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Proliferation, Viability, and Metabolism of Human Tumor and Normal Cells Cultured in Microcapsule

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Abstract

In this study, we investigated the effect of the microenvironment provided by alginate-poly-L-lysine-alginate (APA) microcapsule with liquefied or gelled core on the proliferation, viability, and metabolism of human cells, including anchorage-dependent MCF-7 breast cancer cells and primary fibroblasts, and anchorage-independent K-562 leukemia cells; cells in conventional culture were used as control. The growth pattern of cells in microcapsule was examined by phase-contrast micrography. The cell viability, proliferation, organization, and gene expression were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay, hematoxylin and eosin staining, live/dead staining, 5-bromo-20-deoxyuridine labeling, and immunohistochemistry, respectively. Cell metabolism was determined by measuring glucose and lactate concentrations in medium. The results demonstrate that APA microcapsule with liquefied core provides a microenvironment for both anchorage-dependent and anchorage-independent cells to grow into a large cell aggregate and maintain cell viability at a constant level for a period of time. In conclusion, cells in APA microcapsule are alive and have proliferation potential with lower metabolism rate. APA microcapsule may be a useful tool for in vitro tumor cell modeling and anticancer drug screening as well as for cancer gene therapy. In addition, it lays a solid foundation for the use of microencapsulation in cell culture in vitro and cell implantation in vivo.

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Index Entries: Alginate; microcapsule; cell encapsulation; poly-L-lysine; tumor cell.

Introduction

Since Lim and Sun *(1)* invented alginate-poly-L-lysine-alginate (APA) microcapsule in 1980, microencapsulation has been proven to be an effective strategy for cell implantation and cell-based gene therapy for the treatment of diabetes, metabolic or neurologic, disorders, and cancer *(2–8)*. Cell microencapsulation consists of enclosing cells, such as primary cells; cell lines; and genetically engineered cells to secrete therapeutic product in a semipermeable membrane. The membrane allows bidirectional diffusion of nutrients, oxygen, metabolites, and waste but prevents high molecular weight substances, such as antibodies and immunocytes, from entering the microcapsule, which provides an immune protection for the cells. Microcapsule membrane can be made of natural (alginate, chitosan, agarose, and collagen) or synthetic polymers (polyaminoacids, polyacrylates). APA microencapsulation is one of the most well-studied encapsulation technologies, including entrapment of cells in alginate gel beads, formation of alginate-poly-L-lysine membrane, and liquefying of the alginate gel core to leave the cell floating in the center of the microcapsule *(9)*. A variety of cells, including pancreatic islets, hepatocytes, parathyroid cells, tumor cells, and genetically engineered cells, have been encapsulated in APA microcapsule. Most studies are mainly focused on the in vivo cell implantation or delivery of recombinant gene products using a rodent model *(10–13)*. A better understanding of the microenvironment of microcapsule is of paramount importance for the design and optimization of microcapsule used for cell implantation and cell-based gene therapy. A study by de Haan et al. *(14)* investigating factors that influence the glucose-induced insulin response of encapsulated islets in vitro confirmed that the size and permeability of microcapsule had an effect on cell function. However, little is known about how the liquefied microenvironment of microcapsule affects the viability and proliferation state of the cells in the microcapsule.

Microcapsule is a type of microbioreactor that can provide nutrients and oxygen by diffusion and excrete secreting products out of the microcapsule. The inside of the microbioreactor can be either in liquid (e.g., microencapsulation with liquefying) or in solid alginate gel (e.g., microencapsulation without liquefying). It is important to mention that alginate gel is one of the most used matrices for cell immobilized culture and tissue engineering *(15)*, which can promote cell organization into three-dimensional (3D) structure to proliferate, differentiate, and function. Therefore, questions such as these are raised: Is microcapsule with gelled core more suitable for cell growth? Which provides a better microenvironment for cell growth—microcapsule with liquefied core or microcapsule with gelled core?

To answer these questions, three types of human cells were cultured in microcapsule with or without liquefying and the growth, morphology, viability, proliferation, organization, and metabolism were investigated;

cells in conventional culture were used as control. Human MCF-7 breast cancer cells and primary fibroblasts were chosen as models of anchorage-dependent tumor cells and normal cells, respectively, and human K-562 leukemia cells were used as a model of anchorage-independent tumor cells. Gene expression of human MCF-7 cells in microcapsule with liquefied core was further investigated. To our knowledge, this is the first investigation of the effect of liquid and gel core of APA microcapsule on cell growth. The findings lead to a better understanding of the microenvironment–cell interaction in microcapsule and lay a solid foundation for microcapsule-based cell therapy.

Materials and Methods

Cell Culture

Human MCF-7 breast cancer cells and K-562 leukemia cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 mg/mL of streptomycin, and 100 U/mL of penicillin at 37°C in a humidified incubator with 5% CO_2 and 95% air. Human primary embryonic skin fibroblasts were cultured in RPMI-1640 medium supplemented with 20% FBS. Cells were harvested with 0.25% trypsin after human MCF-7 cells or fibroblasts reached confluence and plated in 24-well plates with a seeding density of 2.5×10^4 cells/well and K-562 with a seeding density of 3×10^4 cells/well.

Cell Encapsulation and Culture

Cells were encapsulated in APA microcapsules prepared as described previously with little modification *(9)*. Briefly, human MCF-7 cells, K-562 cells, or fibroblasts were mixed with 1.5% (w/v) sterilized sodium alginate (from *Macrocystis pyrifera*; low viscosity; Sigma, St. Louis, MO) to form a cell suspension (6 H 10^6 cells/mL) and extruded through a 26-gage needle with an electrostatic droplet generator into 100 m*M* $CaCl_2$ to form calcium alginate gel beads. The microbeads were then suspended in 0.05% (w/v) poly–L-lysine (mol wt = 65,000; Sigma) to form membrane enclosing the alginate gel beads, followed by the addition of 0.15% alginate to neutralize uncoalescent poly–L-lysine. Half of the microbeads was suspended in 55 m*M* sodium citrate to liquefy the alginate gel core, and the other half was allowed to form solid gelled core directly. The diameter of the APA microcapsules was 250–350 mm. Microencapsulated cells within APA membranes were cultured at 37°C in 5% CO_2 in RPMI-1640 medium supplemented with 10 or 20% FBS.

