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# Ultrastructural and immunocytochemical characterization of immortalized odontoblast MO6-G3

**C. Mesgouez[1], M. Oboeuf[2], N. Mauro[2], P. Colon[1], M. MacDougall[3], P. Machtou[1], J. M. Sautier[2] & A. Berdal[2]**

[1]Département d'Odontologie Conservatrice-Endodontie, UFR d'Odontologie, Université Paris 7-Denis Diderot; [2]Laboratoire de Biologie Oro-faciale et Pathologie, INSERM U 714, UFR d'Odontologie, Université Paris 7-Denis Diderot, Institut Biomédical des Cordeliers, Paris, France; and [3]Department of Pediatric Dentistry, University of Texas Health Sciences Center at San Antonio, Dental School, San Antonio, TX, USA

## Abstract

**Mesgouez C, Oboeuf M, Mauro N, Colon P, MacDougall M, Machtou P, Sautier JM, Berdal A.** Ultrastructural and immunocytochemical characterization of immortalized odontoblast MO6-G3. *International Endodontic Journal*, **39**, 453–463, 2006.

**Aim** To investigate an immortalized murine odontoblast cell line as a potential alternative for experimental studies on dentinogenesis.

**Methodology** The MO6-G3 cell line was investigated morphologically over 3, 7, 11 and 42 days of culture, using histochemical localization of dentine sialoprotein (DSP), alkaline phosphatase (AP), type I collagen and actin filaments, histoenzymatic staining and biochemical investigation of AP and finally, transmission and scanning electron microscopy.

**Results** Scanning electron micrographs showed elongated cells. Accordingly, a polarized organization of odontoblasts was observed by transmission electron microscopy, identifying distinct subcellular compartments as described *in vivo*. The secretion apparatus, which includes cisternae of rough endoplasmic reticulum, Golgi apparatus saccules and secretion vesicles and granules, was longitudinally organized in the supranuclear compartment ending distally in the secretory pole. A cellular process was observed. The investigation of the cytoskeleton network revealed that actin microfilaments were organized in parallel stress fibre oriented depending on the longitudinal axis of the cytoplasm. Immunofluorescent labelling showed a continuous expression of type I collagen, DSP and AP. A unipolar distribution characterized intracellular DSP immunoreactivity. Histoenzymology revealed AP active sites increasing from 3 to 11 days albeit with a moderate level of activity comparatively to the *in vivo* situation in dental cells.

**Conclusion** This cell line MO6-G3 not only showed the criteria of odontoblast phenotype as previously reported but also the characteristic morphodifferentiation pattern of polarized odontoblasts at the cellular level but with an apparent random distribution.

**Keywords:** cell culture, dentine sialoprotein, differentiation, odontoblast, polarization.

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## Introduction

Dentineogenesis is the result of successive and ordered epithelial-mesenchymal interactions (Thesleff & Sharpe 1997, Lisi *et al.* 2003). During tooth development, cephalic neural crest cells migrate in the forming jaws and progressively acquire the terminal phenotype of specialized secretory cells (Lumsden 1988, Magloire *et al.* 1992). This process involves several signalling pathways, such as the reciprocal induction of the expression of BMP4 and Msx1 in the epithelium and mesenchyme (Thesleff 2003). Fully differentiated odontoblasts are characterized by a characteristic morphodifferentiation into polarized secretory cells

Correspondence: Catherine Mesgouez-Menez, Département d'Odontologie Conservatrice-Endodontie, UFR d'Odontologie, Université Paris 7-Denis Diderot, 5, rue Garancière 75006 Paris, France (e-mail: catherine.menez@free.fr).

and the acquisition of a biochemical phenotype (Ruch 1998). Odontoblasts produce a set of matrix proteins forming predentine and dentine (for review see Magloire *et al.* 1992, Linde & Goldberg 1993). Numerous proteins are common to bone and dentine (Butler *et al.* 2003), such is the case for type I (Thomas *et al.* 1995) and V collagens, osteocalcin (Bronckers *et al.* 1998), osteonectin (Papagerakis *et al.* 2002), bone sialoprotein (Chen *et al.* 1996) and $DMP_1$ (George *et al.* 1998, MacDougall *et al.* 1998b). DSP and DPP proteins are encoded by the single DSPP gene (MacDougall *et al.* 1997). They result from a unique transcript, which is translated and produce a common propeptide with a N-terminal DSP and a COOH-terminal DPP. The first one, the dentine phosphoprotein or phosphophoryn (DPP) is proposed to play a role in dentine biomineralization by providing phosphates at the mineralization front (Veis 1993). The second dentine-specific protein, dentine sialoprotein (DSP), which contains 30% carbohydrate is also rich in aspartic acid, glutamic acid, serine and glycine (Ritchie *et al.* 1997). It is now established that bone cells also synthesize DSP, and probably DPP, but in a ratio that is estimated to be 1 : 400 of that in dentine (Qin *et al.* 2003).

