Science & Technology Development Journal, 24(4):2204-2214

Open Access Full Text Article

Research Article

Camptothecin can increase the quantity of apoptotic bodies in a conditioned medium from human umbilical cord-derived mesenchymal stem cell culture

Phat Duc Huynh[1,2,*], Vy Quang Nguyen[1,2], Sao Thi Nguyen[2,3], Thuan Minh Le[1,2], Ngoc Bich Vu[2,3,*]

ABSTRACT

Introduction: Recent studies have demonstrated that extracellular vesicles, particularly apoptotic bodies (ABs) from mesenchymal stem cells (MSCs), play an important role in MSC-mediated immune regulation. However, studies on the apoptosis and ABs of umbilical cord mesenchymal stem cells are still limited. This study aimed to investigate the effects of camptothecin on increasing ABs in a conditioned medium consisting of a human umbilical cord-derived mesenchymal stem cell (hUCMSC) culture. **Method:** hUCMSCs were cultured in an expansion medium supplemented with 5 μM camptothecin (apoptosis inducer). The ABs were isolated using the centrifugation approach. The shape of the ABs was confirmed by reverse microscopy and the size of the collected population was confirmed using a particle size analyzer. The protein concentration of ABs was quantified using the Bradford assay. Apoptotic cells and ABs were stained with an optimized procedure using a FITC Annexin V/Dead Cell Apoptosis Kit, and the fluorescent signal was analyzed using ImageJ software and flow cytometry. **Results:** The protein concentrations of the ABs obtained in a conditioned medium without and with camptothecin after 48 h were 3.933 $\pm$ 0.037 μg and 5.567 $\pm$ 0.072 μg, respectively. The fluorescence signal analysis also showed that the number of apoptotic bodies also increased from 24 h (14.87 $\pm$ 1.84%) to 96 h (36.3 $\pm$ 3.99%). **Conclusion:** The results show that camptothecin can trigger hUCMSC apoptosis and increase the number of ABs in the conditioned medium. This research is a foundation for further studies into apoptosis and ABs in hUCMSCs.

Key words: MSC-ABs, ABs of MSC, MSC-EVS, MSC apoptosis, ABs, apoptosis bodies isolation.

[1]*Laboratory of Stem Cell Research and Application, University of Science, Viet Nam National University, Ho Chi Minh City, Viet Nam*

[2]*Viet Nam National University, Ho Chi Minh City, Viet Nam*

[3]*Stem Cell Institute, University of Science, Ho Chi Minh City, Viet Nam*

Correspondence

Phat Duc Huynh, Laboratory of Stem Cell Research and Application, University of Science, Viet Nam National University, Ho Chi Minh City, Viet Nam

Viet Nam National University, Ho Chi Minh City, Viet Nam

Email: hdphat@hcmus.edu.vn

Correspondence

Ngoc Bich Vu, Viet Nam National University, Ho Chi Minh City, Viet Nam

Stem Cell Institute, University of Science, Ho Chi Minh City, Viet Nam

Email: ngocvu@sci.edu.vn

History

- Received: 2021-05-14
- Accepted: 2021-11-21
- Published: 2021-12-31

DOI : 10.32508/stdj.v24i4.2560

Check for updates

INTRODUCTION

Mesenchymal stem cells (MSCs) have been widely used as a potential treatment for many diseases due to their tissue regeneration, immunosuppression, and anti-inflammation abilities. More recently, many studies have shown that the effect of MSCs could, at least in part, be facilitated by their extracellular secretory vesicles, including microvesicles, exosomes and apoptotic bodies (ABs), via apoptosis. However, most studies have focused on MSC-derived exosomes, whereas extensive studies on apoptosis and ABs in umbilical cord mesenchymal stem cells are still limited.

Apoptosis produces a large number of ABs containing many different cellular components, including microRNAs, mRNAs, DNA, proteins, and lipids. The AB size ranges from 1-5 μm, which is the largest form of extracellular vesicles, along with exosomes and microvesicles. ABs are characterized by the presence of phosphatidylserine in the membrane. In addition, they express MHC-II, allowing for direct antigen presentation to CD4+ T cells and the activation of immunological memory[2]. Many studies have shown that ABs are involved in immune regulation[2,3]. For example, the infusion of MSC-derived ABs has been shown to activate phagocytic cells which produce indoleamine 2,3-dioxygenase-secreting phagocytic cells — an immunosuppressant mediator — in a mouse model of graft-versus-host disease[4]. Furthermore, MSC-derived ABs also play an important role in endothelial balance by stimulating cell proliferation, improving tissue regeneration, and promoting the replacement of damaged cells[5]. Mouse ADSC-derived ABs reduce injury and mortality in septic syndrome in mice[1,6]. Although ABs produced in culture for treatment have not been widely used, all three types of extracellular vesicles have potential in regenerative medicine.

