View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by AJOL - African Journals Online

African Journal of Biotechnology Vol. 10(61), pp. 13230-13237, 10 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1980
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Adipose tissue-derived stem cells in oral mucosa tissue engineering: Enhanced migration and proliferation in co-culture with oral keratinocytes *in vitro*

Qin Zhang, Yue-Min Xu*, Hong-Bin Li, Min-Kai Xie and Ji-Hong Wang

Shanghai Sixth People's Hospital, Medical College, Shanghai Jiaotong University, Shanghai, 200233, PR China.

Accepted 5 September, 2011

Tissue-engineered oral mucosa holds a great prospect in urethroplasty and adipose tissue-derived stem cells (ADSCs) may play an important role in this field. In this research, canine oral keratinocytes (OKs) and ADSCs were harvested and cultured *in vitro*. The affinity between the two cell lines was evaluated by analyzing their migration and proliferation patterns in a co-culture environment. The results demonstrate that both canine ADSCs and OKs showed improved migration in the presence of the other cell line as a co-culture when compared to monoculture. Further, conditioned medium using the supernatant of one cell line accelerated the other cell line's proliferation rate. Hence, it was concluded that the affinity between OKs and ADSCs was fitting; the presence of ADSCs accelerated the migration and proliferation of OKs *in vitro.* These results indicate that it is practical to use ADSCs and OKs to construct a tissue-engineered oral mucosa, since the presence of the former could activate the latter *in vitro*, maybe even *in vivo*. This may help to build tissue-engineered oral mucosa, which may be a new method for urethroplasty.

Key words: Urethroplasty, adipose tissue-derived stem cells, oral keratinocytes, tissue engineering.

INTRODUCTION

Defects of the male urethra can be repaired with genital or extragenital skin, bladder mucosa, oral mucosa and colonic mucosa (Xu et al., 2007, 2009; Markiewicz et al., 2007a). In the past few years, oral mucosal grafts (OMGs) have been a popular and reliable substitute used in urethroplasty (Xu et al., 2007, 2009; Markiewicz et al., 2007a). OMGs obviate most of the problems associated with other graft harvesting, including ease of access and a concealed donor site scar (Markiewicz et al., 2007a, 2007b). However, the harvest of OMGs is associated with oral complications such as numbness, tightness of the mouth and motor deficits (Jiang et al., 2005; Dublin and Stewart, 2004; Markiewicz et al., 2008). The emergence of tissue engineering and the repair and regeneration of damaged organs using a combination of cells, biomaterials and growth factors brings new hope to urethral reconstruction. Specifically, tissue-engineered oral mucosa holds great prospect for urethroplasty.

Mesenchymal stem cells within the stromal-vascular fraction of subcutaneous adipose tissue, that is, adipose tissue-derived stem cells (ADSCs), have been used in skin repair with satisfactory results. Although evidence of ADSCs impacting the differentiation of keratinocytes *in vivo* has not been reported, ADSCs accelerate wound healing and exhibit antioxidant effects under various conditions (Kim et al., 2007, 2008; Park et al., 2008). Through a paracrine mechanism, ADSCs produce and secrete growth factors such as basic fibroblast growth factor (b-FGF), keratinocyte growth factor (KGF), transforming growth factor (TGF-β), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF). These growth factors activate keratinocytes and accelerate the repair of skin injury (Kim et al., 2007, 2008; Park et al., 2008). To define the future of ADSCs in oral mucosa tissue engineering for urethroplasty, we assessed the affinity between ADSCs and oral

*Corresponding author. E-mail: xuyuemin2006@yahoo.com.cn. Tel: +86-21-64369181-58372. Fax: +86-21-67647129.

Abbreviations: ADSC, Adipose tissue-derived stem cell; **OK,** oral keratinocyte; **OMG,** oral mucosal graft; **PBS,** phosphate buffer saline.

keratinocytes (OKs) and investigated whether ADSCs activate OKs *in vitro*.

MATERIALS AND METHODS

Canine ADSCs

The experimental protocols were approved by the Animal Care and Use Committee at our institution. The adipose tissue was taken from a 1.5 years old Beagle canine under intravenous anesthesia with remifentanil and propofol. A piece of subcutaneous inguinal adipose tissue about 3 cm × 2 cm × 1 cm in size was carefully dissected through a 3 cm longitudinal incision in the right groin area, which was intermittently sutured with 3-0 silk sutures after the harvest. Antibiotics were administrated intravenously for four days postoperatively to prevent infection. Then the adipose tissue was transferred to a clean bench immersed in sterile phosphate buffer saline (PBS). After two rinses in PBS, the visible small blood vessels were removed with eye scissors, and the remaining tissue was cut into small pieces and digested in 0.1% collagenase I (Worthington, USA) at 37°C for 1 h, with uninterrupted shaking. The suspension was filtered through a 200-μM nylon mesh to separate single cells from undigested tissue. The undigested tissue was discharged and the digestion solution was centrifuged at 2000 r/min for 10 min to isolate the ADSCs. The isolated ADSCs were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution (ADSC medium) at 10^5 cells/100-mm dish at 37°C with 5% CO_2 (Li et al., 2008). Upon reaching 80 to 90% confluence, the isolated cells were trypsinized, counted, and subcultured at a density of 10^5 cells/100-mm dish as passage 1. The cells used for study were passage 3.