Cell Morphology

The growth pattern of cells cultured in APA microcapsule with liquefied or gelled core was observed using phase-contrast micrography for about 2 wk.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide Assay

Cell viability and proliferation was measured using a 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay as described previously *(16)* with minor modifications. Briefly, 200 µL of microencapsulated cells in medium was added to each well of 96-well plates, and then 20 µL of MTT solution (5 mg/mL) was added and incubated at 37°C in a humidified atmosphere (95% air and 5% CO_2) for 4 h. The medium was removed and replaced with dimethyl sulfoxide to solubilize the MTT tetrazolium dye. The absorbance (*A*) was read at 570 nm using a plate reader.

Hematoxylin and Eosin Staining

Microencapsulated human MCF-7 and K-562 cells were washed with phosphate-buffered saline (PBS) followed by fixation with 4% formalin. After processing on a short cycle of alcohols and xylenes, the samples were processed in paraffin for a maximum of 60 min. Blocks were sectioned at 5 µm and stained with hematoxylin and eosin (H&E) according to routine protocols. The samples were examined by microscope.

Live/Dead Viability Assay

Microencapsulated human MCF-7 cells, K-562 cells, and fibroblasts were aspirated in 96-well plates and washed with D-PBS. The cells were incubated with live/dead staining working solution composed of 2 µ*M* calcein AM and 4 µ*M* ethidium homodimer-1 (ED-1) at 37°C for 25 min followed by washing in the dark. Samples were observed under a confocal laser scanning microscope. Live cells were labeled with calcein AM, producing green fluorescence at an excited wavelength of 485 ± 10 nm, and dead cells were labeled with ED-1, emitting red fluorescence at 530 ± 12.5 nm.

5-Bromo-20-Deoxyuridine Incorporation Assay

The proliferation status of cells in microcapsule was determined by measuring 5-bromo-20-deoxyuridine (BrdU) incorporation in DNA using a peroxidase-based immunohistochemical assay. The microencapsulated cell spheroids were incubated with BrdU (10 m*M*) for 12 h prior to harvest and histologic procedure. The paraffin-embedded tissue sections were completely dewaxed and rehydrated with PBS before microwave antigen retrieval. Thereafter they were incubated in 0.3% H_2O_2 in methanol for 10 min, to inhibit endogenous peroxidase activity; rinsed in PBS; and then circled with a PAP pen. After blocking with serum at room temperature, the sections were incubated with primary anti-BrdU antibody (1:50) overnight at 4°C in a humidified chamber followed by washing in PBS. The negative control was incubated with PBS. After incubating with secondary antibody (biotin-conjugated rabbit antimouse IgG) for 1 h at room temperature and washing in PBS, the sections were incubated with horseradish peroxidase (HRP)-linked streptavidin at room temperature for 1 h and washed in PBS.

The sections were shown in color by diaminobenzidine (DAB) and mounted with cover slips.

Immunohistochemistry

The expression of cell growth-related proteins, including hypoxia-inducible factor (HIF)-1α, cyclin D1, vascular endothelial growth factor (VEGF), and p53, was examined using immunohistochemical staining. Briefly, microencapsulated human MCF-7 cell spheroids were embedded in paraffin and thin sectioned on slides. The slides were deparaffinized by incubating for 60 min at 65°C and soaked sequentially for 5 min in each of the following for two cycles: xylene, 100% EtOH, 90% EtOH, and 70% EtOH. After rinsing in PBS twice, 10 min each time, slides were blocked with blocking reagents. Primary antibodies of HIF-1α (1:25), cyclin D1 (1:50), VEGF (1:25), and p53 (1:25) were incubated overnight at 4°C in a humidified chamber. Slides were rinsed in PBS and incubated with EnVision System HRP. The sections were shown in color by DAB and mounted with cover slips.

Determination of Glucose and Lactate Concentration

The concentration of glucose and lactate in the medium was measured using a biosensitive glucose/lactate analyzer (SBA-40C).

Results

Growth Patterns of Different Types of Cells Cultured in APA Microcapsule With Liquefied or Gelled Core

The anchor-dependent human MCF-7 cells distributed inside the APA microcapsule separately after microcapsule was liquefied with sodium citrate (Fig. 1A). After 2 d of cultivation, human MCF-7 cells began to aggregate into a clump (Fig. 1B,C) and proliferated to form a large spheroid with a diameter of 200 μm on day 7 (Fig. 1D). Correspondingly, non-liquefied APA microcapsule containing human MCF-7 cells was filled with alginate gel, and the cells proliferated based on local cells and grew into small multispheroids with a tendency to amalgamation (Fig. 1A1–D1).

Anchor-independent human K-562 cells cultured in APA microcapsule with liquefied core showed a similar growth pattern to that of human MCF-7 cells and formed a large cell spheroid on day 7 (Fig. 1E–H). By contrast, human K-562 cells cultured in microcapsule with gelled core perished with time and turned to dark dots after 7 d of cultivation (Fig. 1E1–H1).

