

Experience in Primary Culture of Human Peritoneal Mesothelial Cell

Kuo-Su Chen^{1, 5}, Wen-Shiang Chen², Huang-Yang Chen³, Chin-Chan Lee¹, Hao-Shi Kao¹,
Yung-Chih Chen⁴, and Ming-Kuo Ting⁴

¹*Division of Nephrology, CGMH, Keelung 20401*

²*Division of Urology, CGMH, Keelung 20401*

³*Division of General Surgery, CGMH, Keelung 20401*

⁴*Division of Internal Medicine, CGMH, Keelung 20401*

and

⁵*Chang Gung Institute of Technology, Taoyuan 33303, Taiwan, Republic of China*

Abstract

To compare the growth condition between different sources and different culture environments, mesothelial cells were isolated from omentum and peritoneal dialysate effluent (PDE), seeded at different densities (5×10^5 , 1×10^5 , 5×10^4 , 1×10^4 , 5×10^3 , 1×10^3 and 5×10^2 cells/cm², respectively), supported with different fetal calf serum (FCS) concentrations (3%, 6%, 10% and 15%) and grown in dishes with and without gelatin pre-coating. Growth condition was evaluated by simple morphological observation. Cells phenotype was examined by immunofluorescent staining. The results showed that omentum-derived mesothelial cells generally showed a uniform growth pattern with good quality. Alternatively, there was a wide patient-to-patient variation in PDE-derived culture. Heterogeneous colonies composed of a mixture of large, small or abortive mesothelial colonies as well as fibroblastoid colonies were frequently observed. A minimum seeding density of 5×10^3 cells/cm² is required for the omentum-derived mesothelial cells to grow to confluent monolayer ($1-5 \times 10^4$ cells/cm² for initial culture from fresh PDE). Appropriate seeding density is always associated with successful culture in omentum-based culture, but not in PDE-based culture. Mesothelial cells could grow to confluency regardless of FCS concentration and gelatin pre-coating. However, growth rate was slower in lower FCS concentrations and on dishes without gelatin coating. Most cells in culture expressed cytokeratin and vimentin, but not VWF. Alpha-smooth muscle actin frequently appeared in cytokeratin+ mesothelial cells, especially in higher FCS concentrations and in PDE-derived culture. Our data demonstrate that PDE, in contrast to omentum, provides a source of mesothelial cells with poor and unstable quality for primary culture. Healthy cell quality and sufficient seeding density seem to be the most important factors for successful culture of mesothelial cells. The frequent occurrence of epithelial-to-mesenchymal transition in cultured mesothelial cells indicates the feasibility of mesothelial cells to undergo phenotype change upon environment changes, especially following chronic exposure to uremic environment and dialysate in peritoneal dialysis patients.

Key Words: mesothelial cell culture, omentum, peritoneal dialysate effluent, epithelium-mesenchyme-transition

Introduction

With the introduction of continuous ambula-

tory peritoneal dialysis (CAPD) and subsequent development of peritoneal fibrosis, there has been much interest in the cell biology of peritoneal

mesothelial cells. Primary culture of mesothelial cell provides us an *in vitro* tool to investigate the physiological function and pathologic process of mesothelium. Omentum is the most common source of human mesothelial cells for primary culture. It is obtained by detaching cells from basal lamina of surgically removed omentum with collagenase or trypsin. Mesothelial cells could also be obtained from peritoneal fluid by simple centrifugation (6, 9). Thus, peritoneal dialysate effluent (PDE) has been considered as an alternative choice. Although the technique of culturing mesothelial cells has been well described in the literature (2, 5, 10, 11, 14, 22), the difference between omentum- and PDE-derived mesothelial cells in culture has rarely been compared. Furthermore, information regarding the impact of different culture environments on mesothelial cell growth is still limited.

Mesothelial cells are generally cultured in basic medium (such as DMEM) containing 10% fetal calf serum (FCS) and plated on dishes pre-coated with gelatin or collagen (14). However, many current commercial culture dishes have been specially treated to improve cell adhesion. Thus, pre-coating of extracellular matrix is no longer necessary. Indeed, many papers published after that of Stylianou *et al.* (14) do not use dishes with gelatin or collagen pre-coating. The necessity of extracellular matrix coating for culture of mesothelial cell deserves re-evaluation.

Epithelial-to-mesenchymal transition (EMT) is a process characterized by the transformation of epithelial cell into fibroblast-like cell. This process could be induced in primary culture of mesothelial cells by treatment with TGF- β and/or IL-1 (20, 21). Given that serum contains cytokine and growth factors which may induce myofibroblast transdifferentiation of mesothelial cells in culture environment, it is better to provide low serum environment to prevent the development of EMT if mesothelial cells could grow in low or serum-free environment. There have been some reports claiming that mesothelial cells could grow in serum-free medium (5). Thus, we also like to investigate the requirement of serum support on mesothelial cell growth.

In this work, we compared the growth conditions between omentum and PDE-derived human mesothelial cells in culture with simple morphological observation. At the same time, we examined the impact of different culture environments on the growth of human mesothelial cells.