For cytometric analysis, the passage 3 ADSCs (approximately 5 × 10^5 cells) were incubated with 10 μl of individual IO test monoclonal primary antibodies coupled to either phycoerythrin (PE) or fluorescein isothiocyanate (FITC) in 50 μl PBS for 30 min in the dark at room temperature. The cells were washed twice with 1 ml PBS, resuspended with PBS, diluted in 200 μl PBS and analyzed with a FACScan flow cytometer (Becton–Dickinson). Data were acquired using CellQuest Pro software and analyzed using FlowJo software. Antibodies for CD34, CD44, CD45, CD90, CD105 and CD106 were obtained from BD Biosciences (Franklin Lakes, NJ, USA).

Canine OKs

A piece of oral mucosa approximately 3 cm × 1 cm in size was harvested from the lower lip of the Beagle canine mentioned above at the same time, and the wound was sutured with 4-0 silk sutures. Then the mucosa was transferred to the clean bench immersed in sterile PBS. The dermis was removed with an eye scissors and the mucosa was cut into 3 strips approximately 0.3 cm × 3 cm in size. The mucosa strips were rinsed with PBS supplemented with 1000 U/ml penicillin and 20 U/ml gentamicin followed by digestion in 0.22% dispase II enzyme at 4°C for 16 h. The epidermis was easily separated from the digested mucosa. The oral keratinocytes (OKs) were harvested from the epidermis, which was digested in a solution containing 0.05% trypsin and 0.02% EDTA (Gibco) at 37°C for 15 min. The isolated OKs were cultured in keratinocyte serum free medium (KSFM) supplemented with 50 μg/ml bovine pituitary extract and 5 ng/ml epidermal growth factor (OK medium) at a density of 10^5 cells/100-mm dish at 37°C with 5% CO_2. For expansion, the OKs were cultured and re-fed every three days until they reached 85 to 95% confluence. Then the OKs were trypsinized, counted, and subcultured at a density of 10^5 cells/100-mm dish as passage 1. The cells used for study were passage 2. Immunofluorescence was performed on methanol-fixed cells using mouse monoclonal cytokeratin AE1/AE3 antibodies (Sigma-Aldrich). After incubation with the primary antibodies for 60 min at room temperature, the slides were washed three times with PBS. FITC-conjugated goat anti-mouse secondary antibody (Millipore) was used as a secondary antibody. Cell nuclei were stained with Hoechst and the slides were viewed on a fluorescence microscope (Nikon, Japan). Flow cytometry, as earlier described for ADSCs, was performed on OKs to detect the expression of cytokeratin AE1/AE3.

Seeding and cell migration

Equal numbers (5 × 10^5 cells) of ADSCs and OKs were plated on scaled Petri dishes (Nalge Nunc International, Rochester, NY), either alone (monoculture) or together (co-culture). The cells were plated just behind the starting line in 1.5 ml of medium and allowed to settle on the dish for 6 h, after which the excess media and un-adhered cells were removed. Care was taken to ensure that all the cells were close to the start line, and successful plating was confirmed by microscopy. The cells were cultured at 37°C, 5% CO_2 and 95% humidity after adding 10 ml of a 1:1 ratio of ADSC and OK medium. The media was changed every other day. The locations of the headmost cells at each of the seven measure lines were recorded everyday (Figure 1), and the mean of these seven measurements was adopted as migration distance. The monocultures were also grown in mixed medium as a control.

Proliferation of ADSCs and OKs in a simulated co-culture environment

When the ADSCs cultures reached more than 50% confluence, the supernatant was collected every day, stored at 4°C and filtered (0.22-μm pore; Millipore Corp). The filtrate and OK medium were mixed at a 1:1 ratio to make conditioned medium from ADSCs cultures (ADSC-CM). Similarly, the supernatant from OK cultures was collected and mixed with ADSC medium to make conditioned medium from OK cultures (OK-CM). Equal numbers of ADSCs or OKs (1,500 cells/well) were seeded in 24 wells of a 96-well cell culture dish. The cells in 12 of the wells of each plate were fed conditioned medium, while the cells in the other 12 wells were fed ADSC or OK medium. The microculture tetrazolium (MTT) assay was performed daily for eight days according to the protocol of the MTT kit (Sigma-Aldrich). Proliferation curves for each cell line in the two media conditions were generated.

