Med & Health Jun 2018; 13(1): 77-87 https://doi.org/10.17576/MH.2018.1301.08

ORIGINAL ARTICLE

Comparative Study on Cartilage Tissue Collected From Less- and Severely-Affected Region of Osteoarthritic Knee

NURZAZLIN BZN[1], SHAMSUL BS[1], YAHYA NHM[2], RUSZYMAH BHI[1,3], ABDUL RANI R[2], CHOWDHURY SR[1]

[1]Tissue Engineering Centre, [2]Department of Orthopaedic and Traumatology, [3]Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.

ABSTRAK

Kondrosit yang dikembang biak hasil isolasi dari kawasan kurang terjejas akibat osteo atritis (OA) dijadikan sumber untuk membentuk konstruk rawan bagi tujuan rawatan. Tujuan kajian ini adalah untuk membandingkan ciri-ciri rawan secara histologi dan mengenalpasti morfologi kondrosit yang telah diisolasi daripada OA lutut manusia yang kurang dan teruk terjejas. Tisu rawan artikular manusia dalam kajian ini diperolehi daripada pesakit OA yang memerlukan pembedahan penggantian lutut. Prosedur dilakukan di Pusat Perubatan Universiti Kebangsaan Malaysia (PPUKM). Tisu rawan artikular dikelaskan mengikut sistem Dougados dan Osteoarthritis Research Society International (OARSI) iaitu kurang terjejas [less-affected (LA); Gred 0-1] dan teruk terjejas [severely affected (SA): Gred 2-3]. Tisu rawan dari kedua-dua kumpulan diwarnakan dengan larutan Safranin O. Sel kondrosit yang diisolasi daripada setiap kumpulan dikultur sehingga ke subkultur ke-4 [passage 4 (P4)]. Perbandingan telah dilakukan untuk mengenalpasti kadar pertumbuhan, luas dan kebulatan kondrosit. Pewarnaan sekata Safranin O atas tisu rawan kurang terjejas (LA) menunjukkan komponen matriks ekstraselular (ECM) masih dalam keadaan baik manakala tisu rawan yang teruk terjejas menunjukkan pengurangan warna ketara dan tidak stabil disebabkan oleh penguraian ECM. Kondrosit-LA menunjukkan pembentukan sel agregasi berbanding dengan kondrosit-SA di mana tiada sel agregasi dapat diperhatikan. Kondrosit-LA mempunyai keluasan dan penyerebakkan sel yang lebih tinggi pada subkultur -0 dan -4 berbanding kondrosit-SA. Kebulatan sel kondrosit juga didapati meningkat seiring dengan peningkatan subkultur. Kadar kebulatan kondrosit-LA di subkultur-3 ketara lebih tinggi daripada kondrosit-SA. Keseluruhan kajian menunjukkan perbezaan ketara di antara ciri-ciri sel kondrosit-LA dan –SA dan implikasi perbezaan dalam sel terapi ini perlu diterokai dengan lebih mendalam di masa akan datang.

Address for correspondence and reprint requests: Shiplu Roy Chowdhury. Tissue Engineering Centre, 12th Floor Clinical Block, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia. Tel: +603 91457679 E-mail: shiplu@ppukm.ukm.edu.my

Kata kunci: kondrosit, osteoartritis, rawan artikular

ABSTRACT

Culture expanded chondrocytes isolated from non-load bearing region of osteoarthritic (OA) joint has been used to construct tissue engineered cartilage for treatment purposes. The aim of the study was to compare the histological properties of the cartilage tissue and morphological properties of the chondrocytes isolated from less and severely affected OA knee. Human articular cartilage was obtained as redundant tissue from consented patients with late-stage OA undergoing total knee replacement surgery at Universiti Kebangsaan Malaysia Medical Centre (UKMMC). Articular cartilage was graded according to Dougados and Osteoarthritis Research Society International (OARSI) classification. Articular cartilage was classified into less affected (LA; Grade 0-1) and severely affected (SA; Grade 2-3). Cartilage tissue from less and severely affected region was stained with Safranin O staining. Isolated chondrocytes from each group were cultured until passage 4 (P4). Their growth patterns, cell areas, and circularity were compared. LA-cartilage tissue shows uniform spread of safranin O staining indicating intact extracellular matrix (ECM) component. However, SA-cartilage shows significant reduction and unstable staining due to its degraded ECM. LA-chondrocytes showed an aggregated growth compared to SA-chondrocyte that remains monolayer. Moreover, LA-chondrocytes have significantly higher cell area with wider spreading at passage 0 and 4 compared to SA-chondrocytes. It was also found that chondrocyte circularity increased with passage, and circularity of LA-chondrocytes was significantly higher than that of the SA-chondrocytes at passage 3. This study demonstrated the considerable difference in the cellular properties for less and severely affected chondrocytes and implication of these differences in cell-based therapy needed to be explored.