All these dentine matrix proteins are elaborated by a secretory apparatus, spatially organized within the dentineogenically active odontoblasts. Indeed, the terminal morphodifferentiation of odontoblasts follows a well-defined temporo-spatial pattern (Magloire *et al.* 1992, Linde & Goldberg 1993, Ruch 1998). The cytodifferentiation occurs in several steps: the cells become post-mitotic, assume an epithelial configuration, become polarized, and finally synthesize and secrete the extracellular predentine, dentine matrix. This spatial organization of odontoblastic cells inside the matrix micro-environment results from successive interactions between the cytoskeletal elements, transmembranous proteins (such as the 165 kDa protein), basement membrane and predentine (Ruch 1998). When they are overtly differentiated, these columnar (50–60 μm) cells contain a well-developed granular endoplasmic reticulum, a prominent Golgi apparatus located in the central and supranuclear compartments. Numerous secretory vesicles and granules are present in the distal cytoplasm. The terminal aspect of odontoblasts corresponds to their secretory pole and a large cytoplasm process.

Dentineogenesis may be controlled by several environmental factors such as metabolites of vitamin A (Block-Zupan *et al.* 1994), vitamin D (Papagerakis *et al.* 2003) and fluoride (Bronckers & Wöltgens 1985). Experimental approaches, *in vivo* and in organotypic cultures, are difficult to interpret regarding the direct effects of these agents on odontoblast because of the known epithelial-mesenchymal interactions leading odontoblast differentiation and activity and cell heterogeneity within the dental pulp.

Various *in vitro* systems have seen set up in order to analyse odontoblast activity. Numerous investigators have reported culture systems which have used odontogenic cells either from outgrowths of total tooth explants (Magloire *et al.* 1981, Takeda *et al.* 1994, Stanislawski *et al.* 1997, Couble *et al.* 2000), or disaggregated foetal, and adult tooth germs and dental pulp (Thesleff 1986, Nakashima 1991, Magloire *et al.* 1992, Kasugai *et al.* 1993, Veron *et al.* 1993, Hao *et al.* 1997, Nakade *et al.* 1999). Immortalized cells lines have also been established (Kasugai *et al.* 1988, Kawase *et al.* 1990, MacDougall *et al.* 1995, Hanks *et al.* 1998a,b, Thonemann & Schmalz 2000a,b, Hao *et al.* 2002).

These culture systems are useful in the study of the physiological effects of intrinsic and extrinsic factors (Sun *et al.* 1998, Ritchie *et al.* 2004). The aim of the present study was to characterize an odontoblast cell line immortalized by viral transformation with the SV 40 (MacDougall *et al.* 1995). MO6-G3 cells were obtained as following: dental papilla mesenchymal cells were enzymatically isolated from Swiss Webster E-18 (vaginal plug day 0) first mandibular molars and immortalized by infection with a recombinant defective retrovirus containing the temperature sensitive SV 40 large T-antigen cDNA. They were expanded, selected and cloned. The MO6-G3 clone was previously shown to express several markers of the odontoblastic phenotype and form Von Kossa positive nodules *in vitro* (MacDougall *et al.* 1995). The MO6-G3 cell line is investigated here over 6 weeks morphologically, by histochemical localization of DSP, alkaline phosphatase (AP), type I collagen and actin filaments, histoenzymatic staining and biochemical investigation of AP and finally, transmission and scanning electron microscopy (SEM). The present data show that the MO6-G3 immortalized odontoblast-like cells provide not only an experimental model system where the phenotypic markers are expressed as previously reported (MacDougall *et al.* 1995) but is also characterized by a polarized morphodifferentiation as described for *in vivo* odontoblasts.

## Materials and methods

### Cell cultures

Cells were plated at density $4 \times 10$ cells $mL^{-1}$ (i) on $100 \times 20$ mm culture dishes (Falcon; Becton Dickinson and Co., Franklin Lakes, NJ, USA) for survey of cellular kinetics, measurement of AP activity and transmission electron microscopy (TEM), (ii) on $35 \times 10$ mm dishes for AP histoenzymology localization and (iii) on glass cover slides for immunochemistry, actin labelling and SEM. The culture medium was a minimum essential medium α (αMEM; Gibco BRL Life Technology, Paisley, UK) supplemented with 15% foetal calf serum (FCS; HyClone, Logan, UT, USA), 100 units $mL^{-1}$ penicillin/streptomycin (Gibco BRL), 50 μg $mL^{-1}$ ascorbic acid (Sigma, la Verpillière, France), and 10 mmol $L^{-1}$ Na-β-glycerophosphate (Sigma). Cultures were incubated at 33 °C in a humidified atmosphere of 95% air and 5% $CO_2$ as previously described (MacDougall *et al.* 1995) for up to 42 days. Media were changed every two days and cells were monitored by phase contrast microscopy. As the behaviour of the cells did not apparently change from 11 days over 42 days, the present study essentially illustrated the most significant data, i.e. until 11 days. All studies are realized three times.

### Scanning electron microscopy

At 7 days, cultures were rinsed with culture medium without FCS, fixed *in situ* for 1 h in Karnowsky's fixative (4% paraformaldehyde and 1% glutaraldehyde), and rinsed three times with 0.2 mol $L^{-1}$ sodium cacodylate buffer at pH 7.4. Cultures were then post-fixed in 1% osmium tetroxide and dehydrated in a graded series of ethanol and amyl acetate before critical point drying. Glass coverslips were coated with 30 nm gold in a Polaron apparatus and examined on a Jeol JMS-35 microscope at 10 or 15 kV.