Camptothecin was discovered from plant extracts in the mid-1950s[7]. It has been shown to inhibit DNA and RNA synthesis, which causes DNA damage[8]. Cell cycle arrest in both the S and G2 phases is necessary for the action of this compound[9,10].

Cite this article : Huynh P D, Nguyen V Q, Nguyen S T, Le T M, Vu N B. **Camptothecin can increase the quantity of apoptotic bodies in a conditioned medium from human umbilical cord-derived mesenchymal stem cell culture**. *Sci. Tech. Dev. J.;* 24(4):2204-2214.

Camptothecin-induced apoptosis involves the induction of mitochondrial membrane depolarization, the activation of proapoptotic Bid and Bax, as well as caspase-9 and caspase-3, protein kinase c delta signaling, and the upregulation of p53 [11].

The different approaches to MSC-derived ABs use different isolation procedures. The resulting 16000 × g for 30 minutes usually refers to a small-size population ranging from 400 to 2000 nm [12]. In another study, after centrifugation at 300× g for 10 min, the supernatant was subsequently filtered through 5 and 1 μm filters, and the supernatant was further centrifuged at 2000× g for 20 min to pellet the ABs [13]. The size of the obtained population was up to 8 μm. However, the desirable size of the final population should range between 1 and 5 μm [14].

Studies regarding MSC-derived apoptosis and ABs are still limited. This experiment was performed to investigate the presence of ABs derived from hUCMSCs in a commercial culture medium. In addition, the study also proposed a procedure for the acquisition and detection of AB-derived hUCMSCs.

METHODS

Human umbilical cord mesenchymal stem cells

The frozen hUCMSCs were produced by the Stem Cell Institute. For thawing, the cells were kept at 37°C in a water bath for 2-3 minutes, added to a similar volume of ThawBest (Regenmedlab, Vietnam), and centrifuged at 500 g for 5 minutes. The pellet was resuspended with MSCCult I (Regenmedlab, Vietnam). The cells were cultured in T25 flasks at 37°C and 5% CO2.

The in vitro differentiation capability of hUCMSCs into osteoblasts, chondroblasts, and adipocytes was evaluated by an induced medium. The hUCMSCs were first cultured in a StemPro® Osteogenesis Differentiation Kit, StemPro® Chondrogenesis Differentiation Kit, and StemPro® Adipogenesis Differentiation Kit (Thermo Fisher Scientific, USA) to induce the differentiation of osteoblasts, chondroblasts, and adipocytes, respectively. After 14 to 21 days, the differentiated cells were evaluated by staining with Alizarin Red S for osteoblasts, Alcian Blue for chondroblasts, and Oil Red O for adipocytes (Sigma Aldrich, USA).

MSC marker expression was evaluated using flow cytometry through a FACSCalibur machine (BD Biosciences, Franklin, Lakes, NJ). In particular, the cells were stained with antibodies against CD14-FITC, CD19-PerCP, CD34-FITC, CD45-APC, CD73-PE, CD90-PerCP, CD105-PerCP, and HLA-DR-FITC (Santa Cruz Biotechnology, Dallas, TX). The cells were then incubated for 20 min at room temperature and analyzed using the BD CellQuest Pro software with 10,000 events.

Proliferation of hUCMSCs

hUCMSCs in MSCCult I and MSCCult I combined with 5 μM camptothecin (Thermo Fisher, USA) were seeded in $5x10^3$ cells/well in a 96-well plate. The experiment was repeated three times at each time point (24, 48, 72, and 96 h of culture). The collected cells were stained with Trypan Blue and counted using the erythrocyte counting method to evaluate proliferation. The cellular size was analyzed using the AxioVision 4.8 Software (Zeiss, USA).