RESULTS

The groin wound had no influence on the mobility of the Beagle canine. Similarly, the mouth wound brought no eating disorder to the Beagle canine. The wound healing was achieved without any signs of infection in six days in the mouth, and in ten days in the groin. More also, after incubation for about seven days, the ADSC population covered approximately 90% of the culture plate. The cells were fusiform, exhibited a spindle-shaped morphology (Figure 2) and could subculture more than 10 generations without any signs of ageing. Flow cytometry analysis showed expression of CD90 (96.13%), CD34 (37.19%), CD44 (98.62%) and CD105 (63.75%) in ADSCs. There was however, no significant expression of CD45 (1.73%)

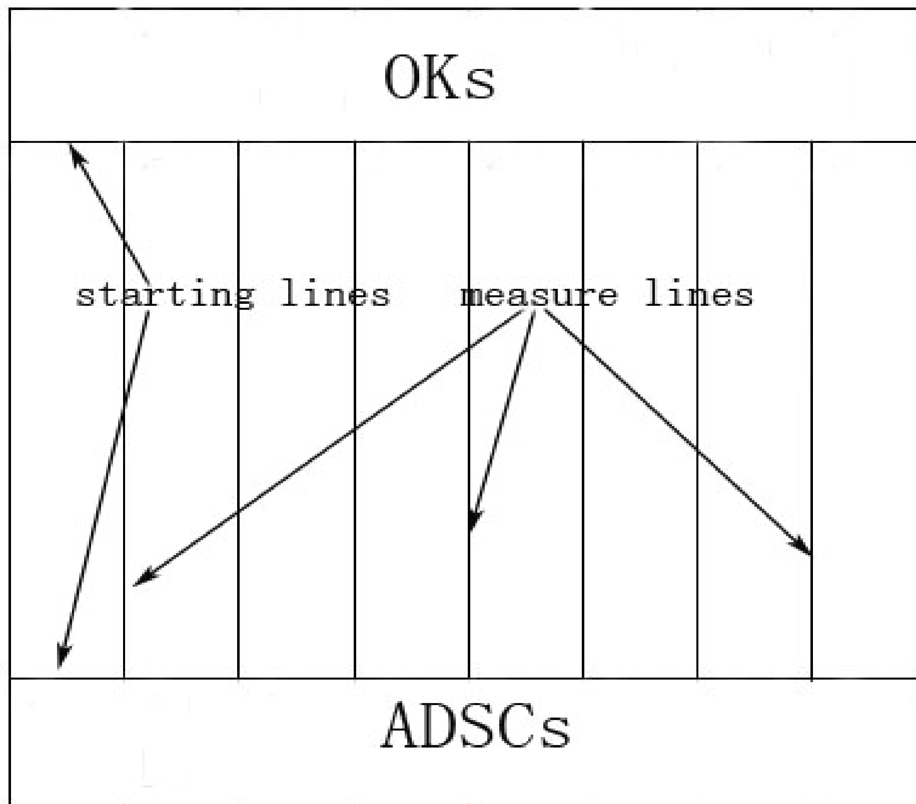

Figure 1. The migration of OKs and ADSCs in a gridded dish; the cells were plated at the starting lines and the location of the headmost cells at each of the seven measure lines was measured every day.

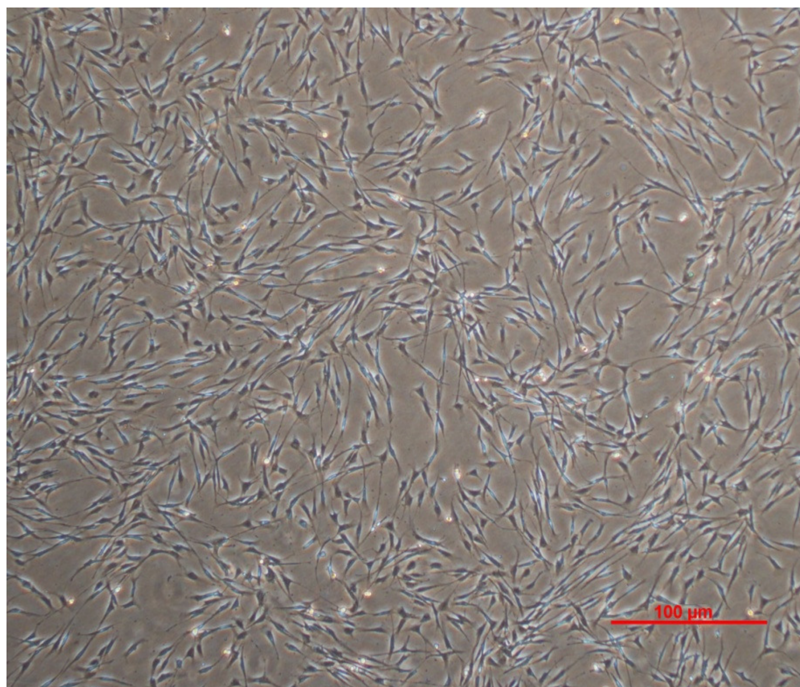

Figure 2. Morphology of ADSCs; the ADSCs were fusiform and exhibited spindle-shaped morphologies (bar =100 µM).