### Transmission electron microscopy

Preliminary follow-up studies were performed during the culture process until 42 days. The data are presented at day 3 because the low cell density enables a clear presentation of their morphology. At day 3, cells were rinsed with culture medium without FCS, fixed in Karnovsky's fixative for 30 min and rinsed three times with 0.2 mol $L^{-1}$ sodium cacodylate buffer at pH 7.4. Cells were scraped off with a rubber policeman to form a pellet. Cells were post-fixed in 1% osmium tetroxide at room temperature for 1 h and dehydrated in a graded series of ethanol. Then, cells were embedded in Epon-Araldite. Semi-thin sections were cut with a diamond knife, mounted on glass slides and stained with methylene blue-Azur II. Ultra-thin sections were collected onto copper grids and stained with 2.5% uranyl acetate in absolute ethanol and lead citrate. All sections were examined using a Philips CM-12 transmission electron microscope.

### Immunohistochemical labelling

All rinsings and incubations were performed with phosphate buffered saline (PBS 0.1 mol $L^{-1}$ pH 7. 4). Polyclonal rabbit antibodies raised to the N-terminal domain of the DSP protein (MacDougall *et al.* unpublished data) and type I collagen (Institute Pasteur, Lyon, France) were used at dilutions of 1/50. Monoclonal mouse primary antibody specific for rat TNAP (M. Vogel, G. Rodan, Merck Research Laboratories, West Point, PA, USA) was used at dilution of 1/50. Cell samples were fixed at day 3, 7 and 11 in 50% methanol/acetone for 5 min. After rinsing during 5–10 min, cells were treated with normal goat serum (NGS) (Sigma) overnight at 4 °C to saturate nonspecific binding sites. Samples were then incubated 16 h at 4 °C with primary antibody. Control coverslips were incubated with primary antibodies omission. After rinsing, cells were incubated with secondary antibody-FITC complex (Sigma) for 30 min at room temperature (dilution: 1/50).

To study actin filaments, cells were fixed at room temperature for 10 min in 3.7% formaldehyde. This treatment was followed by incubation with 0.1% Triton-X 100 (Sigma) in order to permeabilize the cells. Actin microfilaments were visualized with rhodamine-conjugated-phalloïdine (Sigma) diluted to 1/50. After washing three times, coverslips were mounted in hydrophilic fluorescence medium (Biosys, Compiègne, France). Slides were examined under a Leitz-Orthoplan fluorescence microscope (Wild Leitz, Heerbrugg, Switzerland).

### AP histoenzymatic localization

The localization of enzymatic activity was determined as previously described (Loty *et al.* 2001). Cultured cells were fixed at 3, 7 and 11 days for 30 s in fixative solution (citrate buffered acetone 60%) at room temperature and gently rinsed in deionized water for 45 s. The cell samples added with alkaline-dye mixture (solution of fast blue salt RR + naphtol phosphate, Sigma) were incubated at room temperature for

30 min. Samples were protected from direct light. After histoenzymatic reaction, the cells were rinsed thoroughly in deionized water for 2 min, and the samples were studied under phase contrast microscopy.

### Measurement of AP activity

At day 11, cell samples were washed three times in PBS and thenafter rinsed in 0.1 mol $L^{-1}$ sodium carbonate-bicarbonate solution at pH 10.2. The cell layer was then scraped off with a rubber policeman in a solution containing 0.2% nonidet P40 (Sigma) 0.1 mol $L^{-1}$ $MgCl_2$ and sonicated at 4 °C. Lysates were removed by centrifugation at 1400 g for 5 min. AP activity was determined in the supernatant with 15 mmol $L^{-1}$ paranitrophenyl-phosphate as substrate. After 15 min of incubation at 37 °C, the reaction was stopped with NaOH addition and the amount of released p-nitrophenol (PNP) was measured spectrophotometrically at 410 nm and compared with a PNP standard solution (Sigma). Protein concentrations were measured by the BCA protein assay reagent (Pierce, Rockford, IL, USA) using bovine serum albumin as the standard. The enzymatic activity was expressed as nmol of released PNP per minute per milligram of protein at 37 °C.

## Results

### Phase contrast microscopy

Phase contrast microscopy showed the changing morphology of MO6-G3 (Fig. 1a,b,c,d) cell line. Immediately after seeding, MO6-G3 cells spread onto the plastic culture dishes within 4 h (Fig. 1a). At confluence (7 days), they adopted an elongated morphology (Fig. 1b) and became progressively more polygonal at 11 days (Fig. 1c). After 11 days, refractive cells were visible and MO6-G3 apparently formed two cells layers. Such an organization of MO6-G3 cells was maintained throughout culture, even at 42 days (Fig. 1d).

### SEM investigations

Scanning electron microscopy was then performed to further examine the morphological changes of MO6-G3 cells during the cultures. On day 7, cells showed the morphology of an odontoblast attached to the surface and formed a confluent monolayer (Fig. 2a,b). Cells exhibited elongated morphology with numerous filopodiae. In some areas, clusters of columnar cells were observed in parallel lines, with features of polarized cells regarding the nuclear position (Fig. 2c,d).

### Transmission electron microscopy investigations

In order to further analyse an eventual polarization of MO6-G3 cells, an ultrastructural investigation was then performed (Fig. 3). Similarly to the situation observed in phase contrast microscopy, the ultrastructure of MO6-G3 cells was apparently maintained throughout culture, even at 42 days (data not shown). Transmission electron microscopy analysis of the cellular and extracellular compartments suggested the existence of cell polarity, which was also supported by the dissymmetric distribution of DSP immunolabelling (see later Fig. 5).

