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Nasal Fibroblast Conditioned Medium Promotes Cell Attachment and Migration of Human Respiratory Epithelium

(Medium Terkondisi Fibroblas Hidung Menggalakkan Perlekatan dan Migrasi Sel Epitelium Pernafasan)

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ABSTRACT

Endoscopic sinus surgery (ESS) is a well-known surgical treatment for chronic rhinosinusitis disease after failed medical and antibiotics treatment. However, improper wound healing might induce synechiae or adhesion. Conditioned medium from cultured cells is known to promote wound healing and potentially able to accelerate wound healing in ESS and other airway epithelial injuries. This study was to investigate the effect of human nasal fibroblast conditioned medium on the attachment, proliferation and migration of respiratory epithelial cells (RECs) in an in vitro *model. RECs and fibroblasts were co-cultured in Defined Keratinocytes Medium and F-12 and Dulbecco's Modified Eagle's Medium. Once confluent, the fibroblasts were removed, leaving the colonies of RECs to reach confluency. RECs and fibroblasts were cultured separately and the conditioned medium was acquired by culturing fibroblast either in DKSFM or F12: DMEM, denoted as NFCM_DKSFM and NFCM_FD, respectively. RECs were supplemented with 20% conditioned medium for attachment, proliferation and migration assay. The results showed significantly higher cell attachment in NFCM_ DKSFM (3452.77±588.1 cell/cm^2) compared to NFCM_FD (2336.1±440.4 cell/cm^2) and DKSFM alone (2819.8±509.5 cell/cm^2). After 7 days, the specific growth rate was higher in DKSFM (0.019±5.16×10^{-4} h^{-1}) compared to NFCM_DKSFM (0.015±8.94×10^{-4} h^{-1}) and NFCM_FD (0.013±1.03×10^{-3} h^{-1}). The mean of migration rate was significantly higher in NFCM_DKSFM (4341.81±385.7 μm^2/hr) compared to NFCM_FD (1803.38±408.1 μm^2/hr) and DKSFM (1933.48±271.9 μm^2/hr). Hence, NFCM_DKSFM supplementation provides suitable culture conditions for RECs through increased cell attachment and migration, which suggest that the factors secreted in conditioned medium may play a major role in enhancing airway epithelial wound healing.*

Keywords: Conditioned culture media; epithelial cells; fibroblast; wound healing

ABSTRAK

Pembedahan sinus endoskopi (ESS) adalah rawatan yang terkenal untuk penyakit rhinosinusitis kronik selepas kegagalan rawatan perubatan dan antibiotik. Walau bagaimanapun, penyembuhan luka yang tidak sempurna boleh menyebabkan synechiae atau lekatan. Medium terkondisi (MT) daripada sel yang dikultur didapati boleh menggalakkan penyembuhan luka dan berpotensi mempercepatkan penyembuhan luka disebabkan oleh ESS dan kecederaan lain pada epitelium saluran pernafasan. Kajian ini adalah untuk mengkaji kesan MT daripada fibroblas hidung manusia untuk perlekatan, percambahan dan migrasi sel epitelium pernafasan (RECs) dalam model in vitro*. RECs dan fibroblas telah dibiakkan dalam* Defined Keratinocytes Medium (DKSFM) *dan F-12 dan* Dulbecco's Modified Eagle's Medium (FD)*. Selepas konfluen, fibroblas ditanggalkan dan koloni RECs terus dikultur sehingga padat. RECs dan fibroblas dibiakkan secara berasingan dan MT diperoleh dengan mengkultur fibroblas sama ada dalam DKSFM atau FD, dengan dilabelkan sebagai NFCM_DKSFM dan NFCM_FD. RECs telah ditambah dengan 20% medium terkondisi untuk perlekatan, percambahan dan migrasi sel. Keputusan menunjukkan perlekatan sel yang lebih tinggi dalam NFCM_DKSFM (3452.77±588.1 sel/cm^2) berbanding NFCM_FD (2336.1±440.4 sel/cm^2) dan DKSFM sahaja (2819.8±509.5 sel/cm^2). Selepas 7 hari dikultur, kadar pertumbuhan khusus adalah lebih tinggi dalam DKSFM (0.019±5.16×10^{-4}/jam) berbanding NFCM_DKSFM (0.015±8.94×10^{-4}/jam) dan NFCM_FD (0.013±1.03×10^{-3}/jam). Purata kadar migrasi sel jauh lebih tinggi dalam NFCM_DKSFM (4341.81±385.7 μm^2/jam) berbanding NFCM_FD (1803.38±408.1 μm^2/jam) dan DKSFM (1933.48±271.9 μm^2/jam). Oleh itu, penambahan NFCM_DKSFM menyediakan persekitaran yang sesuai untuk RECs melalui peningkatan perlekatan dan migrasi sel, yang menunjukkan bahawa faktor yang dirembeskan dalam MT boleh memainkan peranan utama dalam meningkatkan penyembuhan luka pada epitelium saluran pernafasan.*