Keywords: articular cartilage, chondrocytes, osteoarthritic

INTRODUCTION

Osteoarthritis is a major cause of disability worldwide. It was long believed that wear and tear of joints was the only cause of osteoarthritis (Mow et al. 1992). Pathogenesis of osteoarthritis is more complex. Although cartilage was recorded to be among the first cells to be studied, it is still far off to completely understand the behaviour of this cell (Bhattacharjee et al. 2015).

Chondrocytes which is the specialized cell of articular cartilage naturally has a circular or polygonal morphology and resting within its self-produce matrix (Heidari et al. 2011). Severe trauma or chronic degeneration such as OA is a major problem as cartilage tissue has limited self-repair

ability (Wang et al. 2005). Therefore, treatment at an early stage can help to prevent OA development and reduce the probability for total joint replacement (Chu et al. 2012). Poor healing of lesion on articular cartilage eventually causes OA (Tamaddon et al. 2017). Current treatment includes mosaicplasty, microfracture, and subchondral drilling often leads to the formation of fibrocartilage instead of hyaline cartilage (Lynn et al. 2004).

Autologous Chondrocyte Implantation (ACI) is currently the star of OA treatment regime. In terms of cell source for this procedure, it is logic to choose a readily committed chondrocytes cells instead of stem cells that previously had been proven by Mobasheri et al. (2014) to produce fibrocartilage when treating OA. However, through long term study, Brittberg et al. (2001) found out that some of the grafted chondrocytes isolated from patient's non-load bearing area of the same osteoarthritic knee eventually differentiate into fibrocartilage. It shows that, the knowledge and understanding of human cartilage and osteoarthritis in general have improved through multiple research findings, but the search of a perfect donor cell to replace articular chondrocytes and able to act similarly as the native tissue is still a long way to go.

There were several tissue engineering applications for OA intervention where chondrocytes from OA knee were cultured and transplanted back into the OA affected joint. However, suitable region for harvesting chondrocytes in OA knee for the purpose of treatment was barely studied. In this study, we compared histological properties of different regions namely less and severely affected OA knee, and compare the chondrocytes morphological properties by means of area, circularity and growth patterns to identify the potential difference in cellular properties, which could be helpful to decide suitable region for harvesting chondrocytes for cell-based therapy.

MATERIALS & METHODS

This study received ethical approval from the Universiti Kebangsaan Malaysia Research and Ethics Committee.

SAMPLE PROCESSING AND GRADING

Cartilage samples were collected from consented patients undergoing total knee replacement or knee arthroplasty surgery. Cartilage from human femoral condyle of OA patients was collected and classified into two groups based on Dougados classification assessment; Grade 0-1 (less-affected; LA-C) and Grade 2-3 (severely-affected; SA). LA-cartilage was collected from an unaffected lateral part of primary OA patient's varus knee whereas SA-cartilage was collected from affected medial part of the varus knee.

HISTOLOGICAL STAINING

LA-cartilage and SA-cartilage were embedded in paraffin and sectioned into 5 µm thickness. Tissue section

were then stained with H&E (Sigma-Aldrich, USA) to observe their structure and further validate the grading using OARSI scoring system. To investigate the expression of cartilage ECM, the tissue section were stained with Safranin O (Sigma-Aldrich, USA).