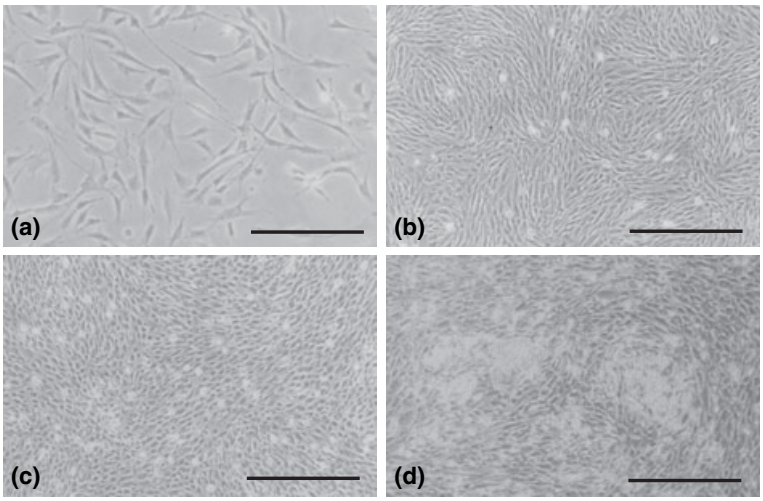


**Figure 1** Follow-up of MO6-G3 cells during culture by phase contrast microscopy. (a) Microscopic aspect 4 h after seeding. Bar = 250 μm. (b) Day 7. Confluence is reached, cells appear to constitute heterogeneous groups in the layer. Bar = 400 μm. (c) Day 11. The cells become polygonal. Bar = 400 μm. (d) Day 42. Refringent groups appear in MO6-G3 cultures. Bar = 400 μm.

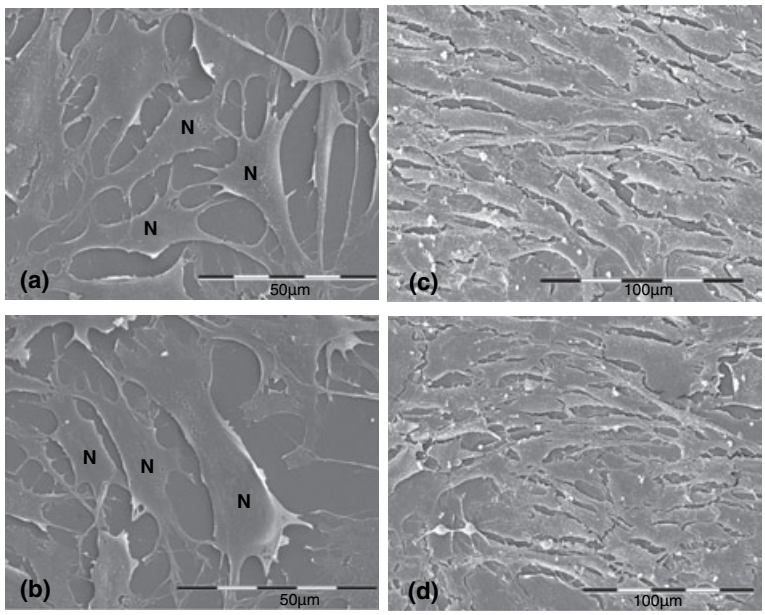


**Figure 2** Scanning electron microscopy study of MO6-G3 cells at day 7. (a) Cells show the morphology of an odontoblast attached to the surface and formed a confluent monolayer. (b) The cells exhibited elongated morphology with numerous fillopadiae. (c–d) In some areas, clusters of columnar cells were observed in parallel lines. N = nucleus.

The odontoblast process extended from the cell body (Fig. 3a) and narrowed gradually from the cell body towards its ending. The process was unique, located at one side of the cell (Fig. 3a). It appeared dense and showed several distal digitations.

The cell body was elongated with an oval nucleus (Fig. 3a,b). Mitochondria were distributed throughout the cytoplasm and usually closely associated with profiles of rough endoplasmic reticulum (Fig. 3a,b). The cell body appeared to contain a small proximal zone under the distended nucleus set in the longitudinal axis of the cell (Fig. 3a,b,c). The proximal pole (Fig. 3b,c) contained only some rough endoplasmic reticulum cisternae and numerous mitochondria but not any Golgi element. The central zone contained a cilia (Fig. 3d). The central and distal zones were characterized by the presence of the secretory apparatus (rough endoplasmic reticulum and Golgi (Fig. 3e) and of numerous secretory vesicles and granules (Fig. 3e). The cells showed a typical Golgi complex with some secretory vesicles and cisternae of rough endoplasmic reticulum parallel to cell axis. The Golgi apparatus was essentially located in the central region of the supranuclear cytoplasm. Cisternae of rough endoplasmic reticulum occupied the rest of supranuclear cytoplasm. The Golgi complex appeared as an aggregation of smooth-walled vesicles and cisternal profiles organized into distinct and separate groups or dictyosomes.

The central zone contained the Golgi saccules framed by the cisternae of the rough endoplasmic reticulum along the longitudinal axis. Higher magnifications (Fig. 3e) identified the Golgi saccules. Some cell–cell contacts were observed (arrow, Fig. 3f).

## Fluorescence microscopy

*Cytoskeleton architecture*

Actin filaments were visualized by fluorescent-labelled phalloïdin (Fig. 4). A well-developed system of microfilaments was diffusely distributed in the cytoplasm of elongated cells and in lamellipodiae (Fig. 4a). In certain areas of the culture dishes, actin microfilaments were organised in parallel stress fibre oriented along the axial axis of the cytoplasm (Fig. 4b).