Kata kunci: Fibroblas; medium kultur terkondisi; penyembuhan luka; sel epitelium

INTRODUCTION

The airway epithelium lining functions as a barrier and provides defence against biological, chemical and physical insults. The epithelium plays a significant role in maintaining and regulating innate immune responses to environmental stimuli that cause inflammation and

epithelial damage (Gropper & Wiener-Kronish 2008; Shin et al. 2013; Sun et al. 2011; Ware & Michael 2000; Zahm et al. 1991).

One of the most common diseases of the nose and sinuses is chronic rhinosinusitis (Carroll et al. 2019; Carter et al. 2019; Kim et al. 2019). It has an estimated prevalence of 1%-12% worldwide (Xu et al. 2016). The number is expected to increase due to climate change and pollution. The first line of treatment is still antibiotics, nasal decongestants and nasal douche; however, these treatments do not solve the problem of chronic inflammation and impaired healing of damaged nasal epithelial layer, which can cause pathological changes and later polyps formation. Endoscopic sinus surgery is often used to treat chronic sinusitis, which does not respond to conservative antibiotics treatment. Removal of the damaged epithelium or polyps causes exposure of raw dermis area would lead to scarring, adhesions and possible recurrence of the disease. Several absorbable biomaterials and chemicals are being used, such as hyaluronic acids and synthetic polymer to promote wound healing and prevent adhesion (Fong et al. 2017; Massey & Singh 2017). However, these materials and chemicals are mainly function as a barrier to prevent adhesion and bleeding, but it is not known to promote epithelial wound healing. Healing of a damaged epithelium requires reparative approaches through the response of wounded cells to a variety of external surrounding cytokines, stimulating factors and growth factors (Zahm et al. 1997). The repair process involves re-epithelialization, which is initiated by cell migration and invasion of neighbouring RECs to restore the damaged surface, followed by cell proliferation and differentiation to improve epithelial barrier integrity (Stripp & Reynolds 2008; Zahm et al. 1991). Alterations in cytoskeletal protein patterns, binding of growth factors and interactions with extracellular matrix proteins (ECM) and metalloproteinases are able to regulate cell migration in the wounded area (Schultz & Wysocki 2009). Failure to restore the epithelial barrier may lead to the activation of a massive inflammatory response, contributed to by the colonization of micro-organisms and the upregulation of innate and adaptive immune responses (Sun et al. 2011). As of today, the underlying mechanisms involved cell migration and cell proliferation in respiratory epithelial wound healing remain elusive.

Various *in vitro* experimental studies have been reported to improve wound healing, focusing on the use of secreted factors produced in conditioned medium. A study by Cantinieaux et al. (2013) suggested that the use of bone marrow stem cell conditioned medium (BMSC-CM) to treat spinal cord injury can lead to improved motor recovery. This raises the possibility of developing a cell-free therapeutic approach. Shen et al. (2015) postulated that the paracrine effects of umbilical cord derived mesenchymal stem cells may enhance stem cell-based tissue repair by promoting the specific homing of progenitor cells to the damaged area. An *in vitro* study by Chowdhury et al. (2012) demonstrated that supplementation with dermal fibroblast conditioned medium (DFCM) significantly enhanced skin keratinocyte expansion by increasing cell attachment and this may be applied in future treatments for skin loss. The fibroblast secretome contains a diverse range of secretory proteins including growth factors, cytokines and ECM proteins (Akram et al. 2014; Chowdhury et al. 2012; Paré et al. 2016). Secretory proteins are known to play a crucial role in many physiological processes (Dowling & Clynes 2011) including cell attachment, proliferation, cell migration and wound healing (Akram et al. 2013; Chowdhury et al. 2012; Walter et al. 2010). Akram et al. (2014) reported that paracrine factors found in the conditioned medium collected from Activin-directed human embryonic stem cells (hESC) differentiated cell significantly enhances airway epithelial cell (AEC) wound repair, through the stimulation of cell migration and proliferation. This report suggested a putative paracrine-mediated epithelial injury healing mechanism by hESC secreted products, which is valuable in the development of novel stem cell-based therapeutic strategies. Akram et al. (2013) also demonstrated that human mesenchymal stem cell-conditioned medium (MSC-CM) facilitates airway epithelial cell (AEC) and small alveolar epithelial cell (SAEC) wound repair in a serum-dependent and independent manner, respectively, by stimulating cell migration. It was found that the hMSC secretome contains an array of proteins, including fibronectin, lumican, periostin, and insulin-like growth factor-binding protein 7 (IGFBP-7), which are capable of influencing AEC and SAEC migration and wound repair. In addition, hMSC also stimulate a strong migratory response in AEC, supported by the observation of rapid and effective AEC wound gap closure by hMSC in a 3D model. All these studies have shown that the secretome from hESC, skin fibroblast dan hMSC are feasible to be used for in *in vitro* wound healing. However, the effects of nasal fibroblast secretome on wound healing of airway epithelium still has not been extensively studied.