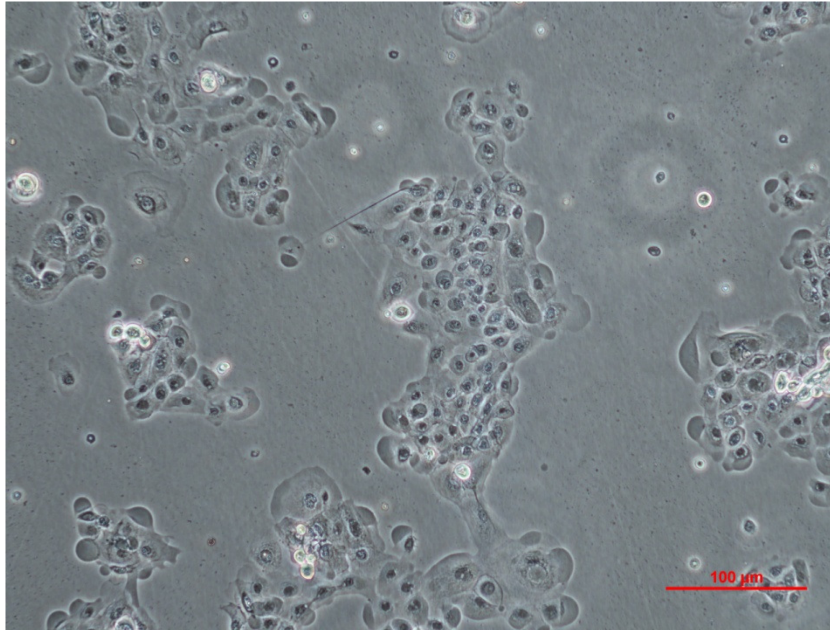

Figure 3. Colonies consisting of passage 0 OKs four days after incubation (bar = 100 μM).

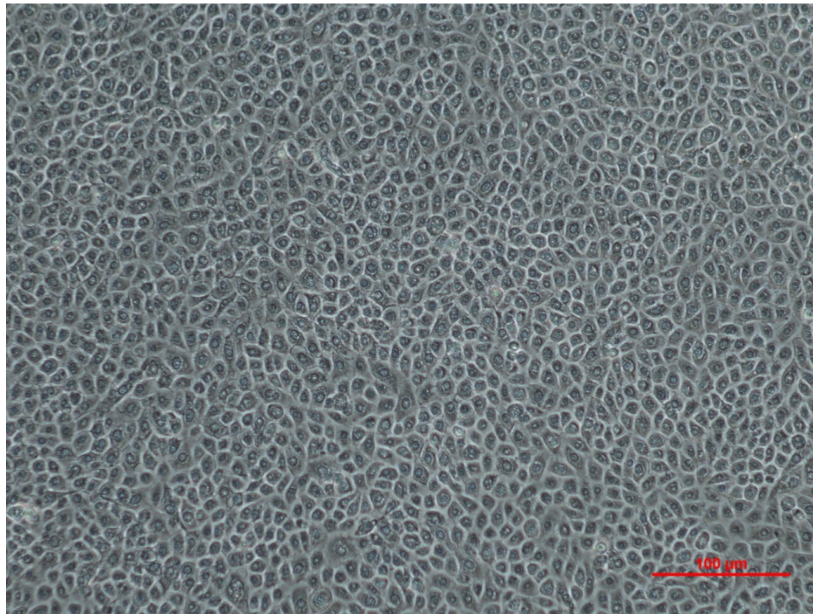

Figure 4. Morphology of OKs; the OKs exhibited characteristic ccobblestone-like morphology (bar = 100 μM).

or CD106 (1.85%).

The OKs were polygonal and exhibited a characteristic cobblestone-like morphology (Figures 3 and 4) and could be subcultured four to five generations before ageing. It took nearly two weeks to cover 90% of the culture plate. Immunofluorescence staining indicated cytokeratin AE1/AE3 expression in the OKs (Figure 5) and flow cytometry results indicate that the expression rate of cytokeratin AE1/AE3 was 98.25%. Moreover, ADSCs grown alone exhibited a robust basal migration rate of between 0.8 and 5.5 mm/day compared with OKs, which had a migration rate of 0 to 1.5 mm/day. The migration rate of ADSCs was enhanced when co-cultured with OKs from 0.8 to 6.8 mm/day. Likewise, the migration of OKs