Human fibroblasts cultured in APA microcapsule with liquefied core began to form clumps after preparation for 4 h, and fibroblasts in gelled core distributed in the microcapsule with the form of many small aggregates. The cells grew slowly in both types of microcapsule (Fig. 2A–C,E–G). The dark blue formazan crystals revealed by MTT viability assay demon-

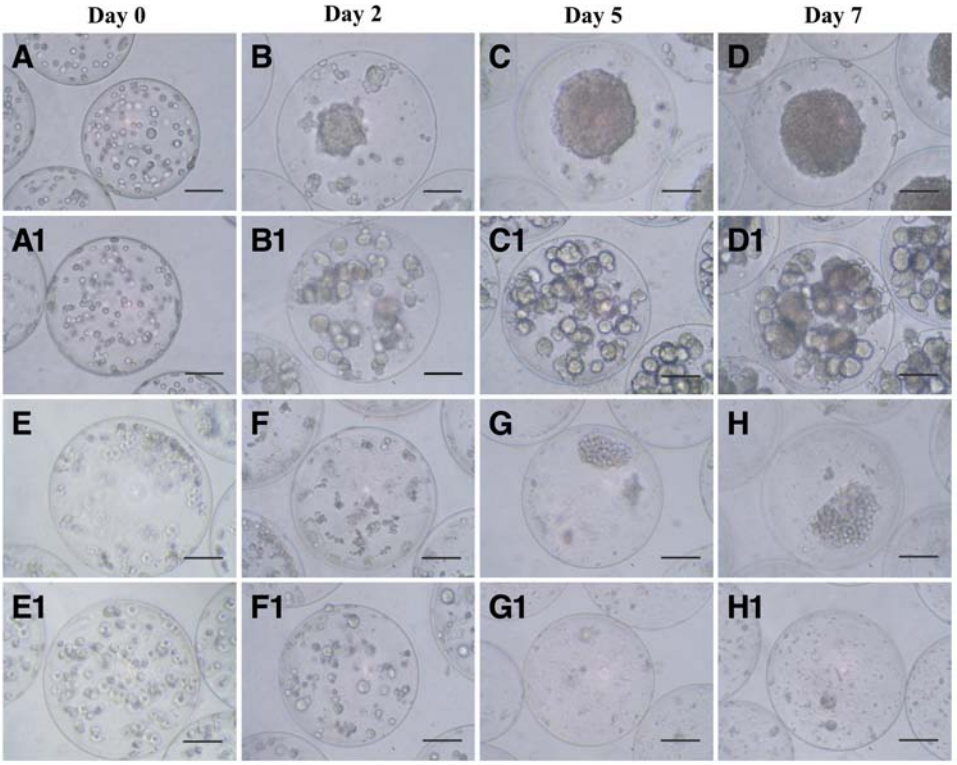

Fig. 1. The growth pattern of human MCF-7 and K-562 cells cultured in APA microcapsule with or without a liquefied microenvironment was observed by phase-contrast micrography at days 0, 2, 5, and 7, respectively: **(A–D)** human MCF-7 cells in microcapsule with liquefied core and **(A1–D1)** solid core; **(E–H)** human K-562 cells in microcapsule with liquefied core and **(E1–H1)** gelled core. Bars = 100 μm.

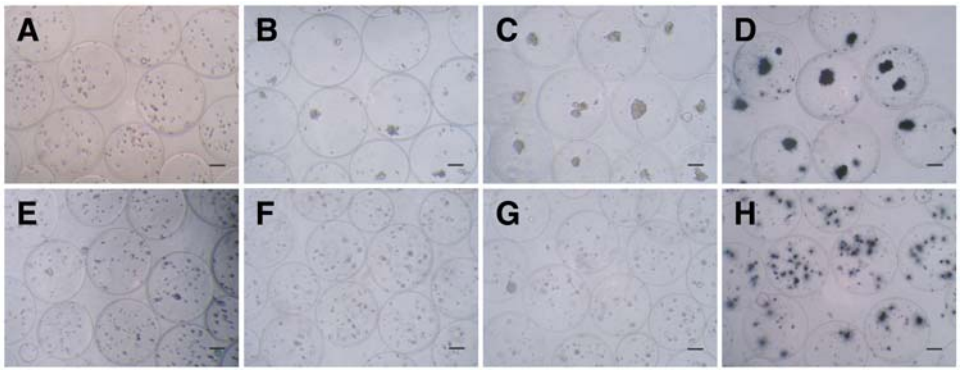

Fig. 2. Growth pattern of human embryonic skin fibroblasts in APA microcapsule with **(A–C)** liquefied core and **(E–G)** gelled core at 0, 4 h, and 5d, respectively. Cell viability was shown by MTT staining, which stained the viable cells with dark blue crystal, after 5 d of cultivation in microcapsule with liquefied **(D)** or gelled **(H)** core. Bars = 50 μm.

strated that the cells were viable and healthy in both types of microcapsule (Fig. 2D,H).

Cell Viability

The viability and proliferation of human MCF-7 cells, K-562 leukemia cells, and fibroblasts cultured in APA microcapsule with liquefied or gelled core were evaluated using MTT assay. Human MCF-7 cells and fibroblasts grown in monolayer and human K-562 cells grown in suspension were used as control, respectively.

For anchorage-dependent human breast cancer MCF-7 cells, there was no significant difference in the viability of cells grown in microcapsule with liquefied core and with gelled core. The viability of cells in microcapsule increased linearly and reached a maximum at about day 4 and 5, similar to cells grown in monolayer, and then decreased and maintained the level of day 6 for three more days, whereas the cells grown in monolayer decreased to almost 0 on day 9 (Fig. 3A).

For anchorage-independent K-562 leukemia cells, the viability of cells grown in microcapsule with liquefied core increased gradually and reached a maximum on day 4, decreased slightly on day 5, and maintained the level constantly thereafter, exceeding the level for cells grown in suspension after 7 d. The viability of cells in microcapsule with gelled core increased gradually and reached a maximum similar to that of cells in liquefied microcapsule on day 4 but decreased gradually thereafter, to a level much lower than that grown in suspension (Fig. 3B).

For feeder-layer human primary fibroblasts in the first 3 d, the viability of cells in two types of microcapsules was similar to that grown in monolayer. After 4 d of cultivation, the viability of cells grown in microcapsule with liquefied core reached a maximum and decreased gradually; the level was higher than that grown in microcapsule with gelled core but much lower than that grown in monolayer (Fig. 3C).