Therefore, in this study, we performed and investigated the effect of the nasal fibroblast conditioned medium on the attachment, proliferation and migration rate of monolayer cultures of RECs using an *in vitro* wound healing model (scratch assay).

Materials and Methods

Nasal turbinate tissue isolation and cell culture

This study was approved by the Universiti Kebangsaan Malaysia Research and Ethics Committee, with the approval code: FRGS/1/2016/SKK08/UKM/03/1. All redundant nasal turbinate tissues were obtained from both consenting male and female patients (n=6), with age ranging from 27 to 56 years old, who underwent turbinectomy procedures. In this study, each patient was randomly selected and they have an equal chance or probability of suffering from associated underlying

respiratory disease, such as turbinate hypertrophy, allergic rhinitis, vasomotor rhinitis or secondary atrophic rhinitis. The tissues were then processed within 24 h after sample collection. Briefly, the tissues were washed three times with Dulbecco's phosphate buffered saline (DPBS) (Sigma Aldrich, UK), cut into 1 mm^3 pieces and digested in 0.6% collagenase type I (Worthington, USA) solution for about 1.5-2 h, until the tissue digestion is visibly complete. After digestion, the cell suspension containing fibroblasts and RECs was centrifuged at 2370 g for 5 min. The cell pellet was washed and centrifuged again. The mixture of RECs and fibroblasts was cultured in Defined Keratinocyte Serum Free Medium (DKSFM) (Gibco, USA) and a 1:1 mixture of Ham's F-12: Dulbecco's Modified Eagle's Medium (DMEM) (FD) (Gibco, USA) supplemented with 10% fetal bovine serum (Biowest, USA) (F: D+10% FBS) at 37°C in a 5% CO_2 atmosphere. Once confluent, the fibroblasts were removed by differential trypsinization, leaving colonies of RECs in the culture dish to reach confluency in DKSFM.

CELL CHARACTERIZATION: GENE EXPRESSION ANALYSIS BY QUANTITATIVE RT-PCR

Total purification of RNA from RECs was obtained using the ready-to-use RNeasy Plus Mini Kit (Qiagen, USA) according to the manufacturer's recommendations for cultured cells. This isolated RNA was then subjected to quantitative real time polymerase chain reaction (qPCR) using iQ™ SYBR Green Supermix (Biorad, USA). The genes of interest were MUC5B, CK14, CK18 and Ki67. The expression of the housekeeping gene, glycerylaldehyde-3-phosphate dehydrogenase (GAPDH) was also used as a loading control for data normalization (Mori et al. 2008). The primer sequences are listed in Table I.

IMMUNOCYTOCHEMICAL ANALYSIS

Monoclonal antibodies against protein markers of interest were used (acetylated β-tubulin; Sigma-Aldrich, USA, Cat. No: T6793; 1:2000), CK14 (Abcam, Cat. No: ab51054; 1:100), MUC5AC (Abcam, Cat. No: ab3649; 1:100) and Ki67 (Abcam, Cat. No: ab16667; 1:250). Cultured RECs in 12-well plates were immunostained as previously described (Walter et al. 2010). Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen) and Alexa Fluor 594 goat anti-mouse (Invitrogen) were used as the secondary antibodies. Stained cells were observed using a Nikon Eclipse Ti fluorescence confocal microscopy system (Nikon, Japan) and the images were processed using NIS-Elements Viewer 3.20 software (Nikon, Japan).

COLLECTION OF CONDITIONED MEDIUM

Fibroblasts (P3) were cultured in FD+10% FBS until confluent. Then, the cells were washed with DPBS and cultured in flask either in DKSFM or serum-free FD for another three days. The waste medium was collected and designated as NFCM_DKSFM and NFCM_FD, respectively. DKSFM was used as a positive control throughout the study.