Immunolocalization for DSP, type I collagen and AP was performed at 3, 7 and 11 days of culture. From day 3 of culture onward, the DSP protein was detected in MO6-G3 cells (Fig. 5a–d). In all samples, this labelling allowed the observation that the vast majority of MO6-G3 (Fig. 5a,b) was in fact unipolar (98% at 3, 7 and 11 days). The relative orientation of DSP-labelled polarity appeared random in the cell layer. The labelling increased from day 3 (Fig. 5c), to day 7 (Fig. 5d) and day 11 (data not shown). DSP labelling was characterized by an unipolar distribution. High magnification allowed the association of this DSP localization to the most prominent cytoplasmic area (Fig. 5c). This labelling was triangular with a large perinuclear basis. Immunolocalization with collagen type I antibodies showed a clear cytoplasmic staining throughout the

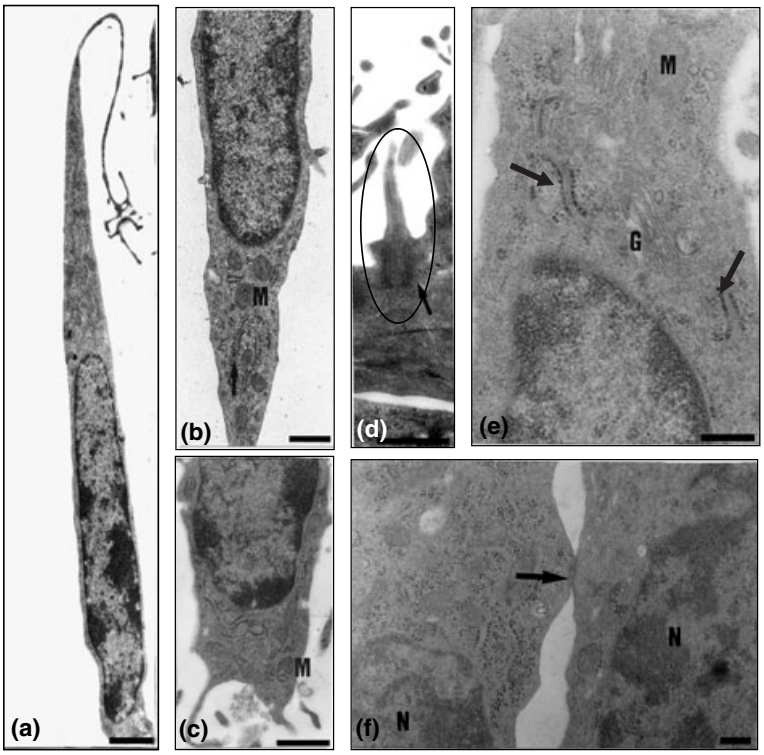


**Figure 3** Transmission electron microscopic investigation of MO6-G3 cell lines. (a) Low magnification of a single cell. The odontoblast appears dissymmetrical with an unipolar cell process. Bar = 1 μm. (b and c) Infranuclear and nuclear compartments. Mitochondria (M) and the cisternae (arrow) of rough endoplasmic reticulum are present in the infranuclear compartment. No Golgi saccules are evidenced. Bar = 0.5 μm. (d) Cilium (arrow) in the central area of MO6-G3 cells. Bar = 0.5 μm. (e) Nuclear and supranuclear compartments. Bar = 0.25 μm. In this area, the Golgi saccules (G) are numerous and inserted between endoplasmic reticulum cisternae (arrows). M, mitochondria. (f) Zone of cellular contacts. N, nucleus. Bar = 0.25.

culture process. A labelling in the extracellular network appeared at 7 days (Fig. 5e). The immunological control did not evidence any reaction (data not shown).

Alkaline phosphatase was analysed with three different methods: immunolabelling (Fig. 6a,b), histoenzymology (Fig. 6c) and measurement of enzymatic activity. The AP immunolocalization was positive very early from the third day of culture onward (Fig. 6a). This protein appeared to form aggregates along cell periphery, thus allowing the delineate cell morphology. In contrast, AP activity appeared to be significant only at 11 days by histoenzymology (Fig. 6c). Aggregates of AP-active cells and isolated AP-positive cells were randomly distributed in the cell layer. Regarding this histochemical data, the biochemical investigation was realized at 11 days. Triplicate biochemical investigation showed that AP activity is $32.66 \pm 1.15$ nmol $PNP^{-1}$ $min^{-1}$ $mg^{-1}$ protein in MO6-G3 cells.

## Discussion

Several cell lines have been obtained from the pulpal mesenchyme (Kasugai *et al.* 1988, Kawase *et al.* 1990, MacDougall *et al.* 1995, 1998a, Hanks *et al.* 1998a,b). The MO6-G3 immortalized odontoblast line (MacDougall *et al.* 1995, 1998a) was studied in this report. Dynamic evolution of the specific markers of differentiation was defined in this cell line and its ultrastructural morphology analysed.

Three approaches have been taken to develop *in vitro* models. The first approach has been to utilize organ explant cultures of isolated late foetal or neonatal molar or incisor dental papillae under defined conditions, which preserve cellular relationships, and to attempt to maintain certain morphological and functional characteristics of odontoblasts (Begue-Kirn *et al.* 1992, Magloire *et al.* 1996). The shortcomings of the organ culture systems are that they cannot be used to produce relatively large quantities of cell-specific molecules, realize biochemical studies and that it is difficult to identify target cells because of various types of cells in the cultured dental papilla.