Organization and Distribution of Cells in Liquefied Microcapsule

As shown in Fig. 1, human MCF-7 and K-562 cells in microcapsule with liquefied core organized into a large cell spheroid after 7 d of cultivation. The cells' organization was furthered revealed by H&E staining. In liquefied microcapsule, K-562 cells aggregated to a spheroid, and the junction between the cells seemed to be loose (Fig. 4A). MCF-7 cells connected with tightness and formed a cell spheroid up to 200 μm in diameter with a blurred necrotic core (Fig. 4B).

K-562 and MCF-7 cells in microcapsule were examined by live/dead staining and observed under a confocal laser scanning microscope, where the green fluorescence showed the calcein AM-labeled viable cells and the red fluorescence showed the ED-1-labeled nonviable cells. The strong green fluorescence that manifested both human K562 and MCF-7 cells maintained high viability after growing in microcapsule for 10 d. Most of the anchor-

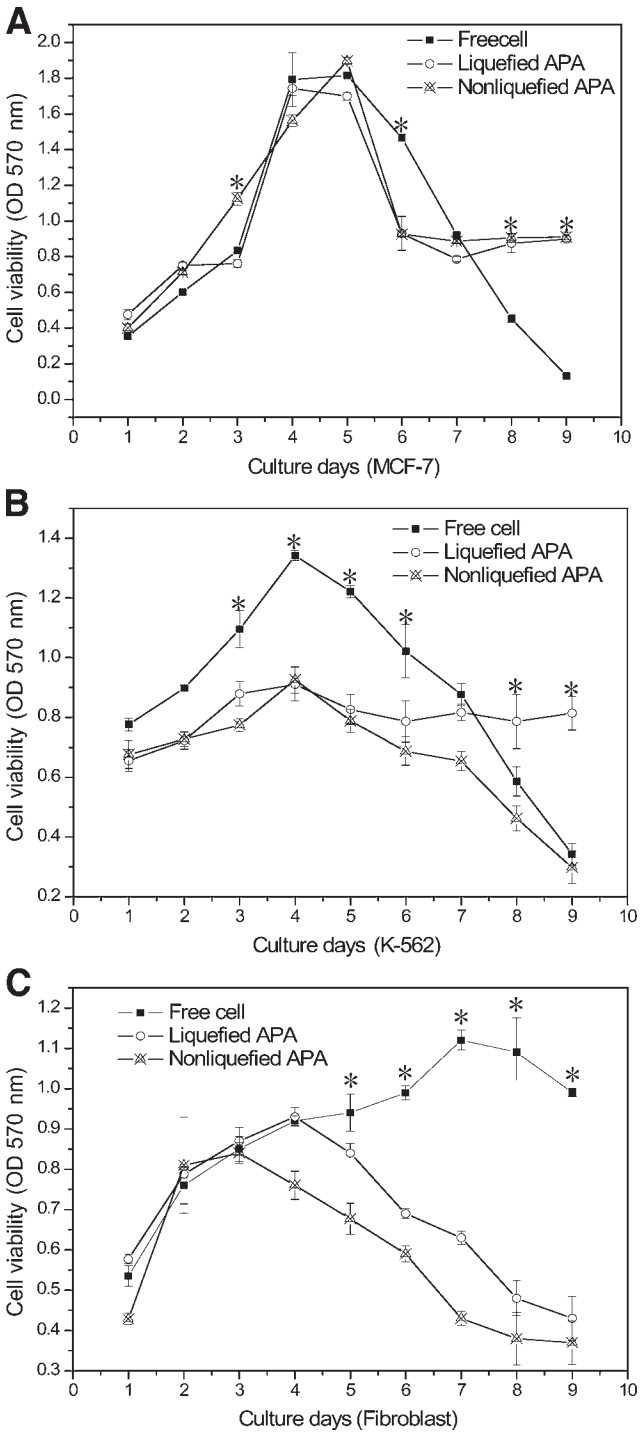

Fig. 3. Time course of cell viability measured by MTT assay. **(A)** Human MCF-7, **(B)** K-562, and **(C)** fibroblast cells were cultured in monolayer, microcapsule with liquefied core, or microcapsule without liquefied core. Each point represents the mean of four wells, and each assay was performed in triplicate. Errors bars are the standard deviation of the mean (SEM) and are shown when greater than the size of symbols (*$p < 0.05$).