Materials and Methods

Design

Omentum was derived from 5 patients who

received gastrectomy or colectomy and fresh PDE was obtained from 26 uremic patients who underwent chronic CAPD therapy. Among these 26 CAPD patients, 9 were male and 7 were victims of DM (diabetes mellitus). Their ages ranged from 27 to 84 years old (mean = 54.55 ± 14.79 yrs), duration of PD ranged from 6 months to 13 years (mean = 4.73 ± 3.22 yrs), peritonitis episodes ranged from 0 to 5 episodes (mean = 1.15 ± 1.38).

Mesothelial cells were detached from omentum by trypsin digestive methods and obtained from PDE by simple centrifugation. Both sources of cells were cultured in DMEM in the presence of 10% FCS on dishes without extracellular matrix coating. Cultured cells were observed daily under phase microscope to compare the difference in growth conditions between these two sources of culture. Success of cell culture was defined as the ability of mesothelial cells to form a confluent monolayer with typical cobble-stone appearance, but not fibroblast, fibroblastoid or mixed morphologies.

To investigate the impact of seeding density on mesothelial cell growth, detached cells from first passage of omentum-derived culture and fresh cells from PDE centrifugation were seeded on 24 well dish at density of 5×10^5 , 1×10^5 , 5×10^4 , 1×10^4 , 5×10^3 , 1×10^3 and 5×10^2 cells/cm², respectively. Two cultures were undertaken for each seeding density from every source of omentum-derived culture (n = 10) and one culture was done for each seeding density in every PDE bag from 26 CAPD patients (n = 26). To complete the test of all seeding densities, a minimum of 1.26×10^6 cells are required. However, cell number per bag of PDE was less than this number in 15 CAPD patients. Thus, only 11 bags of PDE were tested for all the seeding densities in this experiment (Table 1).

To determine the impact of different FCS concentrations and gelatin coating on cell growth, omentum-derived mesothelial cells were suspended in DMEM containing different FCS concentration (3%, 6%, 10% and 15%) and inoculated onto dishes with and without gelatin pre-coating at fixed seeding density (1×10^4 cells/cm² and 1×10^3 cells/cm², respectively) at times of subculture from the first passage. In total, 9 cultures were done for each FCS concentration and gelatin coating condition (Table 2).

Immunofluorescent staining was undertaken to determine the phenotype of the cultured cells for both PDE- and omentum-derived cultures. Proportion of α -SMA+ mesothelial cells in 3%, 6%, 10% and 15% FCS concentrations was counted in 9 slides for each FCS concentration in omentum-derived cultured of second passage. This rate in PDE-derived culture (10% FCS, first passage) was counted in 15 slides which were randomly selected from 15 different

CAPD patients.

Culture of Mesothelial Cell from Omentum

Surgically removed omentum was cut into pieces of $5 \times 5 \text{ cm}^2$ area and washed with PBS, then incubated with a solution containing trypsin/EDTA for 30 min at 37°C and rotated continuously in a hybridization oven. After incubation, the cell suspensions were centrifuged at 500 g for five minutes. The supernatant was discarded and the cell pellet was washed with DMEM containing 10% FCS, resuspended in the same medium and plated in a 75 cm^2 flask. Non-adherent cells were removed 3 days later by two brief washes with medium. The dishes were incubated in a humidified 5% CO_2 atmosphere at 37°C , and the medium was changed every three days. When reaching 80% confluence at each passage, the adherent cells were washed with PBS and treated with PBS containing trypsin/EDTA for 10 min at 37°C until cells were detached as monitored by a phase contrast microscope. Free cells detached from the first passage were suspended, washed in DMEM, centrifuged at 500 g for 5 min, and seeded on 24-well dishes at different culture conditions according to the study protocol. Part of the detached cells were seeded onto a chamber slide for immunostaining at the time of subculture for each passage and culture condition.

Culture of Mesothelial Cell from PDE

PDE was drained into a 50-ml centrifuge tube and peritoneal cells were concentrated by centrifugation at 500 g for five minutes. Cell pellets were suspended in DMEM with 10% FCS and counted with a haemocytometer. Then, suspension cells were seeded onto a 24-well dish at different seeding densities. If total cell number was not enough for all culture protocols, only protocol of lower seeding densities was undertaken.

Immunofluorescent Staining

For immunofluorescent staining, mesothelial cells grown on chamber slides were fixed with 2% paraformaldehyde for 10 min. After washing with PBS containing 0.5% bovine serum albumin (PBS-BSA), 0.2% Triton X-100 was added and the cells were incubated for 5 min. Cells were again washed with 0.5% PBS/BSA three times and blocking buffer was added for incubation for 15 min. Primary antibodies, including anti-cytokeratin (DAKO, Carpinteria, CA, USA), anti-vimentin (Sigma-Aldrich, St Louis, MO, USA), anti- αSMA (Sigma-Aldrich) and anti-VWF (DAKO) antibodies, were then added and incubated for 30 min at room temperature. The

cells were washed three times with 0.5% PBS/BSA and then incubated with an appropriate fluorescein, including FITC, Cy3 and Cy5 (Abcam, Cambridge, UK), conjugated secondary antibody for 30 min in the dark. Cells were then extensively washed with PBS-BSA, mounted and observed with a fluorescent microscope or confocal microscope.

Statistical Analysis

Statistical analysis was carried out with SPSS software version 16.0 for windows (Chicago, IL, USA). Difference between groups was compared by Student's *t*-test or ANOVA test. *Post-hoc* analysis was undertaken with Tukey multiple comparison. Data were expressed as means \pm SD. *P* values < 0.05 were considered statistically significant.