The second approach has involved outgrowths of post-natal pulp tissue explants (Magloire *et al.* 1981, Takeda *et al.* 1994, Couble *et al.* 2000) or disaggregated foetal or adult tooth germs (Thesleff 1986, Nakashima 1991, Kasugai *et al.* 1993) to develop primary cultures and subcultures. A pitfall of this approach is the same quantitative limitation than the one described for organ explants.

In order to set reproducible results, grow large quantities of cells, and manage biochemical investigations, a

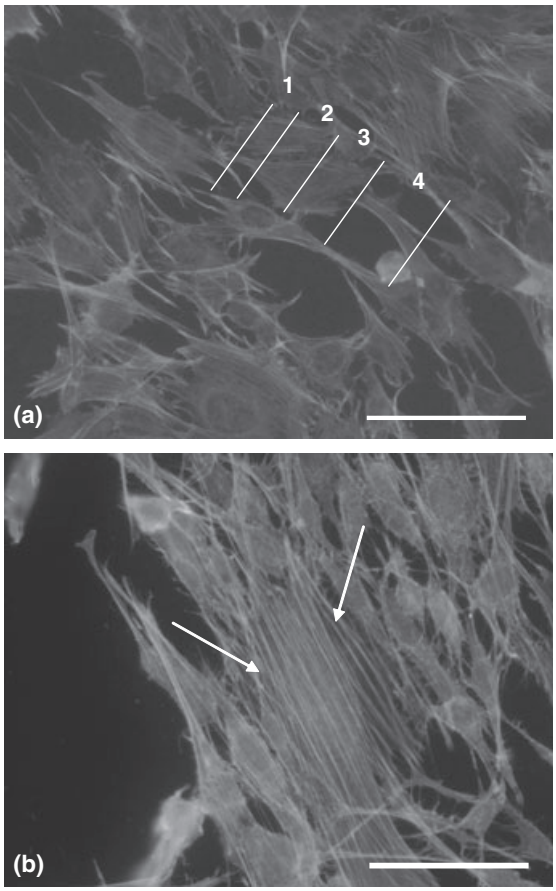


**Figure 4** Cytoskeleton architecture. (a) A well-developed system of microfilaments was distributed in the cytoplasm of elongated cells and in lamellipodiae. The labelling allowed to visualize the organization of MO6-G3 cells in: 1, proximal pole; 2, nucleus; 3, supranuclear zone; 4, process. Bar = 100 μm. (b) Actin microfilaments were organized in parallel stress fibres oriented along the longitudinal axis of the cytoplasm (arrow). Bar = 100 μm.

third strategy has been undertaken. This approach has been to immortalize cell lines from either adult dental pulp or foetal dental papillae. Seven established cell lines have been reported in the literature (Kasugai *et al.* 1988, Kawase *et al.* 1990, MacDougall *et al.* 1995, Hanks *et al.* 1998a,b, Thonemann & Schmalz 2000a,b, Hao *et al.* 2002, Lundquist *et al.* 2002). The present study is devoted to a detailed analysis of MO6-G3 odontoblast cell line.

The odontoblast phenotype is very similar to the one of osteoblast. The major protein of the extracellular matrix expressed by odontoblasts is type I collagen, which was detected in MO6-G3 cell line as described before (MacDougall *et al.* 1995). As shown previously with other antibodies (MacDougall *et al.* 1995) and by RT-PCR in MO6-G3 cells, DSPP expression was confirmed in the present study using an antibody raised to the N-terminal domain of the DSP protein. These findings further support that these cells are, indeed, odontoblastic-type cells. Collagen type I and also noncollagenous DSP/DPP/DSPP proteins were obtained in the other odontoblast systems (Kawase *et al.* 1990, Hanks *et al.* 1998b). The second molecular feature (DSPP) is variably described in other cell lines, which are pulpal cells (Kasugai *et al.* 1988, Kawase *et al.* 1990).

*In vivo*, fully differentiated odontoblasts are not only expressing a unique molecular phenotype but are also morphologically different from cells of the dental pulp. They elongate, polarize and acquire a distal cell process. This feature landmarks a significant morphology of odontoblast terminal differentiation. A primary cilium configuration have been regularly identified in a supra nuclear localization. It can be speculated that the cilium could participate of events leading to the modelling of dentine as well as to the sensory processing in teeth (Magloire *et al.* 2004).

It is interesting to note that DSP protein distribution showed a unipolar and supranuclear distribution. Furthermore, the immunolabelling signal progressively increased: at the confluence stage, a light perinuclear staining was observed. This labelling was more intense at day 7 and became even more pronounced at day 11 of culture.

The present study of the MO6-G3 cells provides a set of evidences for cell polarization. By electron microscopy the topography of the Golgi saccules (Fig. 3e) appeared corresponds to the distribution of immunostaining clearly evidenced by DSP immunofluorescence (Fig. 5a,b,c,d). However, the relative orientation of cell polarity in the cell layer appears to be random as previously assessed by DSP immunolocalization. SEM showed clusters of columnar cells with features of polarized cells (Fig. 2c,d). In some areas of the culture dishes, actin microfilaments were organized in parallel stress fibres oriented in the longitudinal axis of the cytoplasm (Fig. 4b).