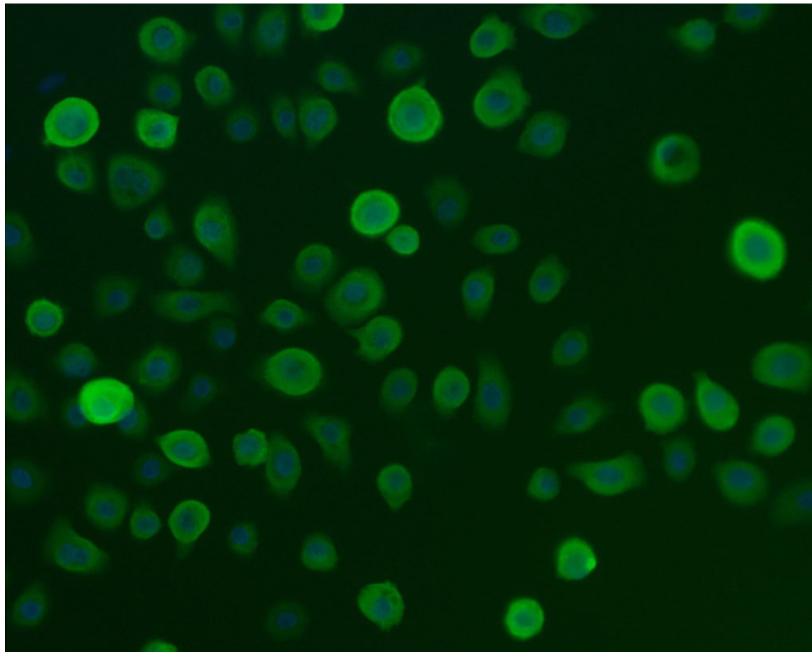

Figure 5. Immunofluorescence staining verifying cytokeratin AE1/AE3 expression in OKs. Cell nuclei were stained with Hoechst.

was increased from 0.2 to 2.5 mm/day in the presence of ADSCs (Table 1 and Figure 6). The two cells lines approached each other in the middle of the plate in approximately ten days, at which point the experiment ended.

In addition, the OD values obtained with the MTT assay of OKs cultured in ADSC-CM increased more rapidly than values obtained from OKs cultured in OK medium. Similarly, the OD values of ADSCs increased more rapidly when ADSCs were cultured in OK-CM than when they were cultured in ADSC medium (Table 2 and Figure 7).

DISCUSSION

The treatment of complex urethral stricture remains one of the most challenging problems in urology, although a lot of methods have been reported to date (Xu et al., 2007, 2009; Markiewicz et al., 2007a). Oral mucosa graft (OMG) has been a wide-accepted substitute used in urethroplasty because of its easy accessibility and a concealed donor site scar. However, OMG harvest is associated with oral complications such as numbness, tightness of the mouth and motor deficits. The emergence of tissue engineering brings new hope for the reconstruction of urethra (Markiewicz et al., 2007b).

Tissue engineering combines the principles and methods of the life sciences with those of engineering, to develop an approach to repair damaged tissue and replace entire tissue structures. During the last decade, tissue engineering using biodegradable materials seeded with cells for urogenital reconstruction has become increasingly common. In our laboratory, epidermal cell-seeded bladder collagen matrix and oral keratinocyte-seeded bladder acellular matrix have been used in urethroplasty in animal studies (Fu et al., 2007; Li et al., 2008). In another study, even tissue-engineered oral mucosa has been studied in urethroplasty clinic, although the results were not satisfactory (Saurabh et al., 2008).

Adipose tissue is derived from embryonic mesodermal precursors and contains multipotent progenitor cells capable of differentiating into mesenchymal tissue (Zuk et al., 2002).The harvest of ADSCs is easier than other types of adult stem cells. Adipose tissue contains 100 to 1,000 times more pluripotent cells on a per-cubic centimeter basis than bone marrow (Rangappa et al., 2003). As multipotential cells, they can differentiate into other mature cell types, such as chondrocytes, osteocytes (Zuk et al., 2002; Fraser et al., 2006), cardiomyocytes (Rangappa et al., 2003), neurons (Ashjian et al., 2003) and smooth muscle cells (Rodríguez et al., 2006). In addition, there has been a report suggesting that ADSCs are immune-privileged both *in vitro* and *in vivo* (Rodriguez et al., 2005), enabling their use as universal donor cells for allogenic transplant. For these reasons, we selected ADSCs to investigate their potential for use in oral mucosa tissue engineering.