Results

Growth Condition of Mesothelial Cells in Different Sources of Culture

In omentum-derived culture, mesothelial cells detached from omentum generally grew well in DMEM medium containing 10% FCS. Suspension cells detached from omentum would adhere to dish within 1-3 days of seeding. The adherent cells usually projected extensive filopodiae to contact with neighboring cells (Fig. 1A) upon adherence to dishes. Active proliferation would occur if the cell number is large enough for the neighboring adherent cells to contact with each other. Confluent monolayer could be reached within 5-10 days at passage 1. Two main types of cell morphology, including fibroblast and polygonal shape, could be recognized before confluency (Fig. 1A). Fibroblast-shape cells were more frequently observed during active proliferation. Once reaching confluency, most cells exhibited cobble stone appearance (Fig. 1B). However, variable numbers of giant cells could usually be observed. The growth condition of omentum-derived mesothelial cell culture was generally stable and predictable.

Alternatively, the growth condition showed great variations in PDE-derived mesothelial cell culture. Wide morphological heterogeneity, from normal to senescent or pathologic (usually large, irregular and broken) morphology could be found upon adherence (Fig. 1C). PDE-derived mesothelial cells could grow normally and form confluent monolayer with typical cobblestone appearance in some patients. However, scattered colonies of variable sizes were more frequently observed. Most colonies were composed of mesothelial cells, despite of the heterogeneous morphology. Mixtures of fibroblastoid and mesothelial colonies could usually be observed (Fig. 1D). Forma-