EFFECT OF CONDITIONED MEDIUM ON CELL ATTACHMENT, PROLIFERATION AND MIGRATION IN RECS CULTURE

RECs at P0 were trypsinized and seeded onto 12-well plate with a seeding density of 4×10^4 cells per well. Cell attachment and proliferation were assessed on RECs cultured in DKSFM supplemented with 20% conditioned medium, at Day 1, Day 3, Day 5 and Day 7, whereas RECs cultured in DKSFM was served as control. Photomicrographs were captured at different intervals using *a* digital *camera* and the attached cells were counted manually. Cell counting after 24 h of cell seeding indicated the cell attachment rate. Furthermore, the morphology of RECs under different culture conditions was observed under inverted microscope. Observation was performed after the cells were washed with DPBS and fresh medium supplemented with conditioned medium was added as described above, in order to eliminate debris and floating cells. Next, the migration rate was assessed using the wound healing assay to evaluate the effects of different conditioned medium on *in vitro* wound healing. Confluent RECs were centrally scratched using 1 μL pipette tips (Eppendorf, Germany), washed three times with DPBS and cultured in DKSFM medium supplemented with 20% conditioned medium. Cells grown only in DKSFM served

TABLE 1. Genes and the sequences that were used for cell characterization by quantitative real time polymerase chain reaction analysis

Genes	Product Size (bp)	Accession No.	Sequences
GAPDH	217	BC020308	F: TCC CTG AGC TGA ACG GGA AG R: GGA GGA GTG GGT GTC GCT GT
CK18	152	NM_000224	F: CTG AGG TTG GAG CTG CTG AGA R: CTG CTC CAT CTG TAG GGC GTA
CK14	150	BC002690	F: AGA ACC GCA AGG ATG CCG AG R: CCT GGA GAT TGA GCT GCA GT
MUC5B	150	U95031	F: GTC AAC AGC CAT GTG GAC AAC R: CTC CTC ACA GGA GTA GCA GCA
Ki67	202	NM_002417	F: GGC CTC ACA GGA GTA GCA GCA R: GCT GAC TGC TAG GGG CTC TTC

as the positive control. REC wound closure under different culture conditions was recorded using the time-lapse imaging tool on the Nikon Eclipse Ti fluorescence microscope, with an interval of 20 min, for up to 72 h. The scratched area was then analyzed using NIS-Elements Viewer 3.20 software.

Statistical Analysis

The data obtained for cell attachment, proliferation and migration were analyzed using the SPSS software package version 16.0 (SPSS Inc., USA). One-way ANOVA was used to determine any statistically significant differences between the groups. A p value of less than 0.05 was considered statistically significant.

Results

Morphological feature of the primary cells

Mixed culture of RECs and fibroblast was obtained from nasal turbinate samples. Co-cultured cells were differentially trypsinized, leaving the RECs colonies to reach 70-80% confluency. At this point, epithelial cells in primary culture were dense and uniformly grew in polygonal shapes, with 76.67±3.53% cell viability (Figure 1(A)).

Cell characterization by gene and protein expression

The qPCR analysis showed expression of all four genes of interest, namely MUC5B, CK14, CK18 and Ki67 by RECs (data not shown). Immunocytochemical analysis showed positive staining for acetylated β-tubulin, CK14 and MUC5AC in the cytoplasm, whereas the cell proliferation marker, Ki67 was positively localized in nucleus of RECs (Figure 1(B)). The positive expression of all markers indicated that the cells were RECs and were actively proliferating.

Effect of NFCM on cell attachment, proliferation and migration

Supplementation of RECs with NFCM_DKSFM led to significantly greater cell attachment (3452.77±588.1 cells/cm^2) compared to NFCM_FD (2336.1±440.4 cells/cm^2), although the mean of NCFM_DKSFM was not significant compared to that of control group (DKSFM) (2819.8±509.5 cells/cm^2) (Figure 2). Based on this result, the analysis indicated that the secretory products provided by NFCM_DKSFM did not have a significant effect on the RECs culture, as the cells grown in NFCM_DKSFM showed almost equivalent levels of cell attachment compared to that of control group (DKSFM). In comparison to the cells cultured in NFCM_FD, both NFCM_DKSFM and DKSFM led to an improvement in cell attachment. In contrast, the growth rate was significantly higher in DKSFM ($0.019\pm5.16\times10^{-4}$ h^{-1}) compared to NFCM_DKSFM ($0.015\pm8.9\times10^{-4}$ h^{-1}) and NFCM_FD ($0.013\pm1.03\times10^{-3}$ h^{-1}) (Figure 3(A) and 3(B)). The wound healing assay showed that RECs in NFCM_DKSFM underwent significantly faster migration into the scratched area with a mean migration rate of 4341.81±385.7 μm^2/h compared to other two conditions, NFCM_FD (1803.38±408.1 μm^2/h) and DKSFM (1933.48±271.9 μm^2/h) (Figure 2(D)). The RECs migrated slowest in NFCM_FD as it took approximately 68 h to close the gap.