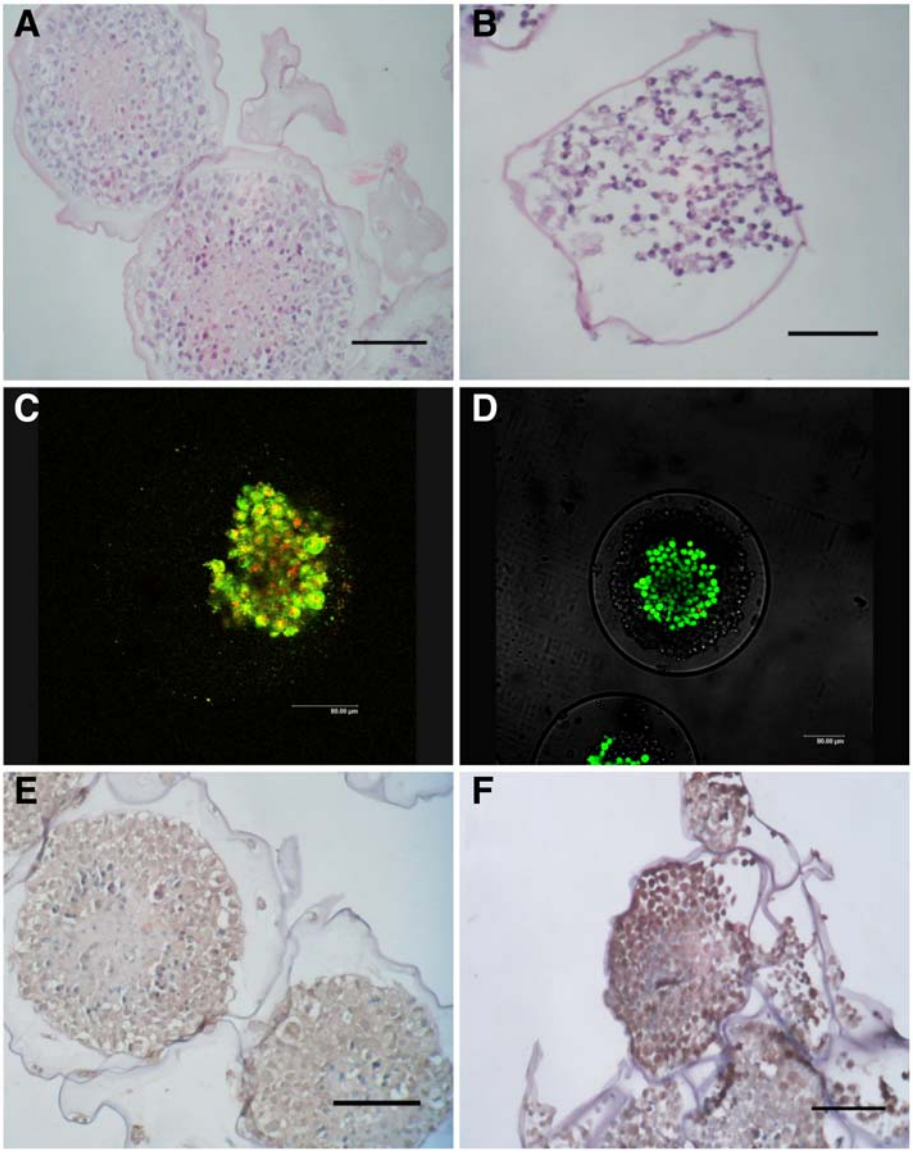

Fig. 4. Characteristics of morphology, viability, and proliferation of **(A, C, E)** human K-562 cells and **(B, D, F)** MCF-7 cells cultured in APA microcapsule with liquefied core. **(A)** H&E staining of human K-562 cells cultured in microcapsule for 10 d. **(B)** H&E staining of human MCF-7 cells cultured in microcapsule for 14 d. The capsule's irregular shape is the result of dehydration during histological processing (bars = 100 µm). Images of fluorescence labeled **(C)** human K-562 cells and **(D)** MCF-7 cells cultured in microcapsule for 10 d revealed by confocal laser scanning microscopy. Viable cells were labeled in green by calcein AM and nonviable cells were labeled in red by ED-1 (bars = 80 µm). Images of **(E)** BrdU-labeled K562 cells and **(F)** MCF-7 cells revealed the proliferating cells with brown nucleus for 10 d of culture (bars = 100 µm).

independent K-562 cells in microcapsule were alive; otherwise, viable MCF-7 cells mainly localized in the periphery of the cell spheroid (Fig. 4C,D).

The cells in proliferating state were further confirmed by BrdU labeling to stain the nucleus in brown. As shown in Fig. 4E,F, K-562 and MCF-7 cells in microcapsule maintained their proliferation potential after 10 d of cultivation, and most of the proliferating cells distributed in the outer layer of the cell spheroid with a brown nucleus.

Cell Growth-Related Protein Expression

The viability, proliferation, function, and apoptosis of human MCF-7 breast cancer cells grown in liquefied microcapsule were further examined by immunohistochemistry (IHC).

Cell growth and viability in microcapsule is compromised by oxygen deprivation (hypoxia) owing to the mass transfer barrier in the membrane of capsule. In response to hypoxia, HIF-1α should be upregulated. From Fig. 5A, one can see that only a few cells expressed HIF-1α, and even some cells in the center of the cell spheroid, where the hypoxia area was supposed to be, did not express HIF-1α, especially in the smaller microcapsule. It is suggested that the MCF-7 cell spheroid formed inside the microcapsule was a highly organized cell community that could deliver oxygen through cell–cell interaction.

Cell proliferation is controlled at specific stages of the cell cycle. Cyclin D1 is one of the G1 cyclins and is expressed in breast cancer cells favoring cell proliferation. Human MCF-7 breast cancer cells grown in microcapsule expressed cyclin D1 that was localized near the center of the cell spheroid (Fig. 5B). This further confirmed that human MCF-7 cells grown in microcapsule retained the proliferation potential.

Tumorigenesis is one of the important functions of tumor cells. VEGF, which is produced by tumor cell lines in vitro, was selected as a marker for the function of human MCF-7 breast cancer cells to facilitate tumor progression. As shown in Fig. 5C, most of the MCF-7 cells grown in microcapsule widely expressed VEGF whether the cells were located in the periphery or in the center.

Cell apoptosis was studied by p53 tumor suppressor gene expression. Overexpression of p53 can induce either cell-cycle arrest or apoptosis. The expression of p53 was not observed in MCF-7 cells grown in microcapsule (Fig. 5D). It may be interpreted that only mutant type p53 could be detected by IHC generally. MCF-7 cells represent the early phase of breast carcinoma and express wild-type p53, so a positive result did not appear in microencapsulated MCF-7 cells. Perhaps MCF-7 cells in the cell spheroid did not undergo apoptosis after 10 d of cultivation.

Cell Metabolism

The growth of mammalian cells requires nutrition, including glucose and oxygen, and produces waste, such as lactate. At the time of cultivation, the concentration of glucose is decreased whereas the concentration of lac-