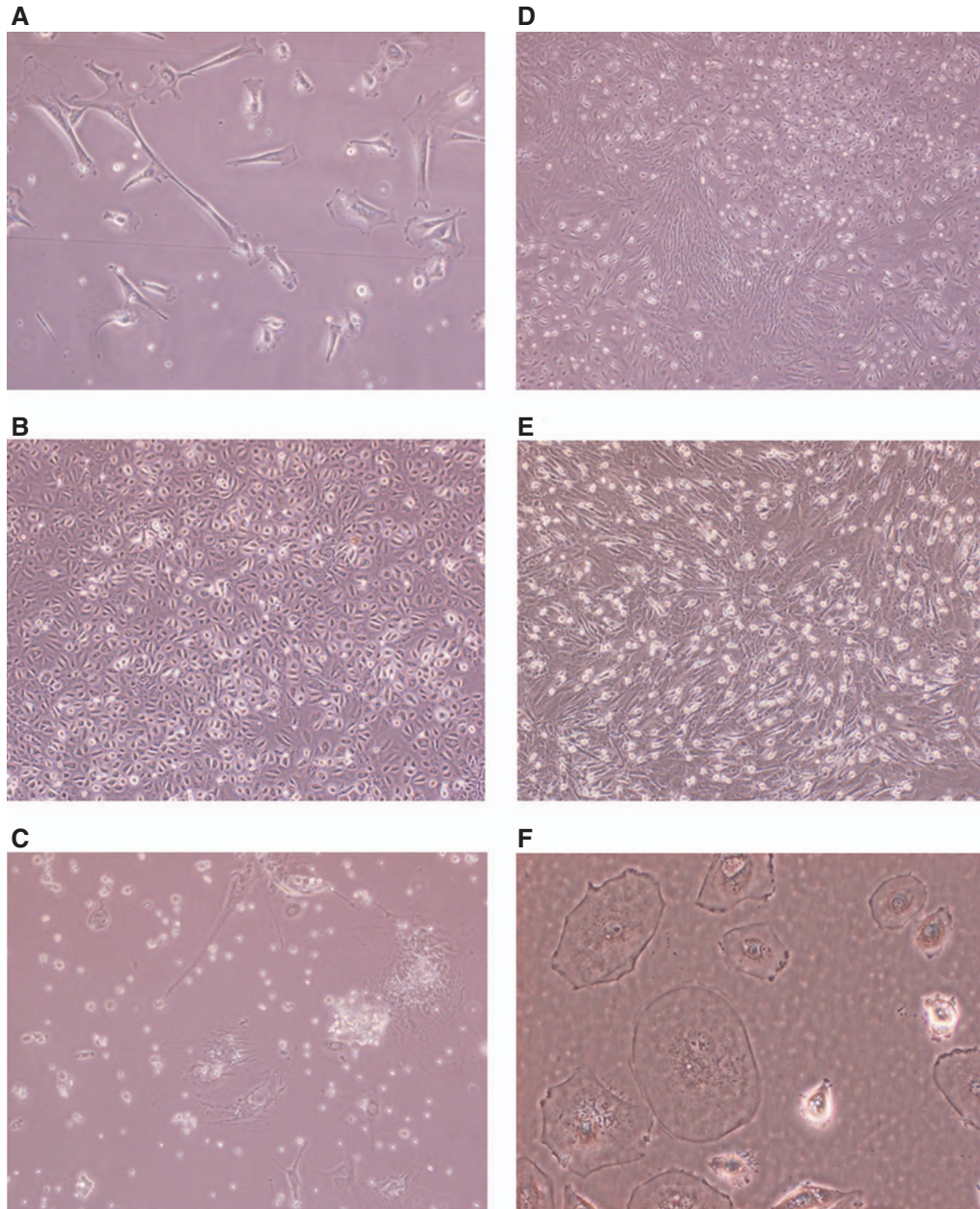


Fig. 1. (A) Filopodiae extends from adherent mesothelial cells to make contact with neighboring cells soon after adherence to dishes. Two main types of cell morphology, including oval and fibroblast shape, could be observed in a healthy mesothelial cells culture. (B) Cells of different morphologies during the proliferative phase all turned into polygonal shape and showed typical cobblestone appearance when reaching confluency. (C) Cells obtained from PDE usually show wide heterogeneity in morphology. Cells with large, broken, irregular shape could usually be found. This is quite distinct from that of omentum-derived mesothelial cells in (A) above. (D) Mixture of mesothelial colonies with fibroblastoid colonies could be found frequently in PDE-culture. (E) Confluent monolayer with pure fibroblastoid morphology could be observed in PDE-derived culture. (F) Cells show senescent changes after 3-4 passages in PDE-derived and 5-7 passages in omentum-derived mesothelial cell culture.

tion of a confluent monolayer of purely fibroblastoid or fibroblast morphologies was not uncommon (Fig. 1E). The growth condition showed great inter-patient variations, but small intra-patient variations. This indicated that the discrepancies in cell morphology and growth condition in PDE-culture could

be attributed to the difference in peritoneal membrane status between patients.

Cell number of each bag of PDE also showed great patient-to-patient variations, ranging from 2×10^4 to 5×10^7 cells per bag. This patient-to-patient variation in cell number further aggravated the dis-

Table 1. Time required to form confluent monolayer with typical cobblestone appearance in different seeding densities cultured in DMEM + 10% FCS and in dish without extracellular matrix coating

Seeding density	Omentum (for maintenance) n = 10	PDE (for initial fresh culture) n = 26
5×10^2 cells/cm ²	all failed (0/10)	all failed (0/26)
1×10^3 cells/cm ²	all failed (0/10)	all failed (0/26)
5×10^3 cells/cm ²	7.1 ± 1.1 days ^{$\pi\omega\theta\phi$} (10/10)	all failed (0/26)
1×10^4 cells/cm ²	4.5 ± 1.0 days ^{$\omega\theta\phi$} (10/10)	10.3 ± 1.5 days (3/25)
5×10^4 cells/cm ²	2.6 ± 0.7 days ^{ϕ} (10/10)	7.2 ± 1.2 days (6/22)
1×10^5 cells/cm ²	1.7 ± 0.4 days (10/10)	5.0 ± 1.6 days (7/16)
5×10^5 cells/cm ²	1.2 ± 0.4 days (10/10)	2.3 ± 1.2 days (7/11)
P value	< 0.001	not done

π : $P < 0.01$ when compared with 1×10^4 cells/cm² group; ω : $P < 0.01$ when compared with 5×10^4 cells/cm² group; θ : $P < 0.01$ when compared with 1×10^5 cells/cm² group; ϕ : $P < 0.01$ when compared with 5×10^5 cells/cm² group.

*The numbers shown in each bracket indicate the number of successful cultures vs. that of total cultures.

crepancy in growth condition of PDE-derived culture between patients. A PDE bag with low cell number was always associated with failure to form mesothelial confluent monolayer. Most cells only grew to form abortive colonies (< 32 cells per colony) in such a condition. However, high cell number per PDE bag did not guarantee successful growth of mesothelial cells. Adherent cells might only undergo limited number of division and proliferation to form colonies of different sizes, including large (> 3 mm), small (< 3 mm, > 32 cells) and abortive colonies. Cells in small or abortive colonies generally showed heterogeneous morphologies, usually senescent change.

Senescent change (Fig. 1F) generally developed after 4 to 5 transfer in omentum-derived mesothelial cell culture. However, it occurred earlier in PDE-derived mesothelial cells, usually at third to fourth passage. Confluent monolayer usually cannot form when senescent changes developed. PDE-derived mesothelial cells could be passaged for about 3-4 generations. However, omentum-derived mesothelial cells could be sustained up to 7-8 passages.

Impact of Seeding Density on Mesothelial Cell Growth

For the maintenance of mesothelial cells in culture following a successful formation of confluent monolayer, a seeding density greater than 5×10^3 cells/cm² was required (Table 1). A higher seeding density was associated with more rapid formation of confluent monolayer (7.1 ± 1.1 , 4.5 ± 1.0 , 2.6 ± 0.7 , 1.7 ± 0.4 and 1.2 ± 0.4 days for increasing seeding densities, $P < 0.001$). A seeding density lower than 5×10^3 cells/cm² always failed to form confluent monolayer. Cultured cells generally became senescent and underwent apoptosis if seeding density was low. Fibroblast colonies more frequently emerged in such

culture. Alternatively, a minimal seeding density of $1-5 \times 10^4$ cells/cm² was required for the successful culture of mesothelial cells from fresh PDE (Table 1). However, a high seeding density did not guarantee success in PDE-based culture. Variable growth conditions could be encountered in PDE-derived culture despite sufficient seeding density. Indeed, only 3/25 cultures seeded at 1×10^4 cells/cm², 6/22 cultures seeded at 5×10^4 cells/cm², 7/16 cultures seeded at 1×10^5 cells/cm² and 7/11 cultures seeded at 5×10^5 cells/cm² grew successfully to form confluent monolayer with typical cobblestone appearance (Table 1). Culture failure was usually encountered if high proportion of adherent cells exhibited sick or pathologic morphology upon initial adherence to culture dish. On the other hand, all 10/10 cultures successfully formed confluent mesothelial monolayer if seeding densities was sufficient in omentum-derived culture (Table 1).