Discussion

Cells that were cultured in different types of growth media have the potential to secrete a number of proteins, including cytokines, chemokines and growth factors, into the surrounding culture environment (Kulasingam & Diamandis 2007; Shin et al. 2013; Thorsell et al. 2008; Walter et al. 2010). These secreted factors are known to promote the rapid migration of cells, followed by cell proliferation both in culture and *in vivo* (Chowdhury et al. 2012; Walter et al. 2010). There have been many identified molecules released into the medium by fibroblasts, including plasminogen activator (Ossowski & Reich 1983), collagen (Goldberg & Green 1964), collagenase (Chua et al. 1985), elastin (Padma & Bhat 2017), thrombospondin (Jaffe et al. 1983) and laminin (Fried et al. 2005).

To date, no study has assessed the secretome from human nasal turbinate cells in a wound healing model and the future use of this conditioned media as a cell-free therapy. In this study, we investigated the efficacy of conditioned medium in terms of enhancing the attachment, proliferation and migration of RECs *in vitro*. Our study shows that the proteins secreted in NFCM_DKSFM may have a role in improving RECs attachment and migration, whereas in the context of proliferation, the number of cells grown in NFCM_DKSFM was slightly increased compared to the control medium, DKSFM. In contrast, cells grown in NFCM_FD in which cell numbers decreased over time, compared to DKSFM and NFCM_DKSFM. This may be due to the presence of high amount of lactate and ammonia, which are known to cause decreased cell growth and growth arrest (Slivac et al. 2010). The attachment and proliferation of cells is also promoted by surface coating with extracellular matrix proteins such as fibronectin and collagen. The observed enhanced cell attachment in NFCM_DKSFM compared to NFCM_FD indicated that NFCM_DKSFM may contain a higher concentration of ECM proteins than NFCM_FD. Besides that, NFCM_FD contain calcium which is known to decrease epithelial cell attachment (Ghani et al. 2016). The supplementation of NFCM_DKSFM increased the efficiency of cell attachment by autologous skin keratinocytes, in correlation with the study by Chowdhury et al. (2012). Dermal fibroblasts cultured in DKSFM could induce the production of extracellular matrix components that increase the efficiency of cell attachment. There are many ECM proteins in the secretomes, such as laminin subunit gamma 1 (LAMC1),