CHONDROCYTES ISOLATION

Cartilage tissue from OA knee was separated from the subchondral bone and washed with Dulbecco's Phosphate Buffered saline (DPBS; Invitrogen, UK). The cartilage was diced finely using a scalpel blade to improve the efficiency of subsequent enzymatic digestion. The diced cartilage tissue was digested for 4 hours at 37˚C in collagenase type II solution (0.6%; Worthington, USA) under continuous agitation (Ruszymah et al. 2005). After digestion, the suspension containing isolated chondrocytes was centrifuged (Hettich Centrifuges, Germany) at 5000 rpm for 5 min to obtain a cell pellet. The cell pellet was washed twice with DPBS to remove remaining digestive enzyme. The cells then re-suspended in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (D-MEM/F12; Biowest USA) medium with 10% Foetal Bovine Serum (FBS; Gibco, USA) and seeded into 6-well culture plate (Greiner Bio-one, USA). The cells were grown at 37˚C in 5% CO_2 incubator (RS Biotech, UK) with culture medium being replaced every 2-3 days. Primary culture of the cells was designated as passage 0 (P0). At 80% confluence, cells at P0 were trypsinized and seeded for subsequent culture i.e. P1. Cells were cultured until passage 4.

CHONDROCYTES AREA AND CIRCULARITY

Chondrocytes at passages 0 to 4 were used to evaluate cells area whereas chondrocytes at passages 2 and 3 were used to analyse circularity. The cell was observed in real time for 48 hours using Nikon microscopic system (Nikon, Japan). Cell area and circularity were evaluated using image processing software, NIS-Element AR 4.2.0 (Nikon). The circularity value was displayed in the scale of 0 to 1, where 0 indicates completely stretch and 1 indicates complete round shape cells.

CONFLUENT CULTURE OF CHONDROCYTES

Chondrocytes of each groups were cultured at 5000 cells/cm^2 in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (D-MEM/F12; Biowest USA) medium with 10% Foetal Bovine Serum (FBS; Gibco, USA) and seeded into 12-well culture plate (Greiner Bio-one, USA) and 0.4 µm cell culture insert (Millipore Corp., USA). The cells were grown at 37˚C in 5% CO_2 incubator (RS Biotech, UK) with culture medium being replaced every day. The chondrocytes were cultured until we achieved 100% confluence and then observed under inverted microscope (Olympus, Germany)

All parameters were analysed using 6 experimental replicates (samples) with 3 technical replicates for each sample unless stated otherwise. Data were expressed as a mean ± standard

error of the mean and analysed using student's *t*-test. $P<0.05$ was considered significantly different.

RESULTS

HISTOLOGICAL ANALYSIS OF CARTILAGE TISSUE

Articular cartilage was graded according to Dougados classification scoring system for a gross tissue. The cartilage samples were classified into two groups i.e. less affected (LA)-cartilage (Grade 0-1) and severely affected (SA)-cartilage (Grade 2-3). The accuracy of sample grading was verified by histological analysis based on OARSI grading system. LA-cartilage showed structural alterations that included a reduction of cartilage thickness of the superficial and the middle zones, and surface integrity was occasionally broken as softening and swelling occurred. The collagen network structure was damaged and dominated with eosin stained acidophilic matrix with collagen type 1 fibres. In contrast, SA-cartilage showed a much thinner superficial layer with small and profound branched extended fissures, cell cloning, non-intact tidemark, and fibrillation. At some parts, the superficial zone of cartilage was totally lost showing surface discontinuity (Figure 1). Moreover, ECM structure of the cartilage tissues was observed via histological staining. Uniform Safranin O staining was seen through all zone in LA-cartilage whereas SA-cartilage showed unstable staining indicating progressive loss in ECM component (Figure 2).

MORPHOLOGY OF CHONDROCYTES ON POLYSTYRENE SURFACE

Chondrocytes from LA-cartilage and SA-cartilage were cultured until passage 4, and their morphological features were observed under phase contrast microscopy. Both LA-chondrocytes and SA-chondrocytes were observed to proliferate, and reach to confluence by 7-10 days (Figure 3A). Upon confluence, LA-chondrocytes were observed to grow in aggregates, while SA-chondrocytes tend to grow in the homogenous layer. Morphologically, chondrocytes at early passage (P0-P2) from both groups demonstrated polygonal shapes. However, at the later passage (P3-P4), SA-chondrocytes became elongated and demonstrated spindle morphology, while cells from LA-chondrocytes maintained the polygonal morphology (Figure 3B).