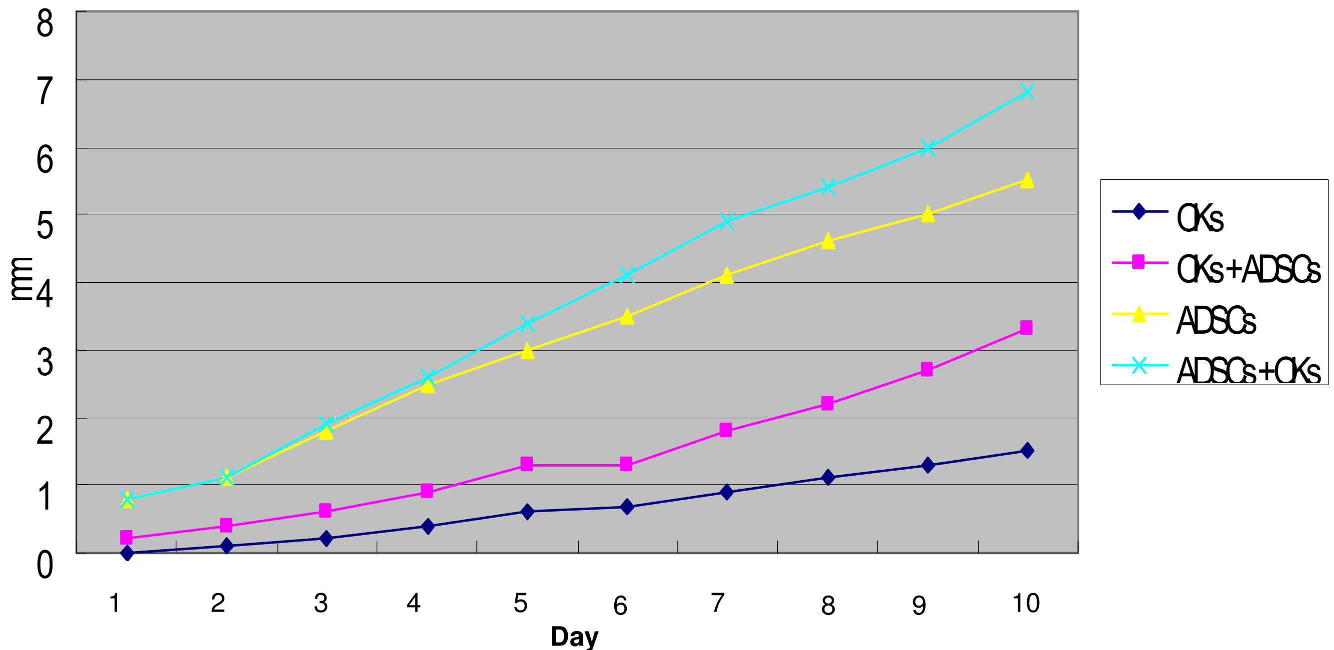

Figure 6. Migration distance (in millimeters) of OKs and ADSCs plated alone and in co-culture measured daily for ten days. The tabulated data illustrates the daily migration distance. OKs + ADSCs is the migration of OKs towards ADSCs in a co-culture environment; ADSCs + OKs is the migration of ADSCs towards OKs in a co-culture environment.

Table 1. Values (mm) of migration distance of OKs and ADSCs plated alone and in co-culture as depicted in Figure 6.

Culture	Day									
	1	2	3	4	5	6	7	8	9	10
OKs	0	0.1	0.2	0.4	0.6	0.7	0.9	1.1	1.3	1.5
OKs + ADSCs	0.2	0.4	0.6	0.9	1.3	1.3	1.6	1.9	2.1	2.5
ADSCs	0.8	1.1	1.8	2.5	3.0	3.5	4.1	4.6	5.0	5.5
ADSCs + OKs	0.8	1.1	1.9	2.6	3.4	4.1	4.9	5.4	6.0	6.8

Since the ADSC phenotype is similar to that of the fibroblast, it is difficult to characterize ADSCs based solely on their morphology in culture. In our study, flow cytometry analysis demonstrated that canine derived ADSCs expressed high levels of stem cell-related antigens (CD90, CD44, CD34, CD105), but do not express CD45 or CD106. Our results are similar to previous reports (Mitchell et al., 2006) suggesting that the population of ADSCs are mesenchymal stem cells. In previous reports (Kim et al., 2009), ADSCs exhibited wound-healing and anti-oxidant effects. The production and secretion of growth factors such as b-FGF, KGF, TGF-β, HGF and VEGF, has been reported to be an essential function of ADSCs. Through a paracrine mechanism, these growth factors may stimulate both collagen synthesis and migration of dermal fibroblasts, and activate dermal fibroblasts and keratinocytes, resulting in accelerated wound-healing in animal models. Also, ADSCs can accelerate the vascularization of tissue-engineered grafts used in the repair of skin defects (Liu et al., 2011). In skin tissue engineering, ADSCs are advantageous not only for the production of bilayered skin substitutes, but also for the reconstruction of trilayered skin substitutes featuring the hypodermal compartment (Trottier et al., 2008).

In view of all these studies, we expect that ADSCs may be useful in oral mucosa tissue engineering for urethraplasty. The presence of ADSCs may activate oral keratinocytes seeded on a scaffold, accelerate the neovascularization of the tissue-engineered graft and reconstruct oral mucosa substitutes that are similar to the oral mucosa in both morphology and function. However,