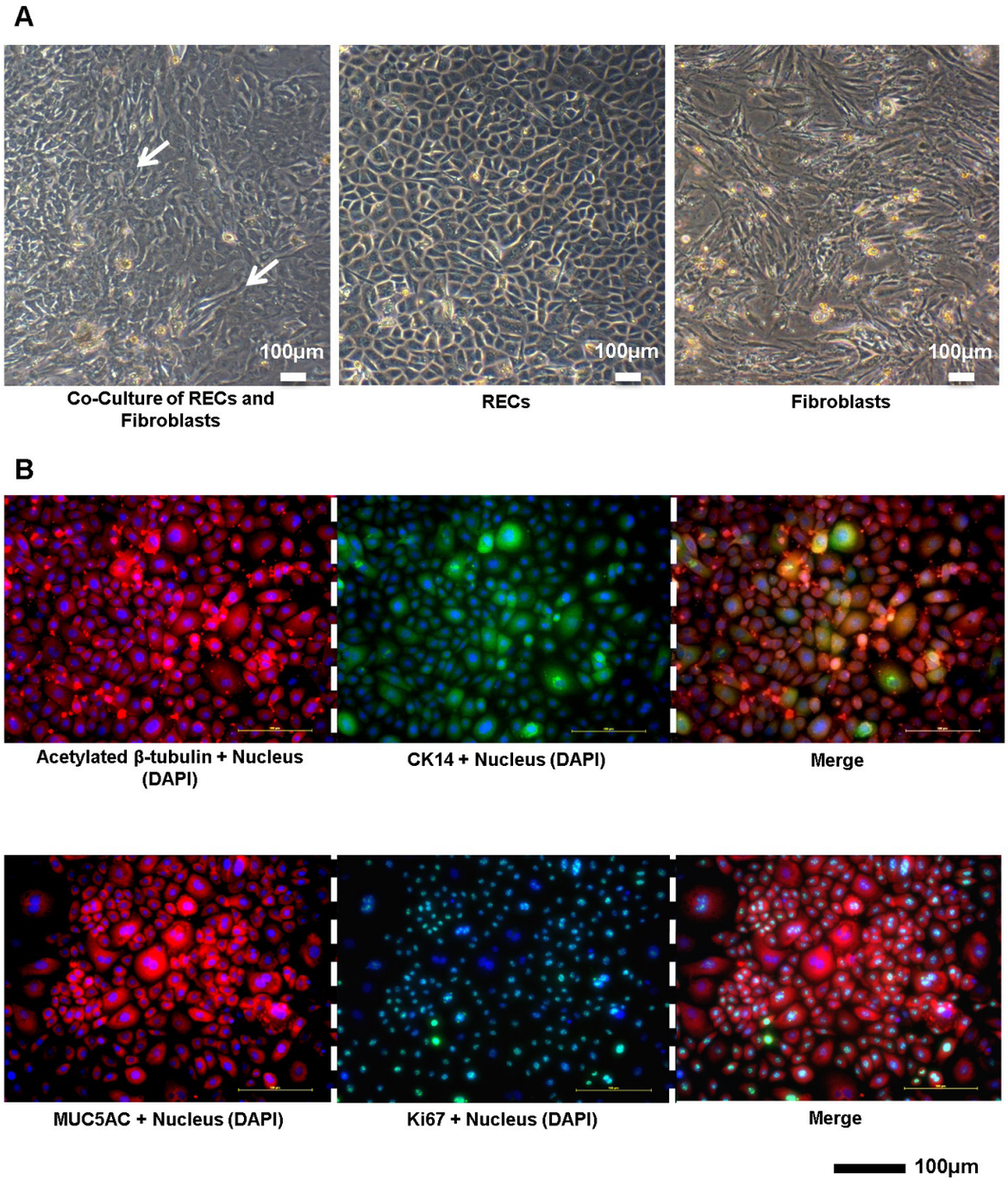

FIGURE 1. (A) Morphology of cells in a mixed culture of RECs and fibroblasts (white arrow). After differential trypsinization, RECs in primary culture grew in compact, dense monolayers and were polygonal in shape, with 76.67% cell viability, whereas nasal fibroblasts had a spindle-like morphology, (B) Cell characterization by immunocytochemical analysis showed positive staining for all four protein markers; three were detected in the cytoplasm (acetylated β-tubulin, CK14 and MUC5AC) whereas Ki67 was detected in the nucleus

laminin subunit beta 2 (LAMB2), nidogen 1 (NID1), immunoglobulin superfamily containing leucine-rich repeat protein (ISLR) and fibrillin-1 (FBN1). However, the effects of the secretory products in NFCM_DKSFM were not significant in terms of cell attachment when compared to DKSFM alone. Cell proliferation was significantly higher in DKSFM compared to NFCM_DKSFM and NFCM_FD. During wound repair, cell surface adhesion molecules play a key role by modulating cell contact with the extracellular matrix or with neighbouring cells (Aragona et al. 2017;

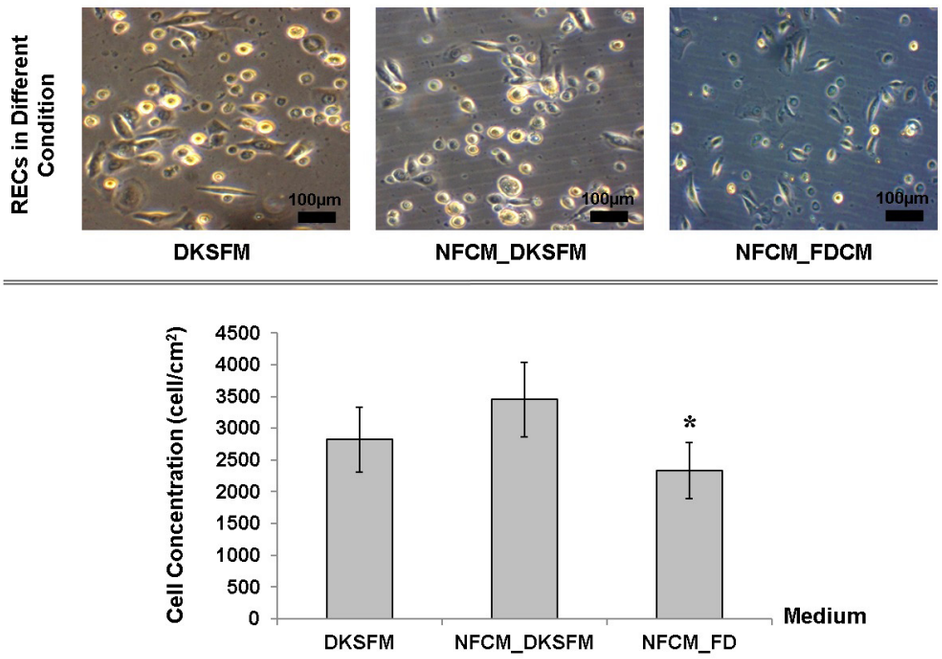

FIGURE 2. The efficiency of cell attachment was recorded 24 h after cell seeding. Cell numbers were analyzed in triplicate, with four images taken from different points of the well. *NFCM_DK vs NFCM_FD. Values of $p<0.05$ were considered statistically significant (n=6)