Impact of FCS Concentration and Gelatin Coating on Mesothelial Cell Growth

At time of subculture from first passage, omentum-derived mesothelial cells could grow to form a confluent monolayer regardless of FCS concentration (3%, 6%, 10% or 15%) if seeded at a sufficient density (1×10^4 cells/cm²). However, the time required to reach confluency was different in different FCS concentrations. A higher FCS concentration was associated with a more rapid formation of confluent monolayer ($P < 0.001$, Table 2).

At a sufficient seeding density (1×10^4 cells/cm²), cells in dishes with or without gelatin pre-coating could both grow to form confluent monolayer. However, the time required to reach confluency was slightly longer in dish without gelatin pre-coating

Table 2. Time required for second-passage omentum-derived mesothelial cells to form confluent monolayer with typical cobblestone appearance in different FCS concentrations and in dishes with or without gelatin pre-coating

Seeding Density	Gelatin Coating	FCS Concentration			
		3%	6%	10%	15%
1×10^4 cells/cm ²	Yes (n = 9)	14.2 ± 1.2	8.2 ± 1.0	4.9 ± 0.8	3.3 ± 0.5
	No (n = 9)	15 ± 1.1	9.0 ± 1.1	6.1 ± 0.6	3.9 ± 0.6
1×10^3 cells/cm ²	Yes (n = 9)	all failed	all failed	all failed	all failed
	No (n = 9)	all failed	all failed	all failed	all failed

Two way ANOVA analysis showed that between-subject difference in FCS concentration and gelatin coating was both statistically significant ($P < 0.001$ for both).

($P < 0.001$, Table 2).

No confluent monolayer could form if the seeding density was low (1×10^3 cells/cm²) even though cells were supported with high FCS concentration (15%) and additional gelatin pre-coating (Table 2).

Phenotype of Cultured Mesothelial Cells by Immunofluorescent Study

The phenotype of mesothelial cell was confirmed by immunohistochemical staining. The immunohistochemical study revealed that cells of fibroblast or polygonal shape or giant-cell morphology were all positively stained by anti-cytokeratin and anti-vimentin antibodies (Fig. 2, A-F), but not by an anti-VWF antibody. This confirmed that cultured cells were mesothelial phenotype.

Of interest, α -SMA+ cells were frequently found in both omentum- (Fig. 3A) and PDE-derived (Fig. 3B) cultures. Almost all these α -SMA+ cells also expressed cytokeratin (Fig. 3, A-F), indicating that these myofibroblasts were transdifferentiated from mesothelial cells. Under confocal microscope, the process of EMT of mesothelial cells could be clearly observed. Initially, α -SMA cytoskeleton appeared at the periphery of mesothelial cells and formed a cortical ring (Fig. 3, A and B). Subsequently, the cortical ring became more prominent and spread toward the nuclear area (Fig. 3, C-E). At the same time, transdifferentiated mesothelial cells became larger and irregular (Fig. 3, C-F). The large irregular cell might exhibit very faint cytokeratin expression, leaving only strong vimentin and α -SMA expression (Fig. 3, E and F). This phenomenon indicates that the cultured mesothelial cells might have lost the mesothelial phenotype and were totally transformed into a myofibroblast phenotype.

The myofibroblasts were more frequently observed in PDE-derived than in omentum-derived culture, and in medium containing higher FCS concentrations. After calculating 9 independent slides in

omentum-derived cultures, the proportion of cells which co-expressed cytokeratin and α -SMA was $3.08 \pm 0.75\%$ in medium containing 3% FCS, $3.88 \pm 1.24\%$ in 6% FCS, $4.35 \pm 1.30\%$ in 10% FCS and $6.40 \pm 2.91\%$ in 15% FCS ($P = 0.003$, Fig. 4-1). Multiple comparisons with Tukey test showed that the 3% and 6% groups had significantly lower percentages of myofibroblast than that of the 15% FCS group ($P = 0.002$ and 0.022 , respectively). When comparing omentum-derived culture (n = 9) with PDE-derived culture (n = 15) at the same 10% FCS support, PDE-derived culture had a significantly higher proportion of myofibroblast than that of omentum-derived culture (19.1 ± 10.9 vs. $4.35 \pm 1.30\%$, $P = 0.001$, Fig. 4-2).