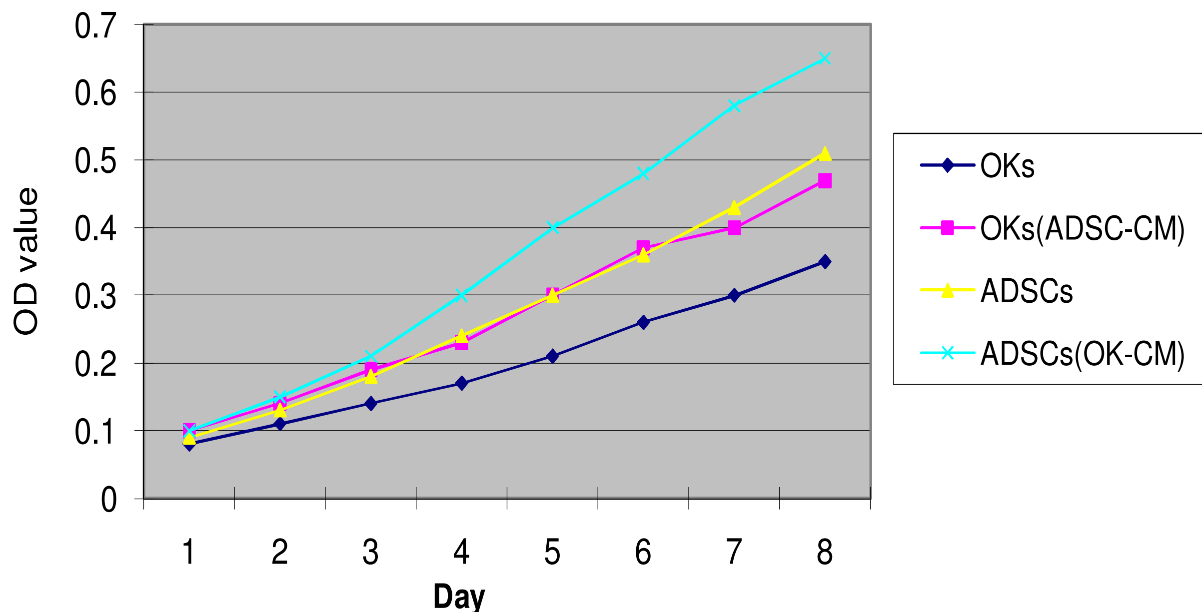

Figure 7. OD values of OKs and ADSCs in normal culture medium and conditioned medium quantitated with the MTT assay. OKs (ADSC-CM) represents the OD values of OKs cultured in conditioned medium from the ADSCs cultures; ADSCs (OK-CM) represents the OD values of ADSCs cultured in conditioned medium from the OKs cultures.

Table 2. OD values of OKs and ADSCs in normal culture and conditioned medium as quantified by MTT assay depicted in Figure 7.

Culture	Day							
	1	2	3	4	5	6	7	8
OKs	0.08	0.11	0.14	0.17	0.21	0.26	0.30	0.35
OKs (ADSC-CM)	0.10	0.14	0.19	0.23	0.30	0.37	0.40	0.47
ADSCs	0.09	0.13	0.18	0.24	0.30	0.36	0.43	0.51
ADSCs (OK-CM)	0.10	0.15	0.21	0.30	0.40	0.48	0.58	0.65

before ADSCs are to be used in oral mucosa tissue engineering, it is necessary to evaluate the affinity between OKs and ADSCs in an *in vitro* environment. Hence, in this study, we successfully isolated and expended OKs *in vitro*. The OKs were polygonal and exhibited characteristic cobblestone-like morphology (Figures 3 and 4). Flow cytometry and immune-fluorescence staining (Figure 5) indicated the expression of cytokeratin AE1/AE3 supporting our claims that we had, in fact, isolated keratinocytes. We also co-cultured ADSCs and OKs to determine whether the individual cell lines showed enhanced migration in a co-culture setting. The migration rates of ADSCs and OKs plated in monoculture were very slow but increased when placed under co-culture conditions. Because the two cell populations were not in direct contact, but grew in the same dish and shared the same medium, the enhanced migration we observed is thought to result from the chemotactic effects from soluble mediators. These results indicate that ADSCs and OKs have a strong growth attraction toward each other.

More also, to evaluate the proliferation rate of the two cell lines in co-culture conditions, we collected the supernatant of one cell line and added it to the other's culture dish to simulate the co-culture condition. When compared to monoculture, the proliferation rate of the OKs was obviously enhanced when the cells were exposed to conditioned medium from the ADSCs (ADSC-CM). Similarly, the proliferation rate of the ADSCs was enhanced when the cells were exposed to conditioned medium from OKs (OK-CM). Because the two cell lines were not in direct contact, the enhanced proliferation rate of the OKs is thought to result from soluble mediators secreted by ADSCs. It appears possible that the OKs can also secrete soluble mediators, therefore activating proliferation of ADSCs.

In this study, we focused on the affinity between OKs and ADSCs, which is necessary to be understood before

the two cell lines can be used in oral mucosa tissue engineering. Our study so far demonstrate that it is practical to use OKs and ADSCs to reconstruct tissue-engineered oral mucosa, which may be a new method for urethroplasty. Future studies will be directed toward constructing tissue-engineered oral mucosa with ADSCs, OKs and scaffold, and then using it in urethroplasty.

REFERENCES

Ashjian PH, Elbarbary AS, Edmonds B, DeUgarte D, Zhu M, Zuk PA, Lorenz HP, Benhaim P, Hedrick MH (2003). In vitro differentiation of human processed lipoaspirate cells into early neural progenitors. Plast. Reconstr. Surg. 111: 1922–1931.