Protein concentration

In a flat-bottom 96-well plate, 10 μl of PBS and 10 μl of each standard BSA dilution were loaded as follows: 0.25, 0.5, 0.75, 1, 1.5 and 10 μl ABs after thawing. Coomassie Blue G-250 dye (200 μl) was added to the wells and lightly mixed. Then the 96-well plate was measured for absorbance at 595 nm using a DTX 880 machine (Backman Coulter 880, U.S.A)

Detection of apoptotic cells and ABs

hUCMSCs were seeded in MSCCult I and MSCCult I combined with 5 μM camptothecin at a density of $3,9x10^5$ cells for each T25 flask. Cells were detached using the Detachment Reagent (Regenmedlab, Vietnam) after 24, 48, 72, and 96 h of culture. The spent culture medium was also collected for protein analysis.

Apoptotic cells

Detached cells were stained with a FITC Annexin V/Dead Cell Apoptosis Kit (Thermo Fisher, USA). The staining procedure followed the manufacturer's instructions. After the incubation, the cells were washed in cold PBS and then recentrifuged. The supernatant was discarded, and the cells were resuspended in 1X annexin-binding buffer. The density of the cells was determined and the cells were diluted in annexin-binding buffer to approximately 1×10^6 cells/mL. Afterward, 5 μl annexin V conjugate and 1 μl of 100 μg/ml PI working solution were added to each 100 μl of cell suspension. Cells were incubated at room temperature for 15 minutes and then washed with 1X Annexin-Binding buffer. Finally, the cells were deposited onto slides and fluorescence was observed.

ABs

The shape of ABs was confirmed by reverse microscopy and the size of the collected population was confirmed using a particle size analyzer (LB550, Horiba) (Institute for Nanotechnology, Vietnam).

The manufacturer's instructions for the FITC Annexin V/Dead Cell Apoptosis Kit for evaluating ABs were applied with some modifications. The medium after culturing hUCMSCs for 24 h, 48 h, 72 h, and 96 h underwent centrifugation at 500 g for 10 minutes to distract cell debris. The medium was centrifuged at 3000 g for 20 minutes to collect the pellet. The cells were resuspended in 1X Annexin-Binding buffer and then 5 μl annexin V conjugate and 1 μl of 100 μg/ml PI working solution were added to each 100 μl apoptotic body suspension. After incubation at room temperature for 15 minutes, the ABs were centrifuged at 3000 g for 20 minutes and washed with 1X Annexin-Binding buffer. The ABs were then resuspended in 100 μl of 1X Annexin-Binding buffer. Finally, 10 μl of the solution was deposited onto a slide for microscopic observation. AB marker expression was evaluated through the application of flow cytometry using FACS Melody.

Statistical analysis

The fluorescent signals were analyzed using ImageJ software. Standard errors ($\pm$) and the mean values of the various treatments were determined through the t-test analysis using the GraphPad Prism 8.4 software (San Diego, CA, USA), set at $p \leq 0.05$.

RESULTS

Characterization of hUCMSCs

After 1 day of culture, the cells started to attach to the plastic surface of the T25 flask. Their morphology was spindle-shaped and resembled fibroblasts (**Figure 1** A).

In the osteogenesis differentiation medium, the hUCMSCs stored and released ion2+, especially Ca^{2+}, which binds to Alizarin Red to form red complexes (**Figure 1** B). On the other hand, the StemPro® Chondrogenesis Differentiation medium induced the hUCMSCs to express aggrecans, which cooperated with the Alcian Blue to form blue complexes (**Figure 1** C). In the StemPro® Adipogenesis Differentiation medium, the hUCMSCs secreted lipid droplets that were cached red with Oil Red O (**Figure 1** D).

The results show that the cells maintained the surface markers of the MSCs after thawing and subsequent culturing to proliferate (**Figure 1**). They were negative for CD14 (**Figure 1** E), CD19 (**Figure 1** F), CD34 (**Figure 1** G), CD45 (**Figure 1** H), and HLA-DR (**Figure 1** I) and positive for CD73 (**Figure 1** J), CD90 (**Figure 1** K), and CD105 (**Figure 1** L).