CHONDROCYTES AGGREGATION ON CELL CULTURE INSERT

To observe chondrocytes growth and formation of cellular aggregates at the confluence, LA-chondrocytes and SA-chondrocytes were cultured on polyethylene terephthalate (PET) membrane of cell culture insert. LA-chondrocytes at confluence state form aggregated on PET membrane whereas, SA-chondrocytes grew in homogenous monolayer until confluent (Figure 4). This indicates that formation of aggregates could be characteristic features of cells from LA-

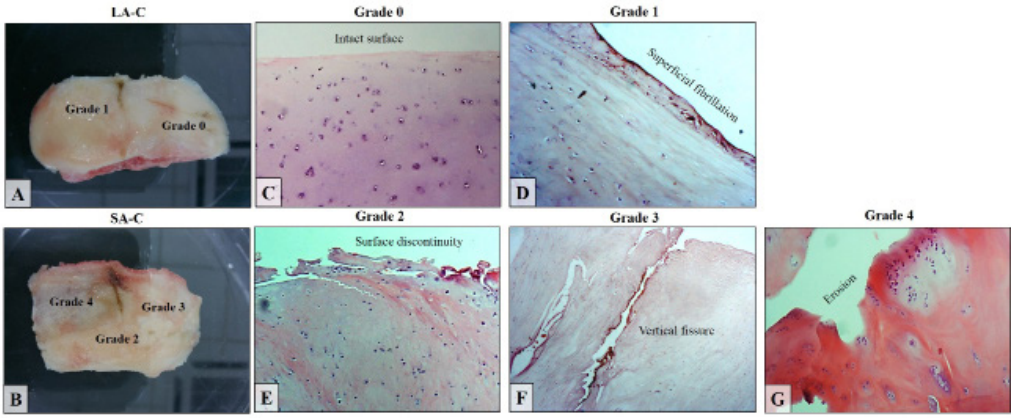

Figure 1: Gross tissue grading based on Dougados classification system; Grade 0-1 as LA-cartilage (A) and Grade 2-4 as SA-cartilage (B). Grade 0: macroscopically normal; Grade 1: softening and swelling; Grade 2: fibrillation and fissuring on surface; Grade 3: fragmentation and fissuring to middle and deep zone; and Grade 4: Exposed subchondral bone. H&E staining of Grade 0(C) and Grade 1(D)shows intact and fibrillated superficial cartilage layer, respectively. Grade 2(E), Grade 3(F) and Grade 4(G) show superficial fissures, deep fissure and total cartilage erosion, respectively.

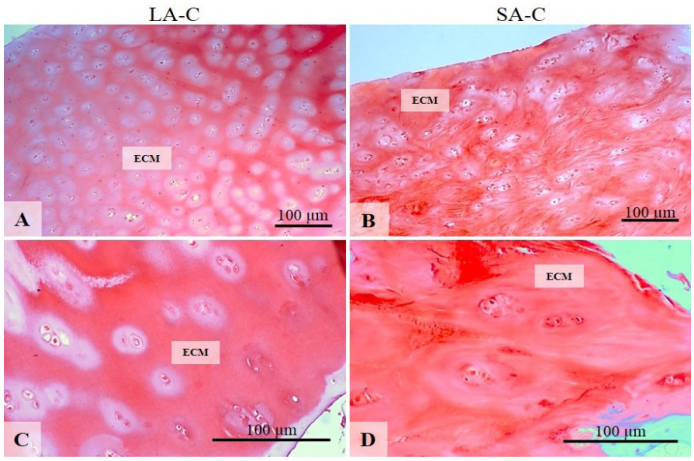

Figure 2: Safranin O showing uniform staining on intact ECM structure of LA-cartilage (A and C), and unstable staining of SA-cartilage (B and D). Progressive loss of ECM was evident in SA-cartilage tissue.

cartilage.

CHONDROCYTES AREA AND CIRCULARITY

Chondrocytes from both LA- and SA-cartilage showed gradual increase of cell area as culture progressed with an exception at P4. At P3 and P4, the area of LA-chondrocytes was significantly higher compared to that from P0 ($p<0.05$) (Figure 5). Moreover, the LA-chondrocytes at P0 and P4 demonstrate significantly higher area compared to that of SA-chondrocytes. There was no noticeable difference observed for the distribution of cell area among LA- and SA-chondrocytes.