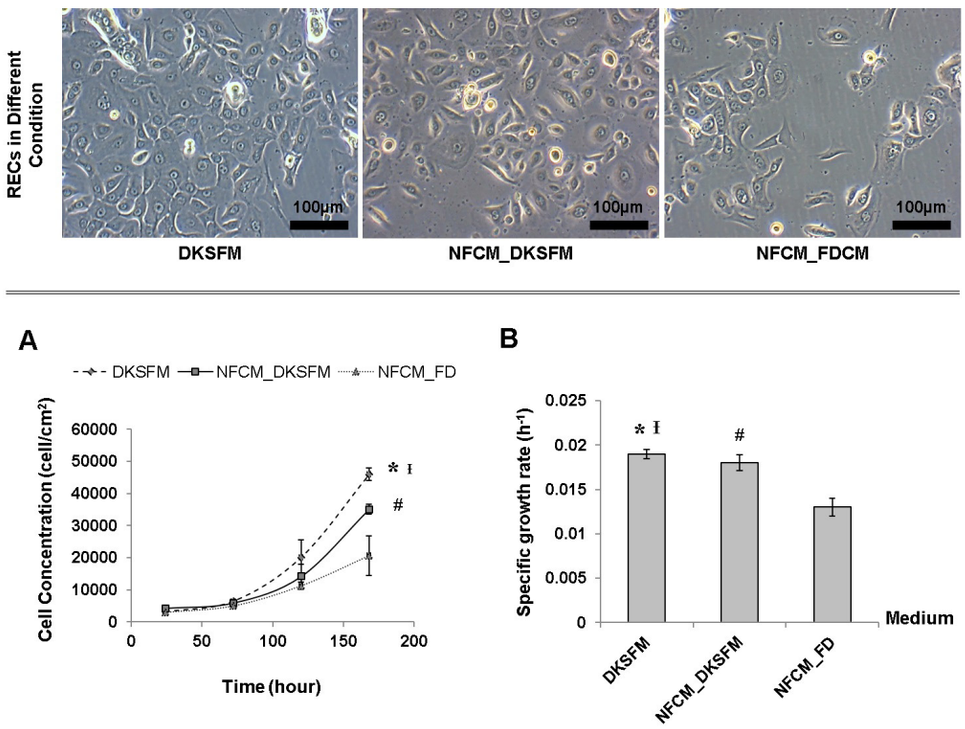

FIGURE 3. Proliferation rate of RECs in different conditions after 72 h of culture. A: Growth curve of RECs supplemented with three different growth media. *DKSFM vs NFCM_DK, ŁDKSFM vs NFCM_FD, #NFCM_DK vs NFCM_FD; B: The growth rate of cultured RECs in three culture conditions. *DKSFM vs NFCM_DK, Ł DKSFM vs NFCM_FD, #NFCM_DK vs NFCM_FD. Values of $p<0.05$ were considered statistically significant (n=6)

