TD, Clifton KH (1993) Primary culture of flow cytometry-sorted rat mammary epithelial cell (RMEC) subpopulations in a reconstituted basement membrane, Matrigel. Exp Cell Res 209:6–20
19. Kleinman HK, McGarvey ML, Hassell JR, Star VL, Cannon FB, Laurie GW, Martin GR (1986) Basement membrane complexes with biological activity. Biochemistry 25:312–318
20. Oliveira PT, Jaeger MMM, Loducca FE, Jaeger RG (1996) Myoepithelioma of minor salivary gland. Case report with immunohistochemical, ultrastructural study and propagation of primary culture. Rev Pós-Grad FOUSP (Brazil) 3:234–241
21. Ronnov-Jessen L, Petersen OW (1996) A function for filamentous α-smooth muscle actin: retardation of motility in fibroblasts. J Cell Biol 134:67–80
22. Schnaper HW, Kleinman HK, Grant DS (1993) Role of laminin in endothelial cell recognition and differentiation. Kidney Int 43:20–25
23. Sciubba JJ, Goldstein BH (1976) Myoepithelioma. Review of the literature and report of a case with ultrastructural confirmation. Oral Surg 42:328–338
24. Sciubba JJ, Brannon RB (1982) Myoepithelioma of salivary glands: report of 23 cases. Cancer 49:562–572
25. Seifert G, Sobin LH (1992) The World Health Organization's histological classification of salivary gland tumors. A commentary on the second edition. Cancer 70:379–385
26. Simpson RHW, Jones H, Beasley P (1995) Benign myoepithelioma of the salivary glands: a true entity? Histopathology 27:1–9
27. Sternlicht MD, Safarians S, Rivera SP, Barsky SH (1996) Characterizations of the extracellular matrix and proteinase inhibitor content of human myoepithelial tumors. Lab Invest 74:781–796
28. Takai Y, Mori M, Dardick I, MacKay A, Leung R, Wattimena D, Christensen H, Burford-Mason A (1994) Myofilament localization and immunoelectron microscopic detection of muscle-specific actin in neoplastic myoepithelial cells in pleomorphic adenomas and myoepitheliomas. Ultrastruct Pathol 18:575–591
29. Takai Y, Dardick I, Mackay A, Burford-Mason A, Mori M (1995) Diagnostic criteria for neoplastic myoepithelial cells in pleomorphic adenomas and myoepitheliomas. Immunocytochemical detection of muscle-specific actin, cytokeratin 14, vimentin, and glial fibrillary acidic protein. Oral Surg Oral Med Oral Pathol 79:330–341
30. Vukicevic S, Kleinman HK, Luyten FP, Roberts AB, Roche NS, Reddi AH (1992) Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. Exp Cell Res 202:1–8
31. Witsell DL, Weissler MC, Livanos E, Bova-Hill C, Gilmer TM (1993) Unique chromosomal rearrangement and mucin production in a novel salivary myoepithelial cell strain. Arch Otolaryngol Head Neck Surg 119:1151–1157
32. Yang J, Flynn D, Larson L, Hamamoto S (1982) Growth in primary culture of mouse submandibular epithelial cells embedded in collagen gels. In Vitro Cell Dev Biol Anim 18: 435–442
33. Yang J, Larson L, Nandi S (1982) Three-dimensional growth and morphogenesis of mouse submandibular epithelial cells in serum-free primary culture. Exp Cell Res 137:481–485