In accordance with the cell area, chondrocyte circularity was also

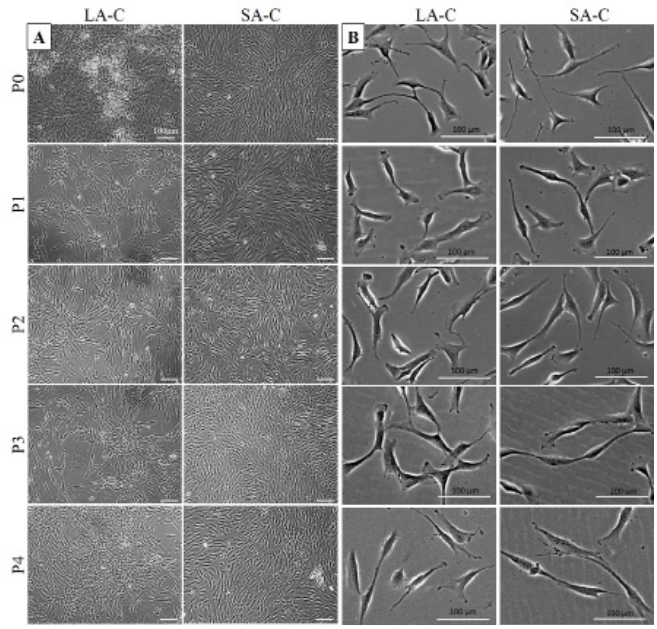

Figure 3: Chondrocytes properties at confluent culture (A). At confluency, for passage 0-4, LA-chondrocytes forms aggregated colonies, while SA-chondrocytes grow homogenously on the surface. Chondrocytes morphology during culture (B). LA-chondrocytes maintain polygonal shapes throughout the passages, while SA-chondrocytes demonstrate spindle shapes at passage 3 and passage 4.

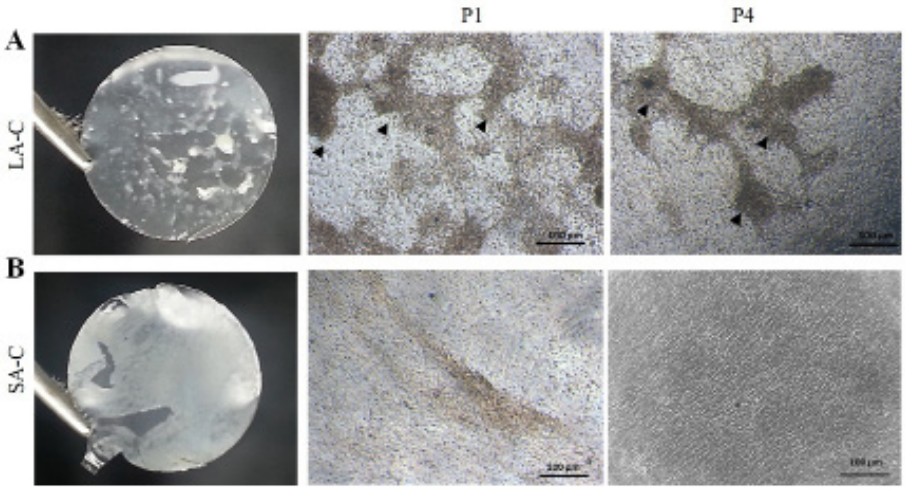

Figure 4: LA-chondrocytes (A) and SA-chondrocytes (B)cultured on PET membrane at passage 1 and passage 4. LA-chondrocytes forms aggregation on the surface (black arrow head), while SA-chondrocytes mostly grow homogenously on the surface (n=3).

increased significantly at passage 3 than that of passage 2 for cells isolated from both region (Figure 6). Circularity of LA-chondrocytes was significantly