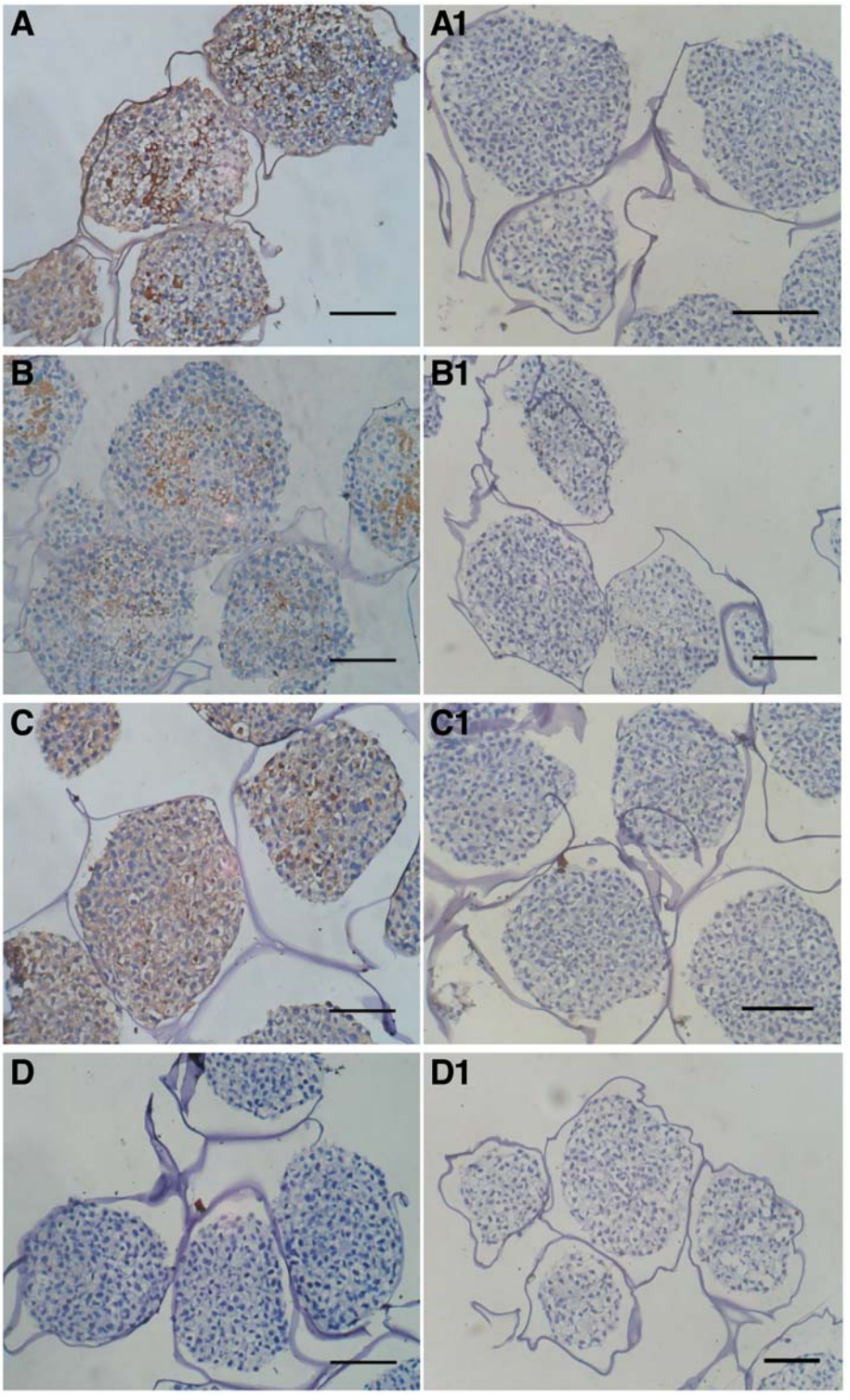

Fig. 5. Immunohistochemical staining of MCF-7 cells in APA microcapsule at d 14 revealed the expression of **(A)** HIF-1α, **(B)** cyclin D1, and **(C)** VEGF in brown but **(D)** no expression in p53: **(A1–D1)** control groups. Bars = 100 μm.

tate is increased. Usually, cell metabolism is measured by the rate of glucose consumption and lactate production. For human MCF-7 cells, the metabolism rate of cells grown in microcapsule with liquefied core was similar to that in gelled core, which was lower than that of cells in conventional monolayer culture (Fig. 6A). For human K-562 cells, the metabolism rate of cells grown in microcapsule with liquefied core was higher than that in gelled core, which was lower than that of cells in conventional monolayer culture (Fig. 6B). The glucose consumption and lactate production curve of human MCF-7 and K-562 cells was correlated with the cell viability curve measured by MTT assay (Fig. 3A,B). For human fibroblasts, the glucose consumption rate in microcapsule with liquefied core was similar to that in gelled core but was a little lower than that grown in monolayer, and the lactate production rate was the same in all three different types of culture.

Discussion

APA microencapsulation is one of the most used encapsulation technologies for in vitro cell culture and in vivo cell therapy. In general, cells in alginate solution are first dropped into a $CaCl_2$ bath to form alginate gel beads and then incubated with poly-L-lysine solution to form poly-L-lysine-alginate membrane, followed by incubation with sodium citrate to liquefy the alginate core inside the capsule membrane. Entrapment of cells in alginate gel beads is widely used in cell culture and tissue engineering. It has been reported that alginate-agarose microcapsules with liquefied core are more mechanically fragile than those with solid gelled core *(17)*. Thus, the question is raised: Is it necessary to liquefy the microcapsule for cell culture? Basically, microcapsules with or without liquefying provide two different types of microenvironment for cell growth. With liquefying, microcapsule provides a semimembrane to allow nutrition to flow in and metabolites to flow sout and liquefied core leaves cells to float freely in the microcapsule. Without liquefying, microcapsule provides a semimembrane and solid gel-like core that immobilizes the cells inside the network of the gel matrix. To our knowledge, the present study is the first to explore the effect of these two types of microenvironment on cell growth, viability, proliferation, function, and apoptosis of anchorage-dependent and anchorage-independent cells.

The human MCF-7 breast cancer cell line was used as a model for anchorage-dependent tumor cells. In conventional monolayer culture, human MCF-7 cells attach to the surface of the plate and stretch out first,

Fig. 6. *(opposite page)* Glucose consumption and lactate production of **(A)** human MCF-7, **(B)** K-562, and **(C)** fibroblast cells cultured in conventional culture, microcapsule with liquefied core, or microcapsule without liquefied core. Each point represents the mean of four wells, and each assay was performed in triplicate. Errors bars are the standard deviation of the mean (SEM) and are shown when greater than the size of symbols (*$p < 0.05$).