Dublin N, Stewart LH (2004). Oral complications after buccal mucosal graft harvest for urethroplasty. Bju Int. 94: 867–869.

Fraser JK, Wulur I, Alfonso Z, Hedrick MH (2006). Fat tissue: an underappreciated source of stem cell for biotechnology. Trend Biotechnol. 24(4): 150–154.

Fu Q, Deng CL, Liu W, Cao YL (2007). Urethral replacement using epidermal cell-seeded tubular acellular bladder collagen matrix. Bju int. 99: 1162-1165.

Jang TL, Erickson B, Medendorp A, Gonzalez CM (2005). Comparison of donor site intraoral morbidity after mucosal graft harvesting for urethral reconstruction. Urology, 66(4): 716–7120.

Kim WS, Park BS, Kim HK, Park JS, Kim KJ, Choi JS, Chung SJ, Kim DD, Sung JH (2008). Evidence supporting antioxidant action of adipose-derived stem cells: protection of human dermal fibroblasts from oxidative stress. J. Dermatol Sci. 49(2): 133-1342.

Kim WS, Park BS, Sung JH, Yang JM, Park SB, Kwak SJ, Park JS (2007). Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. J. Dermatol Sci. 48(1): 15-24.

Kim WS, Park BS, Sung JH (2009). The wound-healing and antioxidant effects of adipose-derived stem cells. Exp. Opin. Biol. Ther. 9(7): 879-887.

Li C, Xu YM, Song LJ, Fu Q, Cui L, Yin S (2008). Urethral reconstruction using oral keratinocyte seeded bladder acellular matrix grafts. J. Urol. 180(4): 1538–1542.

Liu S, Zhang H, Zhang X, Lu W, Huang X, Xie H, Zhou J, Wang W, Zhang Y, Liu Y, Deng Z, Jin Y (2011). Synergistic angiogenesis promoting effects of extracellular matrix scaffolds and adipose-derived stem cells during wound repair. Tissue Eng. 17(5-6): 725-739.

Markiewicz MR, Lukose MA, Margarone JE 3rd, Barbagli G, Miller KS, Chuang SK (2007a).The oral mucosa graft: a systematic review. J. Urol. 178(2): 387–394.

Markiewicz MR, Joseph E. Margarone III, Guido B, Frank AS (2007b).Oral mucosa harvest: an overview of anatomic and biologic considerations. EAU-EBU Update Series 5: 179–187.

Markiewicz MR, DeSantis JL, Margarone JE 3rd, Pogrel MA, Chuang SK (2008).
Morbidity associated with oral mucosa harvest for urological reconstruction: an overview. J. Oral. Maxillofac. Surg. 66: 739–744.

Mitchell JB, McIntosh K, Zvonic S, Garrett S, Floyd ZE, Kloster A, Di Halvorsen Y, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM (2006). Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells. 24(2): 376–385.

Park BS, Jang KA, Sung JH, Park JS, Kwon YH, Kim KJ, Kim WS(2008).Adipose-derived stem cells and their secretory factors as a promising therapy for skin aging. Dermatol. Surg. 34(10): 1323-1326.

Rodríguez LV, Alfonso Z, Zhang R, Leung J, Wu B, Ignarro LJ (2006). Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. Proc. Natl. Acad. Sci. USA. 103(32): 12167–12172.

Rangappa S, Fen C, Lee EH, Bongso A, Sim EK (2003). Transformation of adult mesenchymal stem cells isolated from the fatty tissue into cardyoiomcytes. Ann. Thorac. Surg. 75(3): 775–779.

Rodriguez AM, Pisani D, Dechesne CA, Turc-Carel C, Kurzenne JY, Wdziekonski B, Villageois A, Bagnis C, Breittmayer JP, Groux H, Ailhaud G, Dani C (2005). Transplantation of a multipotent cell population from human adipose tissue induces dystrophin expression in the immunocompetent mdx mouse. J. Exp. Med. 201: 1397–1405.

Saurabh B, Jacob MP, Richard DI, Sheila M, Christopher RC (2008). Tissue-engineered buccal mucosa urethroplasty-clinical outcomes . Eur. Urol. 53: 1263-1271.

Trottier V, Marceau-Fortier G, Germain L, Vincent C, Fradette J (2008). IFATS collection: using human adipose-derived stem/stromal cells for the production of new skin substitutes. Stem Cells, 26(10): 2713–2723.

Xu YM, Qiao Y, Sa YL, Wu DL, Zhang XR, Zhang J, Gu BJ, Jin SB(2007).Substitution urethroplasty of complex and longsegment urethral strictures: a rationale for procedure selection. Eur. Urol. 51(4): 1093–1098.

Xu YM, Sa YL, Fu Q, Zhang J, Si JM, Liu ZS (2009).Oral mucosal grafts urethroplasty for the treatment of long segmented anterior urethral strictures. World J. Urol. 27(4): 565-571.

Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH (2002). Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell. 13(12): 4279–4295.