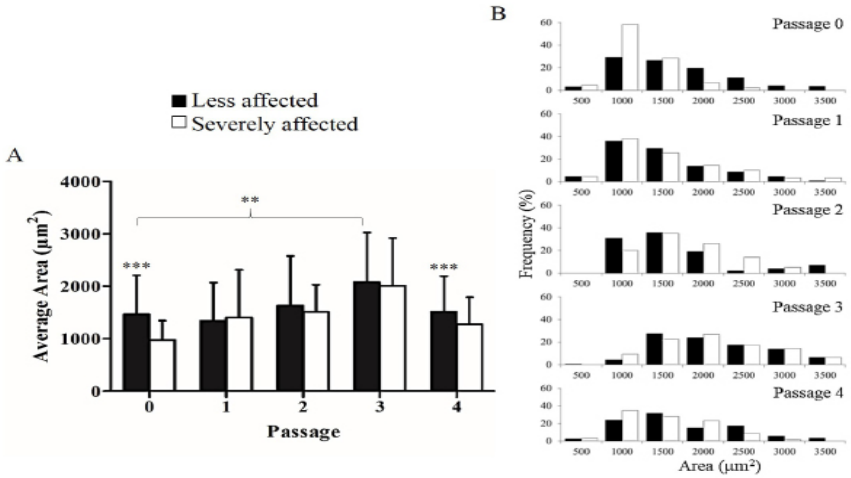

Figure 5: Area of chondrocytes during in vitro culture. Average cell area (A) and distribution of cell area (B) at passage 0 until passage 4. ** indicates significantly higher area of LA-chondrocytes at passage 3 compared to passage 0. *** indicates significantly higher area of LA-chondrocytes at passage 0 and 4 compared to SA-chondrocytes. $p<0.05$ is considered as significant difference.

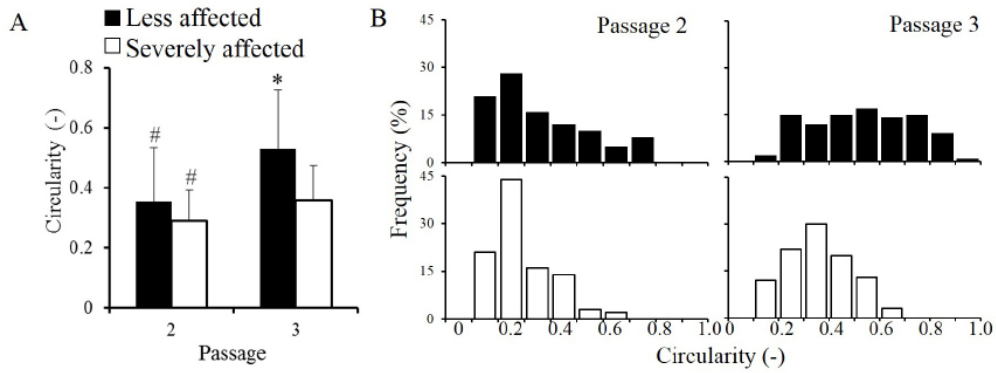

Figure 6: Average circularity(A) and distribution of circularity(B) of LA- and SA-chondrocytes at passage 2 and 3. *indicates significantly higher circularity for chondrocytes from less affected region than severely affected region. #indicates significantly lower circularity at passage 2 than passage 3 for chondrocytes from both less and severely affected region. $p<0.05$ is considered as significant difference

higher than that of the SA-chondrocytes at passage 3. Chondrocytes from LA region showed a widely distributed frequency of circularity compared to chondrocytes from SA region for passage 2 and 3. Spindle morphology of chondrocytes from LA region was observed at passage 3.

DISCUSSION

Articular cartilage is a highly-specialized connective tissue, and has an organized layered structure that is divided into four zones: superficial, middle, deep, and calcified cartilage layer. The chondrocytes that make-up

approximately 10% of the wet weight of articular cartilage are a specialized metabolically active cell that are responsible for the development of the articular cartilage and the maintenance of the ECM (Sophia Fox et al. 2009). Collagens are the most abundant macromolecules of the ECM that provide tensile and shear strength to the tissue (Cohen et al. 1998). Proteoglycans molecules in articular cartilage form a highly hydrated, gel-like ground substance that provide tensile strength to the surrounding collagen network. This ECM acts as an absorbent and helps in load distribution throughout the joint surface. Disruption of the ECM balance caused from the damage of the articular surface collagen network is shown to significantly change the load bearing ability of the articular cartilage, and increase the chance to OA conditions (Akkiraju, & Nohe 2015; Setton et al. 1994; Setton et al. 1993). It results in progressive cartilage degradation characterized by the softening, fibrillation, and erosion of the articular surface (Sandell & Aigner 2001). In this study, we compared the morphological properties of chondrocytes, which were isolated from different region of OA knee.