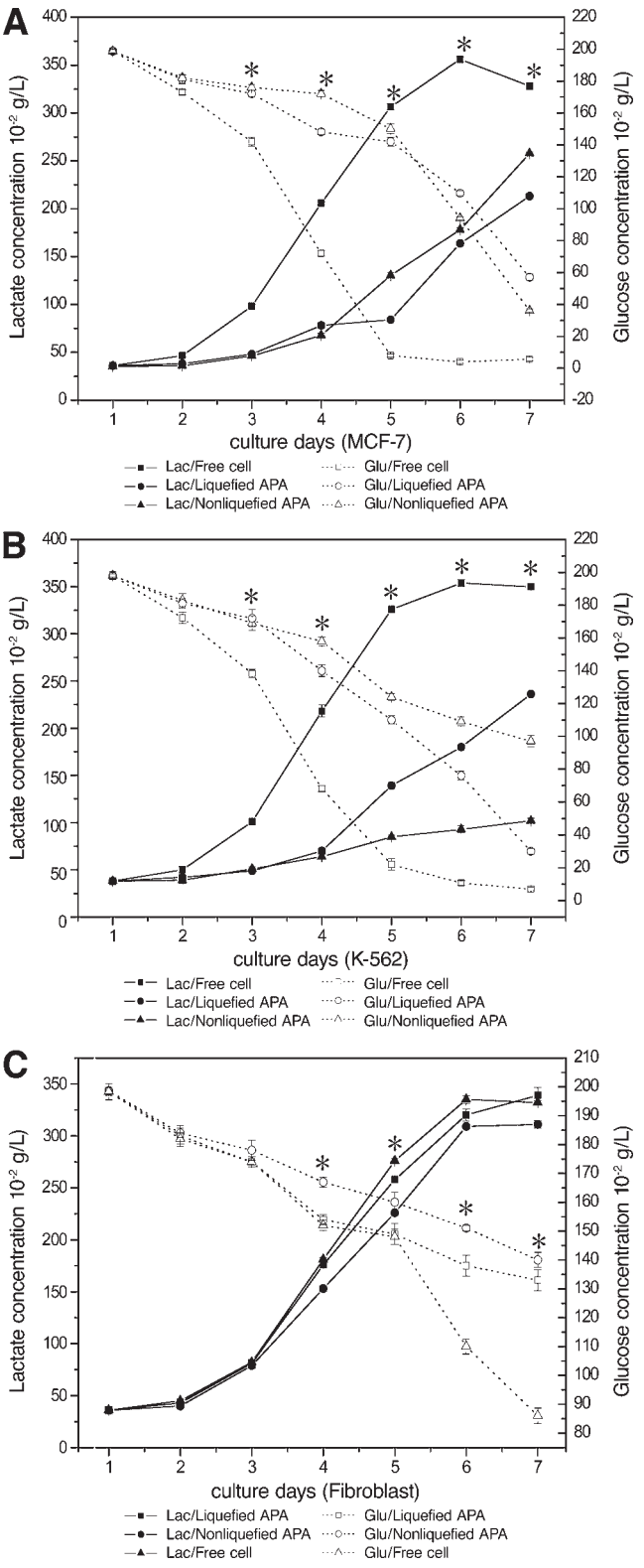
A
Lactate concentration 10-2 g/L)
Glucose concentration 10-2 g/L)
culture days (MCF-7)
Lac/Free cell
Lac/Liquefied APA
Lac/Nonliquefied APA
Glu/Free cell
Glu/Liquefied APA
Glu/Nonliquefied APA
B
Lactate concentration 10-2 g/L)
Glucose concentration 10-2 g/L)
culture days (K-562)
Lac/Free cell
Lac/Liquefied APA
Lac/Nonliquefied APA
Glu/Free cell
Glu/Liquefied APA
Glu/Nonliquefied APA
C
Lactate concentration 10-2 g/L)
Glucose concentration 10-2 g/L)
culture days (Fibroblast)
Lac/Liquefied APA
Lac/Nonliquefied APA
Lac/Free cell
Glu/Liquefied APA
Glu/Nonliquefied APA
Glu/Free cell

Fig. 6.

then engender a cobblestone pattern after confluence. Liquefied microcapsule facilitated human MCF-7 cells to form a single large aggregate floating in the center of the microcapsule. By contrast, human MCF-7 cells formed many small aggregates filled uniformly in the microcapsule with solid gel-like core (Fig. 1). There was no significant difference in cell viability and proliferation and metabolism rate of cells grown between the two types of microcapsule (Figs. 3A and 6A). Microencapsulated cell culture retained a lower metabolism rate and maintained the cell viability and proliferation potential constantly after 7 d of cultivation, superior to conventional culture. This suggests that microcapsule provides a 3D microenvironment for cell organization to facilitate cell–cell interaction and communication and maintain cell viability, whereas cells grown in monolayer lack the 3D cell connection to retain cell viability. The viability and proliferation of cells in liquefied microcapsule were confirmed by H&E staining, live/dead staining, and BrdU labeling (Fig. 4). The human MCF-7 cells in liquefied microcapsule were organized into a cell spheroid of about 200 μm in diameter, which is similar to a "multicellular tumor spheroid," in which the proliferating and viable cells are focused on the margin of the cell spheroid. The lower expression of HIF-1α in smaller microcapsule and the expression pattern in larger microcapsule demonstrate that human MCF-7 cell spheroid formed in liquefied microcapsule could facilitate oxygen transfer itself in spite of the transfer barrier of the microcapsule membrane (Fig. 5A). Microencapsulated human MCF-7 breast cancer cells express tumor-specific functional marker of cyclin D1 and VEGF, which is similar to the expression of tumor in vivo (Fig. 5B,C). These data demonstrate that the microenvironment provided by microcapsule is suitable for anchorage-dependent human MCF-7 tumor cells to grow, proliferate, and function. Microencapsulation may be a useful tool to form tumor cell spheroid in controlled size for anticancer drug screening. In addition, microencapsulation of tumor cells has potential for use in tumor cell-based gene therapy.