Proliferation of hUCMSCs

The hUCMSCs cultured in the MSCCult I medium exhibited a steady accumulation of proliferation throughout each stage of observation (**Figure 2** A). Due to the strong proliferation but limited culture surface, the cells at the edge of the culture surface peeled off in clusters at 96 h. Meanwhile, the cells in the MSCCult I medium combined with 5 μM camptothecin remained at a nearly unchanged density throughout the study (**Figure 2** A). The analysis of the hUCMSC proliferation through the erythrocyte counting method also provided a similar result. The density ($x10^4$) of the hUCMSCs in the MSCCult I medium at 24 h was 4.25 $\pm$ 0.25 cells/ml. This increased to 7.58 $\pm$ 0.38 cells/ml at 48 h and 13.83 $\pm$ 1.37 cells/ml at 72 h. At 96 h, this density decreased to 9.4 $\pm$ 0.87 cells/ml. Meanwhile, with the same initial culture density, after 24 hours, the hUCMSCs decreased to 1.75 $\pm$ 0.25 cells/ml, a slight increase at 48 h with 2.33 $\pm$ 0.52 cells/ml, but then slightly decreased to 2.25 $\pm$ 0.43 cells/ml at 72 hours and 1.92 $\pm$ 0.38 cells/ml at 96 h (**Figure 2** B).

The cellular size of the hUCMSCs in the MSCCult I medium supplemented with camptothecin was also larger than that in the MSCCult I medium during the experiment (**Figure 2** C). The hUCMSCs cultured in the MSCCult I environment maintained size stability with small variations, with 21.67 $\pm$ 1.02 at 24 h, 21.7 $\pm$ 0.76 at 48 h, 24.1 $\pm$ 1.7 at 72 h, and 23.4 $\pm$ 2.16 at 96 h. The difference was not statistically significant. Meanwhile, the size of the hUCMSCs in the MSCCult I medium supplemented with camptothecin increased over time. In particular, the cell size was 24.7 $\pm$ 1.02 at 24 h, 32.2 $\pm$ 1.05 at 48 h, 34.12 $\pm$ 2.03 at 72 h, and 37.17 $\pm$ 1.05 at 96 h.

Detection of apoptotic cells

Annexin V/PI was used to measure the number of apoptotic cells cultured in the MSCCultI medium supplemented with camptothecin. At 24 h of culture, only Annexin V-positive cells were detected (pink arrow). However, after 48 hours, 72 hours, and 96 hours of culture, there were cells that stained positive for both Annexin V and PI (purple arrow) (**Figure 3**). Meanwhile, no positive signals or weak signals for Annexin V and PI were observed among the MSCs cultured in the camptothecin-free MSCCult I medium during the experiment (**Figure 4**).