Discussion

Our results showed that mesothelial cells could be successfully cultured from surgical-removed omentum as well as from PDE. However, the quality and stability were quite different between these two sources. Culture of mesothelial cells from surgically-removed omentum was almost exclusively successful. These cells generally showed a homogenous and healthy quality. Alternatively, success rates of PDE-derived culture were variable and unpredictable. Cells morphology and growth condition usually showed great heterogeneity in PDE-derived culture. Poor mesothelial cell quality seems to be the contributing factor for the discrepancy in growth condition between these two sources of mesothelial cell culture. Given that mesothelial cells obtained from PDE had been chronically exposed to high concentrations of glucose and low pH environment (3, 15, 19) as well as chronic inflammation status of uremia (18, 23), it is expected that these cells were in a sick or pathological status (13). Thus, many PDE-derived cells could not grow well and healthily in culture environment. Low cell number per bag of PDE might further influence the growth condition of PDE-derived mesothelial cell

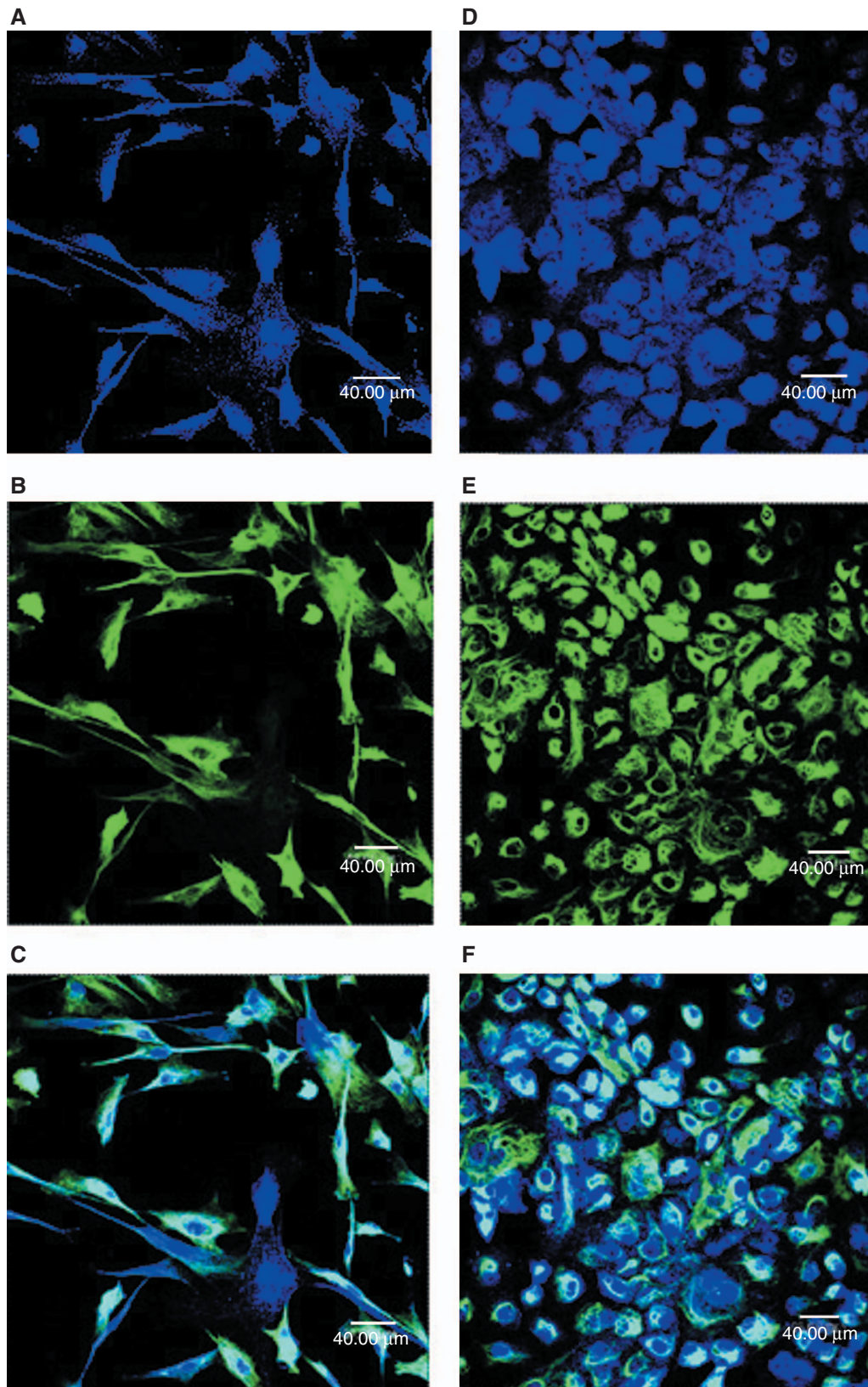


Fig. 2. In omentum-derived culture at passage 2, cells of either oval or fibroblast shape in early proliferative phase (A-C) and polygonal shape after reaching confluency (D-F) all exhibit mesothelial markers cytokeratin and vimentin. Blue and green colors indicated positive staining with an anti-vimentin antibody or an anti-cytokeratin antibody respectively. C and F are merged pictures of A + B and D + E. (Culture condition: DMEM + 10% FCS, dish without gelatin pre-coating)