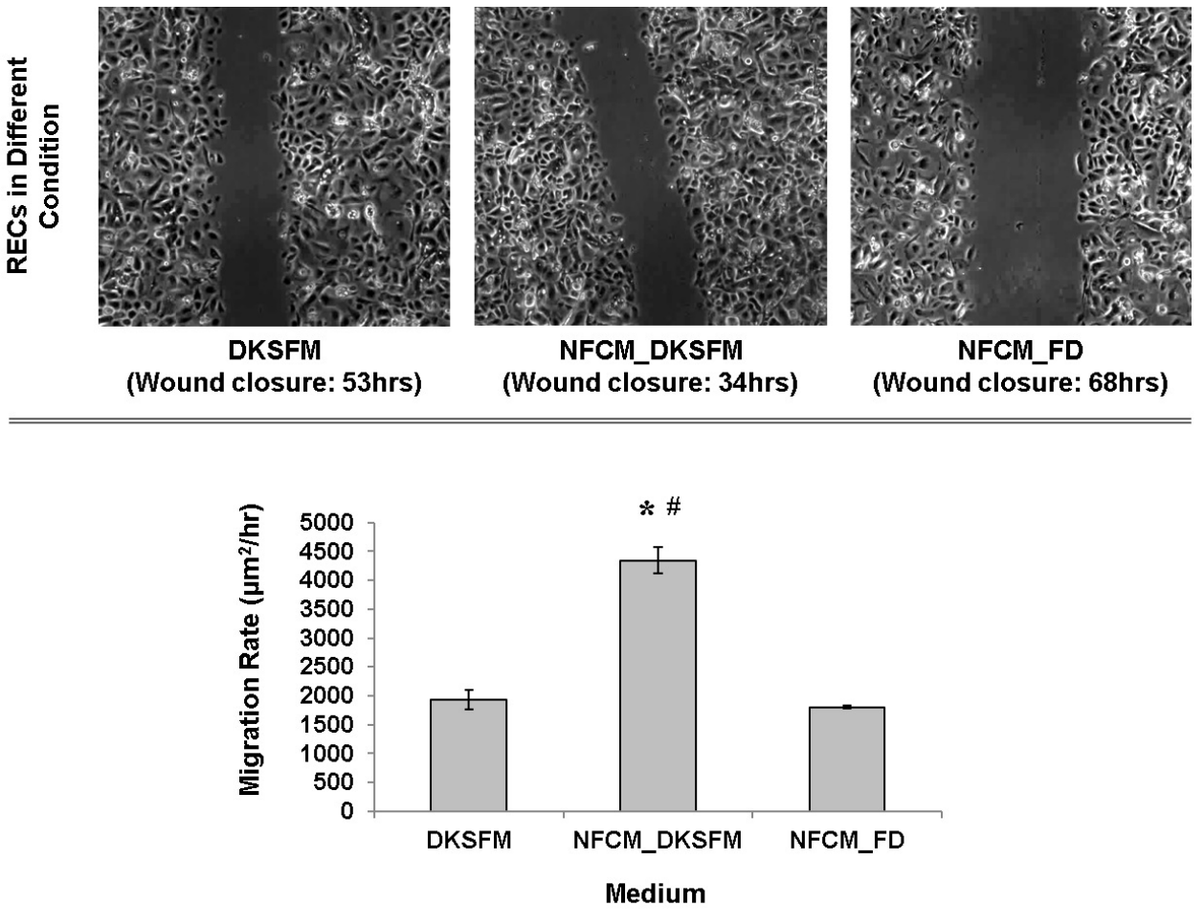

FIGURE 4. Cell migration assay under three culture conditions. Photomicrographs of RECs under different culture conditions were taken immediately after cell wounding, with time-lapse video recordings with an interval of 20 min. *DKSFM vs NFCM_DKSFM, #NFCM_DKSFM vs NFCM_FD. Values of $p<0.05$ were considered statistically significant (n=6)

Sahu et al. 2017). Time-lapse video recordings of the progressive closure of the wound suggested that early re-epithelialization had occurred by a cell migration process, indicated by an increased migration rate in NFCM_DKSFM. In contrast, cells in NFCM_FD showed slower proliferation and migration rates as compared to those of in other media, suggesting that the secreted proteins in this conditioned medium may have induced cell differentiation, leading to slower cell growth and cell migration. A study reported by Walter et al. (2010) suggested that the use of mesenchymal stem cell derived conditioned medium enhances skin wound closure by affecting the migration of both dermal fibroblasts and keratinocytes, along with a contribution to the formation of extracellular matrix. It has been shown that serum-free mesenchymal stem cell conditioned medium (MSC-CM) significantly enhances the wound closure rate of fibroblasts and keratinocytes, separately and in co-culture, whereas conditioned medium from L929 or HaCaT cultures had no significant effects on wound closure. This enhancement of wound closure in the presence of MSC-CM was found to be due to accelerated cell migration rather than increased cell proliferation. These investigators also successfully identified secreted cytokines, chemokines and ECM components including TGF-β1, IL-6, IL-8, MCP-1, RANTES, collagen type I, fibronectin, SPARC and IGFBP-7 which may have influenced cell growth and migration. However, in this study, there are two potential limitations that should be noted. The first limitation concerns on the sample variation that includes the age groups, gender and different underlying conditions, which eventually may influence content of the secretome. The second limitation was that the secretome were collected from sample obtained from non-healthy subjects as it was not possible to collect nasal turbinate samples from healthy individuals. Thus, the secretome from unhealthy subject might have some level of difference with that of from healthy subject. The secretome from healthy subject might show a better outcome in promoting epithelial wound healing and also important in translating this discovery into clinical therapies.

Hence, supplementation of NFCM_DKSFM provides suitable culture conditions for RECs through increased cell attachment and migration, which suggest that the factors

secreted in conditioned medium may play a major role in enhancing airway epithelial wound healing. However, more extensive studies should be done to identify the specific proteins that enhance RECs growth and migration, the proteins upregulated by supplementation with conditioned medium, and also to validate the role of the underlying mechanism predicted to promote wound healing by RECs in this study. Hopefully, these findings will encourage the use of secretomes and derivatives in promoting the regeneration of the nasal epithelium in cases of chronic infection or inflammation and, most importantly, following post sinus surgery.

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