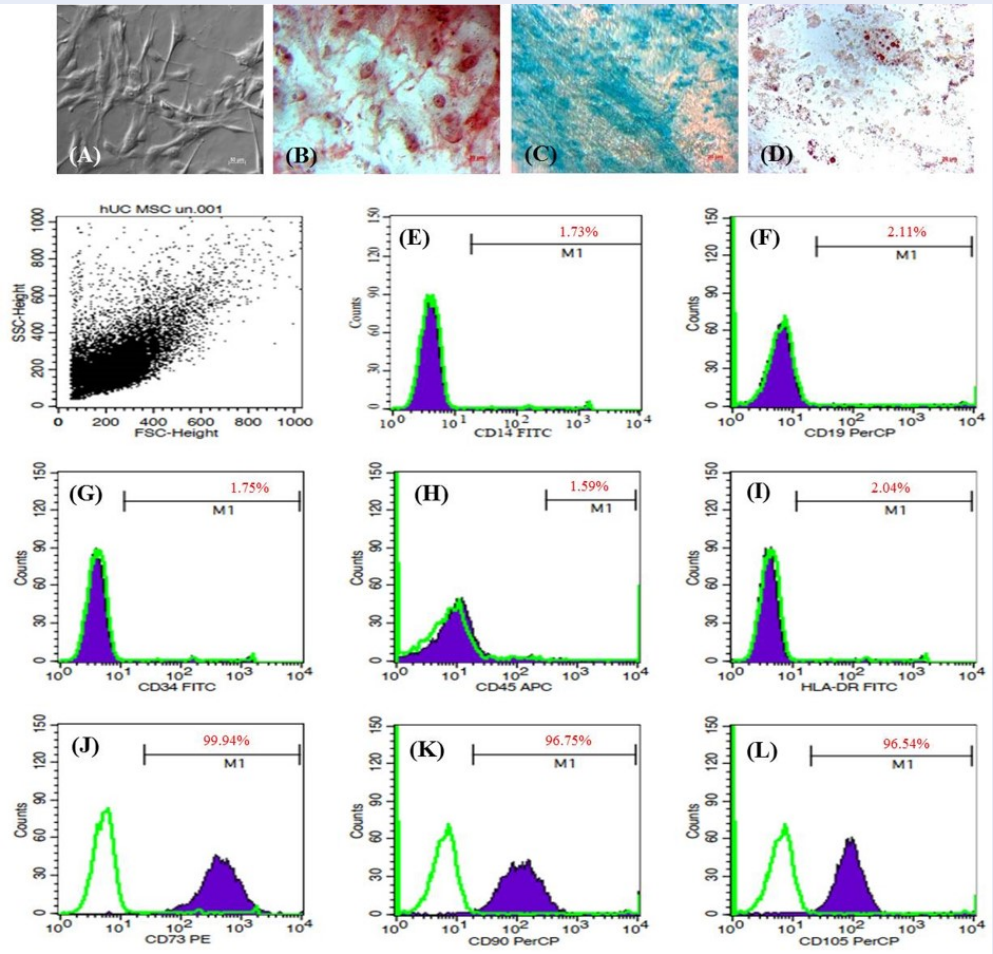

Figure 1: **Characterization of the hUCMSC proliferation**. The hUCMSCs exhibited fibroblast-like morphology (**A**); in vitro differentiation into osteoblasts (**B**), chondrocytes (**C**), and adipocytes (**D**); the cells had positive surface markers such as CD73 (**J**), CD90 (**K**), and CD105 (**L**) and negative surface markers such as CD14 (**E**), CD19 (**F**), CD34 (**G**), CD45 (**H**), and HLA-DR (**I**).

ABs derived from the hUCMSCs

The ABs that were stained with annexin V gave a green signal (white arrow) (**Figure 5** A). The collected population was confirmed using a particle size analyzer (LB550, Horiba) which showed that the size of the ABs varied from 1 μm to less than 6 μm, with no signal below 1 μm. In particular, the number of apoptotic bodies obtained below 5 μm in size accounted for nearly 97%, and other bodies in the range of 5 – 6 μm accounted for only 3%. (**Figure 5** B). The seed size in the population was concentrated in the range of 2.5 - 4.5 μm (accounting for more than 65% of the population) (**Figure 5** B). This result was also confirmed by reverse microscopy. The apoptotic bodies had a round seamless shape and existed in a single, non-cluster form after the centrifugation process (**Figure 5** C).

The protein concentration of the MSCCult I medium was 1.085 $\pm$ 0.0141 mg/ml, which increased to 1.239 $\pm$ 0.03 mg/ml after 48 h of culture. Meanwhile, the total protein concentration in MSCCult I supplemented with camptothecin after 48 h of culture was 1.116 $\pm$ 0.04 mg/ml, which was not much different from that at 0 h of culture. The ABs obtained in the MSCCult I medium supplemented with camptothecin at 48 h were 5.567 $\pm$ 0.072 μg/ml, and the concentration was higher than that of the ABs obtained in the MSCCult I medium at 3.933 $\pm$ 0.037 μg/ml (**Figure 5** F). This result is similar to the analysis of the fluorescence signal of the ABs. In addition, the results of flow cytometry showed that in the particle population obtained in MSCCult 1 medium at 48 h, only 0.12% of the total particles were ABs, while 1.49% were candidates for ABs (**Figure 5** D).