However, routine phase-contrast microscope observations of MO6-G3 cells did not allow any polarity to be evidenced as was the case for RPC-C2A (Kasugai *et al.* 1988) and RDP4-1 (Kawase *et al.* 1990) and in contrast clear epitheloid morphology of MDPC-23 cells (Hanks *et al.* 1998b) and explant cultures (Couble *et al.* 2000). In this study, in fact, DSP distribution was the first landmark of a polarized organization of MO6-G3

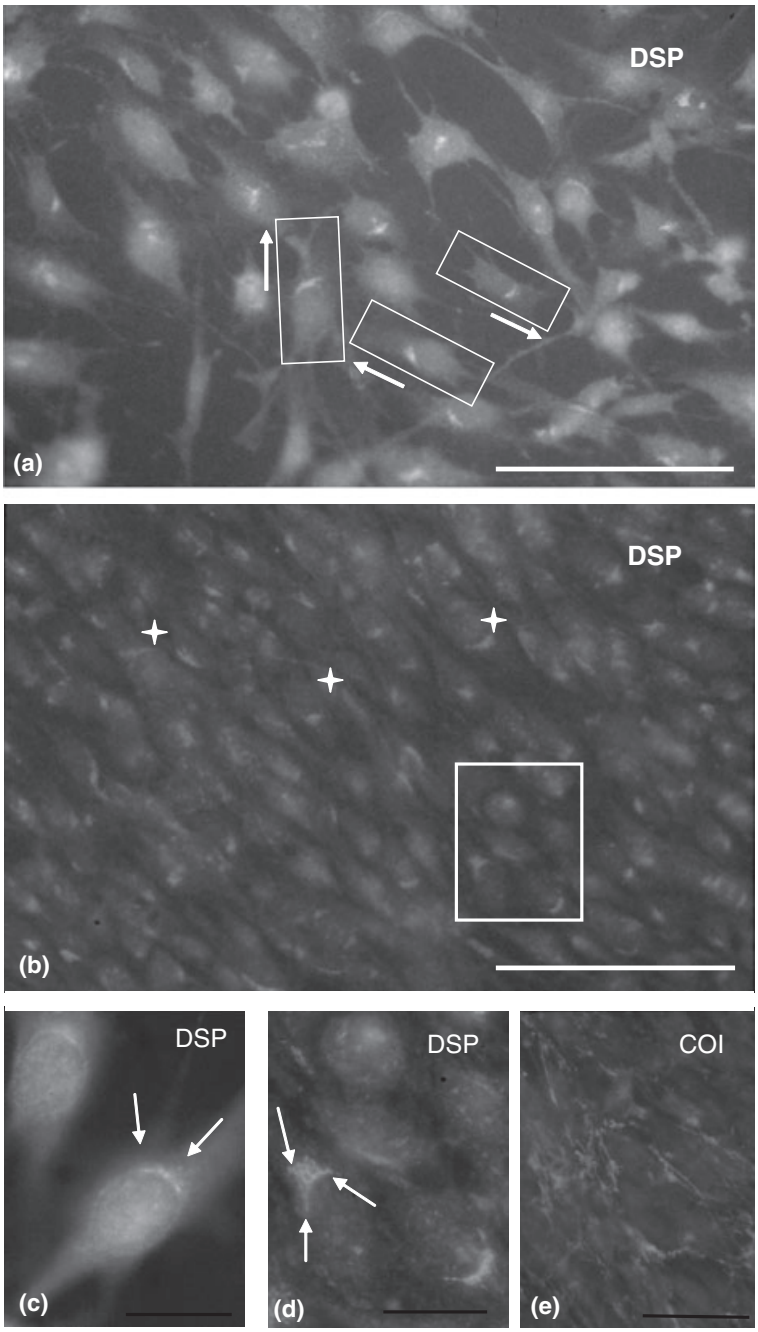


**Figure 5** Immunolocalization of matrix proteins in MO6-G3 cells. (a) DSP labelling at day 3. The relative orientation of DSP-labelled cells appears random in the layer as exemplified by cells marked with white orientated rectangles. Bar = 100 μm. (b) DSP labelling at day 7. The labelling allows observing that the vast majority of cells (98%) appear unipolar. Significant examples are illustrated by white stars. The white rectangle determine the cell area enlarged on figure C. Bar = 160 μm. (c) DSP labelling at day 3. High magnification allows to associate this DSP localization to the most prominent cytoplasm area. A unipolar staining of DSP is present with a triangular basis set up on the nucleus (arrows). Bar = 15 μm. (d) DSP labelling at day 7. The intensity and the intracellular extensions of the staining increase (arrow). This labelling allows to locate a supranuclear compartment and therefore to evidence cell polarity. Bar = 15 μm. (e) Type I collagen labelling at day 11. Collagen type I is found in the cells and extracellular compartment. Bar = 30 μm.

cells. The ultrastructural analysis confirmed the organization of a cellular body organized in proximal, central and distal compartments and the existence of a cellular process, which was apparently similar to odontoblasts elaborating orthodentine *in vivo* (Linde & Goldberg 1993, Butler 1995).