The stage and severity of OA was commonly assessed via different grading system. Cartilage samples from patients with knee replacement surgery in this study were classified into two groups i.e. less affected cartilage (LA-cartilage; Grade 0-1) and severely affected cartilage (SA-cartilage; Grade 2-3). The grading was performed based on the methods proposed by Dougados et al. (1994). This assessment is either a continuous variable (scoring) or a semi-quantitative variable (grading) related to the three key features of cartilage damage: localization, depth, and surface. The accuracy of OA cartilage sample graded via Dougados scoring was further verified by Osteoarthritis Research Society International (OARSI) assessment system, which was proposed in 1998 to standardize grading system based on the histopathological analysis of cartilage tissue (Pritzker et al. 2006). Microscopic signs of OA cartilage through H&E and Safranin O staining demonstrated minimum structural alterations with intact ECM of LA-cartilage. Whereas, SA-cartilage showed a deep surface cleft, and significant reduction of ECM component. These findings were in line with the observation made by Musumeci et al. (2013).

In a synovial joint, the articular cartilage, which consists of chondrocytes, is directly affected during the progression of Osteoarthritis (OA). Chondrocytes from articular cartilage can be isolated from its surrounding matrix via enzymatic digestion and cultured in vitro. Cultured chondrocytes have served as useful models for studying the differentiation of chondrocytes, and the effects of cytokines and growth factors which control the maintenance or suppression of cartilage phenotypes (Goldring 2000). The morphology of cultured chondrocytes strongly correlate with the differentiation status and expression pattern of ECM protein. Chondrocytes at early passage resembled polygonal morphology,

driven in part by the cortical organization of the actin cytoskeleton (Nurminsky et al. 2007). After serial passage, the cells switched into spindle morphology and became elongated. It resembles a fibroblast-like morphology which also commonly termed as cells dedifferentiation (von der Mark et al. 1977).

In this study, we showed that LA-chondrocytes exhibit relatively bigger area and polygonal morphology, which was equivalent with normal articular cartilages harvested from non-OA samples. Similar observation was reported by Heidari et al. (2011). Moreover, LA- and SA-chondrocytes show an increment of cell area with increasing passage, which indicated the transition phase of dedifferentiation of chondrocytes when cultured on a plastic surface. The cells start to become spindle in shapes and projecting its morphology towards fibrocartilage.

One of the distinct features that can be seen between LA-and SA-chondrocytes was their growth patterns. LA-chondrocytes tends to form aggregates when cultured on the plastic surface as well as PET membrane. However, SA-chondrocytes tends to grow in monolayer when grown on the same surfaces. This feature can be seen clearly when the cultured cells reached 90% to 100% confluence.

CONCLUSION

In conclusion, it was proven that chondrocytes harvested from the different severity of osteoarthritic cartilage tissue have several differences in terms of cell morphology, growth patterns, and cell circularity at different passages. This study can be a stepping stone for future works in understanding the behaviour of osteoarthritis cartilage to be used for clinical applications and development of *in vitro* cartilage model for research and pharmaceutical industry.

ACKNOWLEDGEMENT

This research was financially supported by Ministry of Science, Technology and Innovation (MOSTI) (02-01-02-SF0961) and Universiti Kebangsaan Malaysia Medical Centre (UKMMC) (FF-2014-215).

REFERENCES

Akkiraju, H., Nohe, A. 2015. Role of chondrocytes in cartilage formation, progression of osteoarthritis and cartilage regeneration. *J Dev Biol* **3**(4): 177-92.

Bhattacharjee, M., Coburn, J., Centola, M., Murab, S., Barbero, A., Kaplan, D.L., Martin, I., Ghosh, S. 2015. Tissue engineering strategies to study cartilage development, degeneration and regeneration. *Adv Drug Deliv Rev* **84**: 107-22.

Brittberg, M., Tallheden, T., Sjögren-Jansson, B., Lindahl, A., Peterson, L. 2001. Autologous chondrocytes used for articular cartilage repair: an update. *Clin Orthop Relat Res* (391 Suppl): S337-48.

Chu, C.R., Williams, A.A., Coyle, C.H., Bowers, M.E. 2012. Early diagnosis to enable early treatment of pre-osteoarthritis. *Arthritis Res Ther* **14**(3): 212-21.

Cohen, N.P., Foster, R.J., Mow, V.C. 1998. Composition and dynamics of articular cartilage : structure , function , and maintaining healthy state. *J Orthop Sports Phys Ther* **28**(4): 203-15.