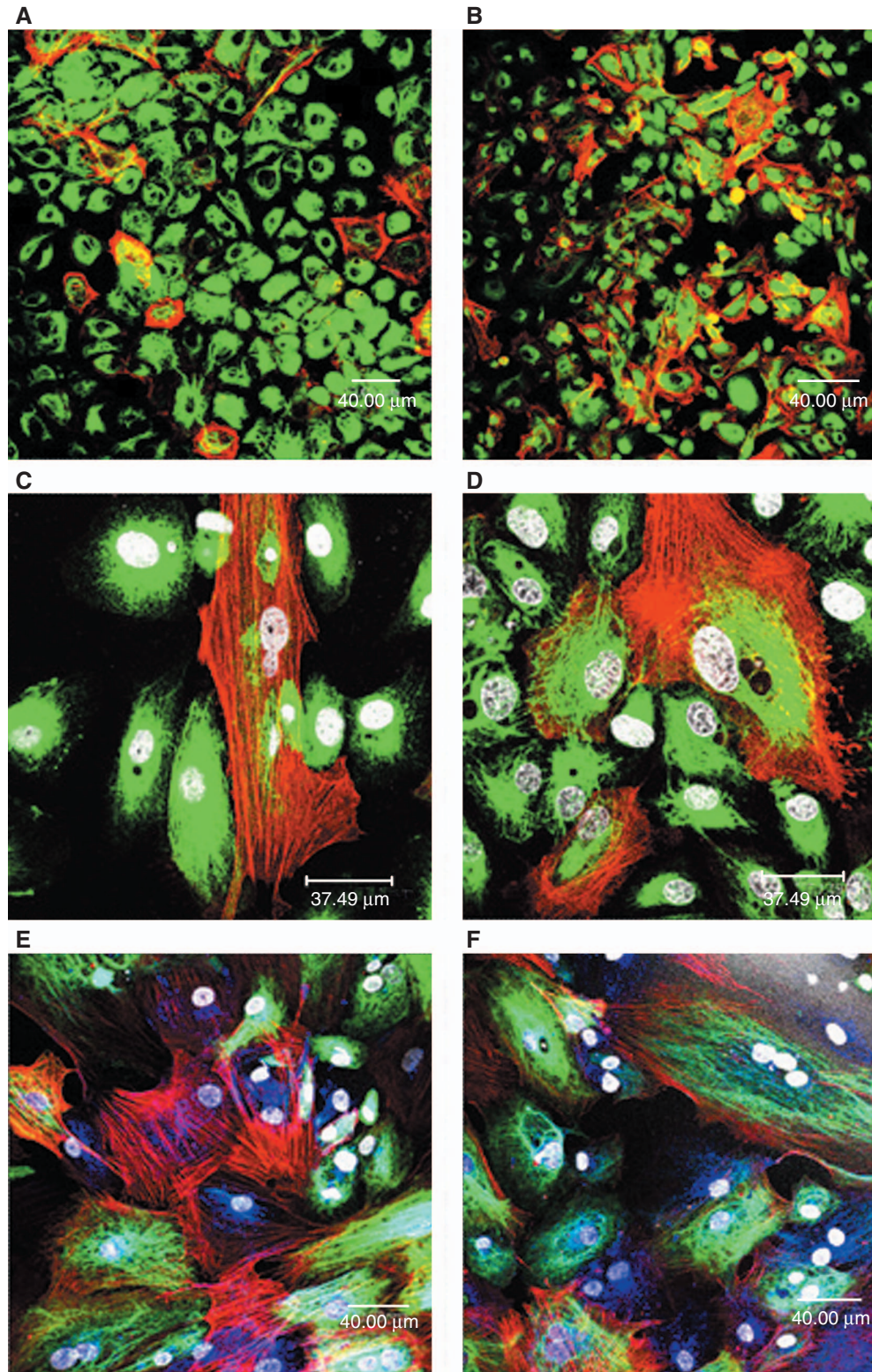


Fig. 3. α -SMA⁺ mesothelial cells could be observed in either omentum-derived mesothelial cells culture (A) or PDE-derived culture (B). α -SMA cytoskeleton appears in periphery of cells initially (A, B) and then spread toward nuclear area (C, D). Large, irregular or broken cell found in PDE-derived culture usually shows strong α -SMA and vimentin, but weak cytokeratin staining (E-F). This indicates that these cells gradually lost the mesothelial phenotype and were transdifferentiated into a myofibroblast phenotype. Blue, green and red colors indicated positive staining with anti-vimentin, anti-cytokeratin and anti- α -SMA antibodies respectively. (Culture condition: second passage in omentum-derived culture and first passage in PDE-derived culture, both in DMEM with 10% FCS).

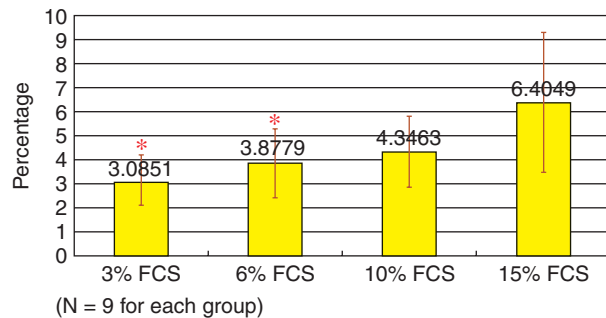


Fig. 4-1. Comparison of the proportion of α -SMA+ mesothelial cells in different FCS concentrations in omentum-derived culture at passage 2. It shows that EMT occurred in all FCS concentrations. However, the proportion tends to be higher in medium containing higher FCS concentration ($P = 0.003$). Proportion of myofibroblast transdifferentiation in 15% FCS culture is significantly higher than that of 3% FCS and 6% FCS cultures ($P = 0.002$ and 0.022 , respectively). All 4 groups came from the same source of mesothelial cells. $*P < 0.05$ when compared with the 15% FCS group.

culture. Our results showed that cell number per PDE bag may be as low as 2×10^4 . Such low cell number could not support the growth of mesothelial cells in culture environment. Thus, primary culture of mesothelial cell from PDE might encounter multiple problems, such as insufficient cell number, longer waiting time, failure of monolayer formation and wide variety in cell phenotype. It is, therefore, not advisable to use PDE as a source of mesothelial cells culture unless the investigators intend to study the impact of uremia or dialysate on mesothelial cells.