Human embryonic skin fibroblasts are another example of anchorage-dependent cells. They are primary cells from normal human tissue, which is usually used as a feeder layer for stem cell maintenance. Human fibroblasts in liquefied microcapsule formed a clump after 4 h of cultivation (Fig. 2). However, the proliferation rate was much lower than that of tumor cells. This could mean that fibroblasts from human skin excrete many more extracellular matrix molecules, accelerating cell adherence and aggregation with a diploid growth state of tardiness in vitro. For the first 3 d of cultivation, the viability of cells in microcapsule with or without liquefying was the same as for cells grown in monolayer. After 5 d of cultivation, the viability and proliferation of human fibroblasts in liquefied microcapsule was higher than that in microcapsule with gelled core, but much less than that in conventional monolayer culture (Fig. 3C). It is suggested that the microenvironment of APA microcapsule is not favorable for long-term human embryonic skin fibroblast culture, which is consistent with the findings of Shimi et al. *(18)*. This may be owing to the fact that primary fibro-

blasts prefer to grow in monolayer, unlike tumor cells, which prefer to grow in multilayers.

The human K-562 leukemia cell line was used as a model for anchorage-independent tumor cells. Conventionally, human K-562 cells were cultured in suspension with roundness and brightness. The liquefied microcapsule promoted anchorage-independent human K-562 cells to form a large cell aggregate (Fig. 1). Different from human MCF-7 cells, K-562 cell aggregates preferred the microcapsule membrane rather than the center of the microcapsule. The gelled core of microcapsule prevented K-562 cells from forming large aggregates. The cell viability of human K-562 cells in liquefied microcapsule was higher than that in microcapsule with gelled core (Fig. 3B). The possible reason is that cells in microcapsule with liquefied microcapsule more closely resemble those in suspension than in microcapsule with gelled core. Originally, the viability of cells grown in microcapsule with liquefied core was lower than that of cells grown in suspension, probably because of the nutrition limitation in microcapsule, but after reaching a maximum on day 4, the viability of K-562 cells in suspension decreased whereas that in liquefied core maintained some level owing to the formation of cell aggregate in microcapsule. Although the cell junction was loose in microcapsule for human K-562 cells (Fig. 4A), most of the cells inside a large cell aggregate remained viable and healthy (Fig. 4C), and in the proliferating state (Fig. 4E).

Conclusion

Cells in APA microcapsule are alive and have proliferation potential with a lower metabolism rate. APA microcapsule with liquefied core provides a microenvironment for both anchorage-dependent and anchorage-independent cells to grow into a large cell aggregate and maintain the viability of tumor cells at constant level for a period of time. Recently, we also attempted to make an anticancer drug screening test using microencapsulated tumor cells based on relative research and found that APA microcapsule may be a useful tool for in vitro tumor cell modeling and anticancer drug screening as well as for in vivo cancer gene therapy *(19).* In addition, the difference in the behavior of anchorage-dependent cells between human MCF-7 cells and fibroblasts suggests that it is essential to select a suitable cell line for secretion of the therapeutic product when using a microcapsule strategy for cell implantation or cell-based gene therapy.

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References

1. Lim, F. and Sun, A. M. (1980), *Science* **210(21),** 908–910.
2. Sambanis, A. (2003), *Diabetes Technol. Ther*. **5(4),** 665–668.
3. Sun, T., Chan, M. L., Zhou, Y., et al. (2003), *Tissue Eng*. **9(Suppl. 1),** S65–S75.
4. Basta, G., Sarchielli, P., Luca, G., (2004), et al. *Transpl. Immunol.* **13,** 289–296.
5. Luca, G., Basta, G., Calafiore, R., Rossi, C., Giovagnoli, S., Esposito, E., and Nastruzzi, C. (2003), *Biomaterials* **24,** 3101–3114.
6. Marc, R. G., Robert, C. H., and Emmanuel, C. O. (1998), *J. Surg. Res*. 1998; **76,**7 –10.
7. Ross, C. J. D., Ralph, M., and Chang, P. L. (2000), *Exp. Neurol*. **166,** 276–286.
8. Tadanobu, U., Henry, B., Josef, P., Thorsteinn, L., Thordis, K., Betty, M. T., and Alessandro, O. (1996), *J. Control. Release* **40,** 251–260.
9. Ma, X. J., Vaccie, I., and Sun, A. M. (1994), *Art Cells Blood Subs. Immob. Biotech*. **22(1),** 43–69.
10. Bjerkvig, R., Read, T. A., Vajkoczy, P., et al. (2003), *Acta Neurochir. Suppl*. **88,** 137–141.
11. Aristides, D. T., Ivan, A. D., Ian, R. G., et al. (2005), *BBA – Mol. Cell. Biol. L* **1686,** 190–199.
12. Barsoum, S. C., Milgram, W., Mackay, W., et al. (2003), *J. Lab. Clin. Med.* **142,** 399–413.
13. Patricia, L. C. (1996), *Transfus. Sci*. **17,** 35–43.
14. de Haan, B. J., Faas, M. M., and de Vos, P. (2003), *Cell Transplant*. **12(6),** 617–625.
15. Molly, M. S., Hala, F. Q., Robert, L., and Shastri, P. V. (2004), *Biomaterials* **25,** 887–894.
16. Hasan, U. and Michael, V. S. (1990), *Biomaterials* **11(11),** 708–712.
17. Orive, G., Hernandez, R. M., Gascon, A. R., Igartua, M., and Pedraz, J. L. (2003), *Eur. J. Pharm. Sci*. **18,** 23–30.
18. Shimi, S. M., Hopwood, D., Newman, E. L., and Cuschieri, A. (1991), *Br. J. Cancer* **63,** 675–680.
19. Zhang, X. L., Wang, W., Yu, W. T., Xie, Y. B., Zhang, X. H., Zhang, Y., and Ma, X. J. (2005), *Biotechnol. Prog*. **21,** 1289–1296.