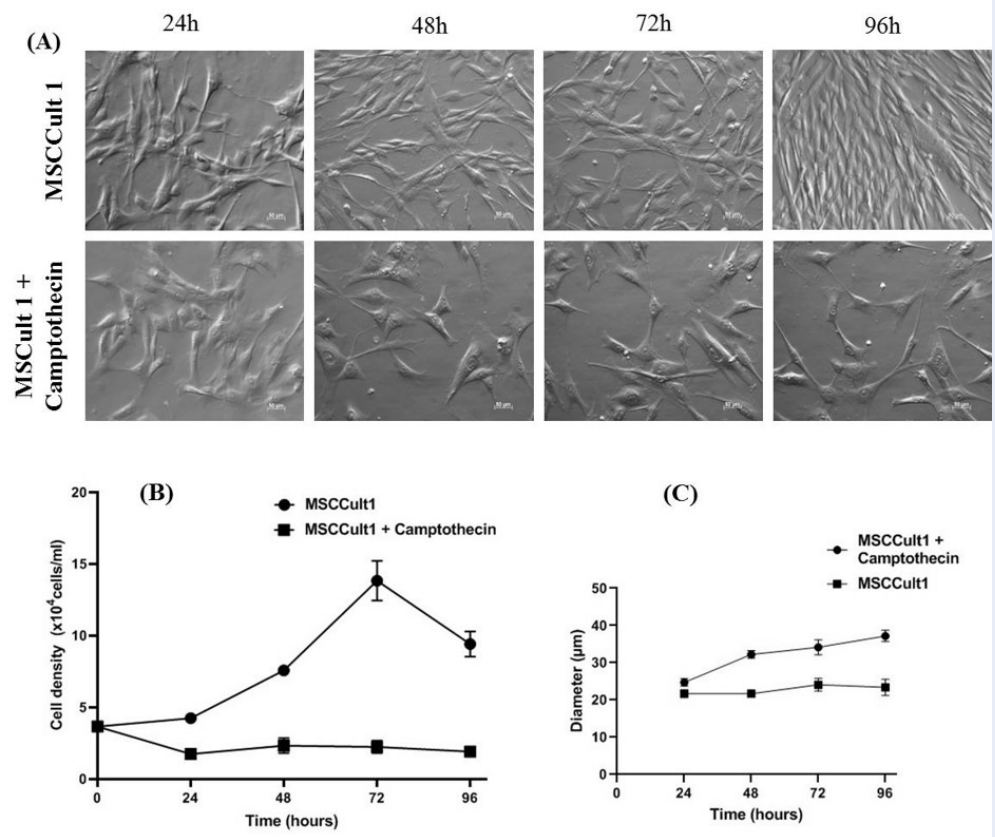

Figure 2: **The proliferation of the hUCMSCs in the MSCCult I medium and MSCCult I + 5 μM camptothecin for 24 h, 48 h, 72 h, and 96 h**. (**A**) The morphology and proliferation of HUCMSCs; (**B**) hUCMSC proliferation assessed using the RBC count method; (**C**) The size of hUCMSCs after being detached and analyzed using the Axiovision 4.8 software (Zeiss, USA).

All samples of the ABs collected in both media over time exhibited a green signal of annexin V (**Figures 6 and 7**). However, the ratio of the green signal of annexin V to the black background signal of each sample was different. The analysis of this rate through the ImageJ software showed that the annexin V signal of the hUCMSC ABs in the MSCCult I medium supplemented with camptothecin increased linearly from 24 h (14.87 $\pm$ 1.84%) to 96 h (36.3 $\pm$ 3.99%). On the other hand, the rate of ABs in the MSCCult medium was maintained at a low level at 24 hours (7.9 $\pm$ 2.46%), 48 hours (11.86 $\pm$ 0.67%), and 72 hours (12, 15 $\pm$ 3.18%). In general, the annexin V green fluorescence signal in this treatment increased slightly but this increase was not found to be statistically significant. Surprisingly, this rate dropped to only 3.8 $\pm$ 2.03% at 96 h (**Figure 8**).

DISCUSSION

The topic of extracellular vesicles derived from MSCs is drawing increasing attention in terms of their therapeutic potential. The ABs from the MSCs are one of the three types of extracellular vesicles with the largest size while also being the easiest to collect and containing the most content. Furthermore, the standardized protocol for the isolation and purification of ABs is still a challenge. The isolation method for the ABs is generally a universal protocol that is applied to all cell types. Therefore, a specific and clear process to isolate the ABs derived from hUCMSCs is truly necessary.

After thawing, the hUCMSCs adhere and proliferate on the culture surface. Their morphology was similar to that of fibroblasts. They differentiated into chondroblast , adipocytes, and osteoblasts. Furthermore, the hUCMSCs positively expressed CD73, CD90, and CD105 and negatively expressed CD14, CD19, CD34, CD45, and HLA-DR. This result is in line with the Mesenchymal and Tissue Stem Cell Committee of the