Seeding density is an important factor for the successful culture of mesothelial cells. Mesothelial cells are one kind of polarized cells which need cell-cell contact and signal communication to support healthy cell growth (4, 7, 16, 17). Our observation revealed that mesothelial cells usually projected extensive filopodia to make contact with neighboring cells soon after adherence to the culture dish. Insufficient seeding density prevented such cell-cell contact and communication. Thus, confluent monolayer could not form. Under such a circumstance, adherent mesothelial cells only underwent limited number of proliferation and formed a loose, small abortive colony. Cells in the abortive colony generally become senescent and undergo apoptotic change. In this experiment, a minimum seeding density of 5×10^3 cells/cm² was required for the maintenance of omentum-derived mesothelial cells and $1-5 \times 10^4$ cells/cm² for the successful culture of mesothelial cells from fresh PDE. While less than 20% of cells in PDE are mesothelial cells (1, 8, 12), it is reasonable that seeding density required for PDE-based culture

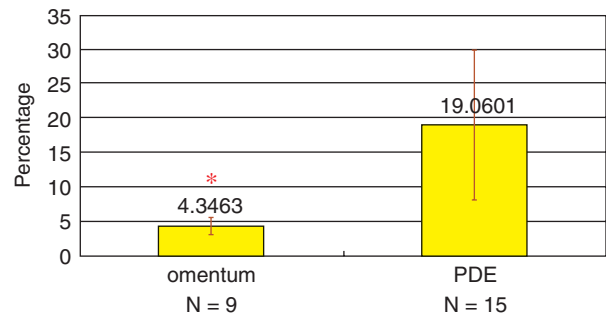


Fig. 4-2. When comparing rates of α -SMA+ mesothelial cells between omentum and PDE-derived culture (both in DMEM + 10% FCS), the PDE-derived culture had a higher rate of myofibroblast conversion than that of omentum-derived culture ($P = 0.001$). Omentum-derived cells were of second passage and PDE-derived cells were of first passage. $*P < 0.01$ when compared with the PDE group.

is about 5 times that of omentum-based culture. However, a sufficient seeding density could not guarantee successful culture from fresh PDE due to the pathologic status of mesothelial cells in PDE.

Adhesion of cells to dish pre-coated with extracellular matrix compounds is crucial for cell differentiation and maintenance of mesothelial cells in culture (14). Traditionally, human peritoneal mesothelial cells are cultured in dishes pre-coated with gelatin or collagen. Our results showed that mesothelial cells could successfully grow in dish without extracellular matrix coating despite time required to reach confluency was slightly longer. As many current commercial culture dishes had been specifically treated to improve cell adhesion, coating with extracellular matrix seems to be unnecessary for culturing mesothelial cell.

Serum is a necessary element to support cell growth in culture. Traditionally, 10% FCS is used in culturing mesothelial cells. Our results revealed that mesothelial cells could grow and form a monolayer in medium containing FCS concentration as low as 3% despite the growth rate and cell morphology was not optimal. This is compatible with the report of Hjelle *et al.* (5) who showed that mesothelial cells could grow in serum-free media. Thus, 10% FCS is still the best for normal culture of mesothelial cells. However, low or serum-free media could also be employed in studies which intend to deprive the effect of serum on mesothelial cells, such as the occurrence of EMT.

Cells of different morphologies, including fibroblast-like, polygonal shape, oval giant cells and large irregular cells all expressed the mesothelial markers cytokeratin and vimentin. It is possible that different shapes of mesothelial cells represent different cytoskeleton composition and expression and, thus,

different cell phenotypes. An in-depth immunofluorescent study in the future is required to confirm this postulation.

It is now well recognized that EMT process is involved in the aberrant healing and progressive peritoneal fibrosis during peritoneal injury (20). Our observation revealed that myofibroblast transdifferentiation of mesothelial cells was a common phenomenon in culture environment, even in first passage omentum-derived mesothelial cells with healthy growth. The higher rates of occurrence of EMT in medium containing higher concentration of FCS indicate that serum is an important triggering factor for the development of EMT in culture.

The findings of more frequent occurrence of EMT in PDE-derived mesothelial cells indicated that chronic exposure to dialysate and uremia environment may induce transdifferentiation of mesothelial cell. This is consistent with the findings of previous investigators that chronic PD therapy causes mesothelial cells injury and changes in phenotype of mesothelial cells (3, 13, 15, 18-20, 23). Subsequently, myofibroblast conversion and peritoneal fibrosis may occur.

In brief, PDE is neither a good nor a reliable source for the culture of mesothelial cells. It takes longer waiting time and shows great variabilities in quality and growth condition, in addition, having much higher rates of EMT. For successful culture of mesothelial cells, seeding density and cell quality are the most important factors.

Acknowledgments

This work was supported by a grant from the Chang Gung Memorial Hospital (CMRPG260611).

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