However, the present culture conditions in contrast to explants cultures (Couble *et al.* 2000) and MDPC-23 cells (Hanks *et al.* 1998b) did not allow epithelioïd organization which is the key feature of an odontoblast row. In the present study, cultures were not conducted on collagen or polystyrene scaffolds, which could mimic the matrix scaffold required for tissue morphodifferentiation in an odontoblast layer. Another study has isolated an odontoblast stem cell from human dental pulp (Gronthos *et al.* 2000). The data showed

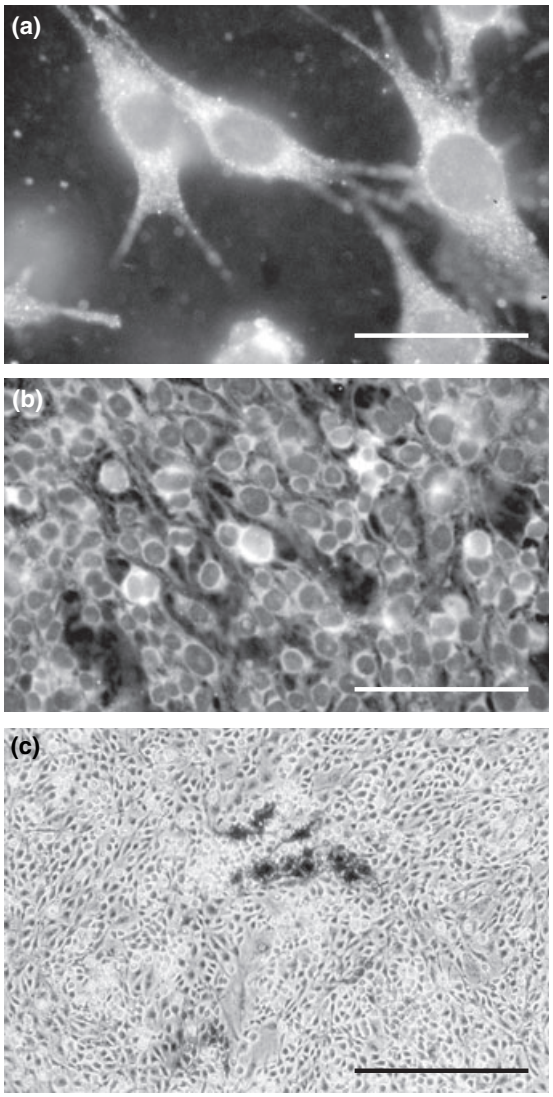


**Figure 6** Immunocytochemical and histoenzymatic studies of alkaline phosphatase. (a) Immunolabelling of alkaline phosphatase is distinctly distributed as multiple aggregates in cell periphery. Bar = 40 μm. (b) Immunolabelling of alkaline phosphatase shows more irregular distribution inside the cell population. Bar = 75 μm. (c) Histoenzymology of alkaline phosphatase shows a restricted distribution of the activity inside cell groups. Bar = 350 μm.

that the organization of odontoblast row and generation of dentine-like structure could not be obtained in primary cultures but only in an *in vivo* situation in immunocompromised mice (Gronthos *et al.* 2000). The evidences shown here of a structural and functional polarity for MO6-G3 cell line indicates that despite an individual odontoblast morphodifferentiation at the cellular level, an organized odontoblast row could never be obtained.

These immortalized odontoblast cell lines may be valuable tools for molecular investigations, but their ability to undergo cell division is at variance with the post-mitotic nature of odontoblasts under physiological conditions. The significance of theses cell cycle differences remains to be established (Goldberg & Smith 2004).

Alkaline phosphatase is an enzyme which is expressed by both the pulpal cells and odontoblasts *in vivo* (Hotton *et al.* 1999). It has been shown that it plays a role in mineralization process of bone and dental tissues (Beertsen *et al.* 1999). It is, therefore, an important phenotype marker of the formative cells of hard tissues. This enzyme has been widely used as a marker for odontoblast-type (Nakashima 1991, Kasugai *et al.* 1988), and also bone cells (Wlodarski & Reddi 1986) *in vitro*. By using *in vivo* data as landmarks of odontoblast life-span (Hoshi *et al.* 1997, Hotton *et al.* 1999), the presently observed levels of AP activity were extremely low. The relative quantities were: $32.66 \pm 1.15$ nmol $PNP^{-1}$ $min^{-1}$ $mg^{-1}$ proteins in MO6-G3 cells *versus* $7145 \pm 214$ nmol $PNP^{-1}$ $min^{-1}$ $mg^{-1}$ proteins in rat microdissecred epithelial dental cells and $15\,184 \pm 911$ nmol $PNP^{-1}$ $min^{-1}$ $mg^{-1}$ proteins in rat microdissecred mesenchymal dental cells (Hotton *et al.* 1999). This biochemical feature suggests that MO6-G3 corresponds to odontoblasts at the stage of orthodentine deposition. Indeed, the alkaline phosphatase transcripts and protein are actively synthesized in odontoblast during the mantle-dentine stage and then drastically down-regulated (Hotton *et al.* 1999). During orthodentine formation, their apparent levels are significantly reduced while sub-odontoblastic cells are expressing high AP levels. Ultrastructural observations showing (i) no evidences for matrix vesicles, (ii) the presence of a long and thin distal process and (iii) this described low level of AP activity may suggest that MO6-G3 cell line correspond to the developmental stage of orthodentine odontoblast.

## Conclusion

The present study was dedicated to the ultrastructural and immunocytochemical characterization of a cell line already identified as differentiated odontoblast (MacDougall *et al.* 1997). DSP immunolocalization, investigation of the cytoskeleton network, electron microscopy data shows that the immortalization

process might surprisingly enable their polarized morphodifferentiation.

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