Dougados, M., Ayral, X., Listrat, V., Gueguen, A., Bahuaud, J., Beaufils, P., Beguin, J.A., Bonvarlet, J.P., Boyer, T., Coudane, H., Delaunay, C., Dorfmann, H., Dubos, J.P., Frank, A., Kempf, J.F., Locker, B., Prudhon, J.L., Thiery, J. 1994. The SFA system for assessing articular cartilage lesions at arthroscopy of the knee.

Arthroscopy **10**(1): 69-77.

Goldring, M.B. 2000. Osteoarthritis and cartilage: the role of cytokines. *Curr Rheumatol Rep* **2**(6): 459-65.

Heidari, M., Naghi Tahmasebi, M., Etemad, S., Salehkhou, S., Heidari-Vala, H., Akhondi, M.M. 2011. In vitro human chondrocyte culture ; a modified protocol. *Middle East J Sci Res* **9**(1): 102-9.

Lynn, A.K., Brooks, R.A, Bonfield, W., Rushton, N. 2004. Repair of defects in articular joints. Prospects for material-based solutions in tissue engineering. *J Bone Joint Surg Br* **86**(8): 1093-9.

Mobasheri, A., Kalamegam, G., Musumeci, G., Batt, M.E. 2014. Chondrocyte and mesenchymal stem cell-based therapies for cartilage repair in osteoarthritis and related orthopaedic conditions. *Maturitas* **78**(3): 188-98.

Mow, V.C., Ratcliffe, A., Poole, A.R. 1992. Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures. *Biomaterials* **13**(2): 67-97.

Musumeci, G., Castrogiovanni, P., Trovato, F.M., Di Giunta, A. Di, Loreto, C., Castorina, S. 2013. Microscopic and macroscopic anatomical features in healthy and osteoarthritic knee cartilage. *OA Anat* **1**(3): 30.

Nurminsky, D., Magee, C., Faverman, L., Nurminskaya, M. 2007. Regulation of chondrocyte differentiation by actin-severing protein adseverin. *Dev Biol* **302**(2): 427-37.

Pritzker, K.P., Gay, S., Jimenez, S.A., Ostergaard, K., Pelletier, J.P., Revell, P.A., Salter, D., van den Berg, W.B. 2006. Osteoarthritis cartilage histopathology: grading and staging. *Osteoarthritis Cartilage* **14**(1): 13-29.

Ruszymah, B.H., Chua, K., Latif, M.A., Hussein, F.N., Saim, A.B. 2005. Formation of in vivo tissue engineered human hyaline cartilage in the shape of a trachea with internal support. *Int J Pediatr Otorhinolaryngol* **69(11)**: 1489-95.

Sandell, L.J., Aigner, T. 2001. Articular cartilage and changes in Arthritis. An introduction: cell biology of osteoarthritis. *Arthritis Res* **3**(2): 107-13.

Setton, L.A., Mow, V.C., Müller, F.J., Pita, J.C., Howell, D.S. 1994. Mechanical properties of canine articular cartilage are significantly altered following transection of the anterior cruciate ligament. *J Orthop Res* **12**(4): 451-63.

Setton, L.A., Zhu, W., Mow, V.C. 1993. The biphasic poroviscoelastic behavior of articular cartilage: role of the surface zone in governing the compressive behavior. *J Biomech* **26**(4-5): 581-92.

Sophia Fox, A.J., Bedi, A., Rodeo, S.A. 2009. The basic science of articular cartilage: structure, composition, and function. *Sports Health* **1**(6): 461-8.

Tamaddon, M., Burrows, M., Ferreira, S.A., Dazzi, F., Apperley, J.F., Bradshaw, A., Brand, D.D., Czernuszka, J., Gentleman, E. 2017. Monomeric, porous type II collagen scaffolds promote chondrogenic differentiation of human bone marrow mesenchymal stem cells in vitro. *Sci Rep* **7**: 43519.

von der Mark, K., Gauss, V., von der Mark, H., Müller, P. 1977. Relationship between cell shape and type of collagen synthesised as chondrocytes lose their cartilage phenotype in culture. *Nature* **267**(5611): 531-2.

Wang, Y., Kim, U.J., Blasioli, D.J., Kim, H.J., Kaplan, D.L. 2005. In vitro cartilage tissue engineering with 3D porous aqueous-derived silk scaffolds and mesenchymal stem cells. *Biomaterials* **26**(34): 7082-94.

Received: 26 May 2017
Accepted: 5 Jul 2017