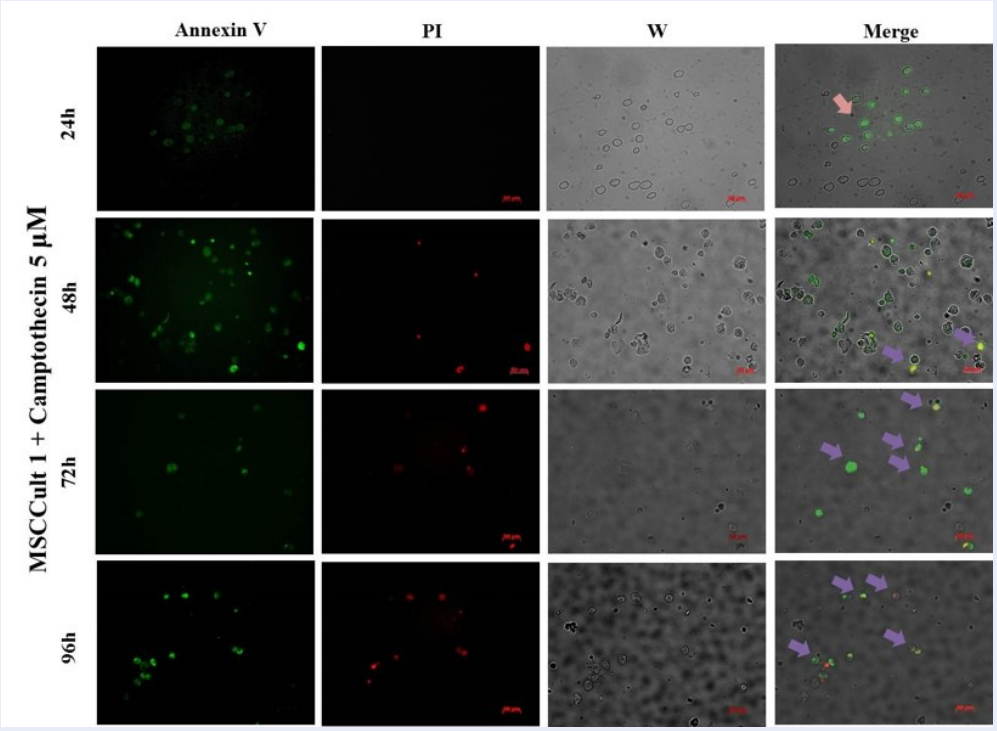

Figure 3: **Representative images of the Annexin V/PI staining of the hUCMSCs cultured in MSCCult I + 5 μM camptothecin for 24 h, 48 h, 72 h, and 96 h**. Annexin V/PI staining was detected in early apoptosis (pink arrow) and late apoptosis (violet arrow).

International Society for Cellular Therapy, which proposes minimal criteria to define MSCs[15]. These results are also equivalent to the research by Phuc *et al.*[16].

The MSCCult I commercial medium was selected for this study due to its appropriate nutritional conditions, osmotic pressure, and pH value for MSC growth and development (http://cipp.com.vn/). Camptothecin is an anticancer compound that causes cell cycle arrest at the S and G2 phases[17], inhibiting the activity of the DNA enzyme topoisomerase-I (topoisomerase-I)[11,17]. Arrested cells are unable to grow and initiate apoptosis, and hUCMSCs are no exception to this (**Figure 2** B). For the cells that entered apoptosis, the nucleus was compressed and the volume of the cell decreased. However, the cellular size of the MSCs increased from 24 to 96 h of culture. Meanwhile, early apoptotic cells appeared at 24 h (**Figure 3**). The cells in which apoptosis was induced by camptothecin were in phase S and phase G2[17], at which point the hUCMSCs had synthesized enough DNA and/or protein. This explains why the size of the cells during this investigation period was larger than that of the control group. At 96 h, we noted that the cells were also transitioning to late apoptosis, some of which were still in the early stage of apoptosis. Depending on the signal, apoptosis can last only a few hours or even days[2,18].

The size of the ABs ranges from 1-5 μm[14]. The protocol for the AB collection used in this experiment was able to isolate the ABs with high purity with particle sizes ranging from 1 to 5 μm accounting for 97%. The phosphatedinserin expression on the lipid membrane surface is a characteristic feature of both ABs and apoptotic cells. Therefore, it can be easily detected with Annexin V (**Figure 5**). The procedure used in this study showed better results than in the previous studies[12,13]. There are numerous studies on MSC-derived exosomes that have had a positive impact on many pathological animal models[19,20]. If the obtained population is smaller (microvesicles <1 μm, exosomes 30-150 μm[18]), the results of the investigation into the impact of the ABs on pathology will be less reliable. Therefore, the procedure in this study lays the groundwork for further studies on ABs derived from MSCs.

In this study, we observed the AB ratio 48 h after the initiation of the culture. The percentage of observable

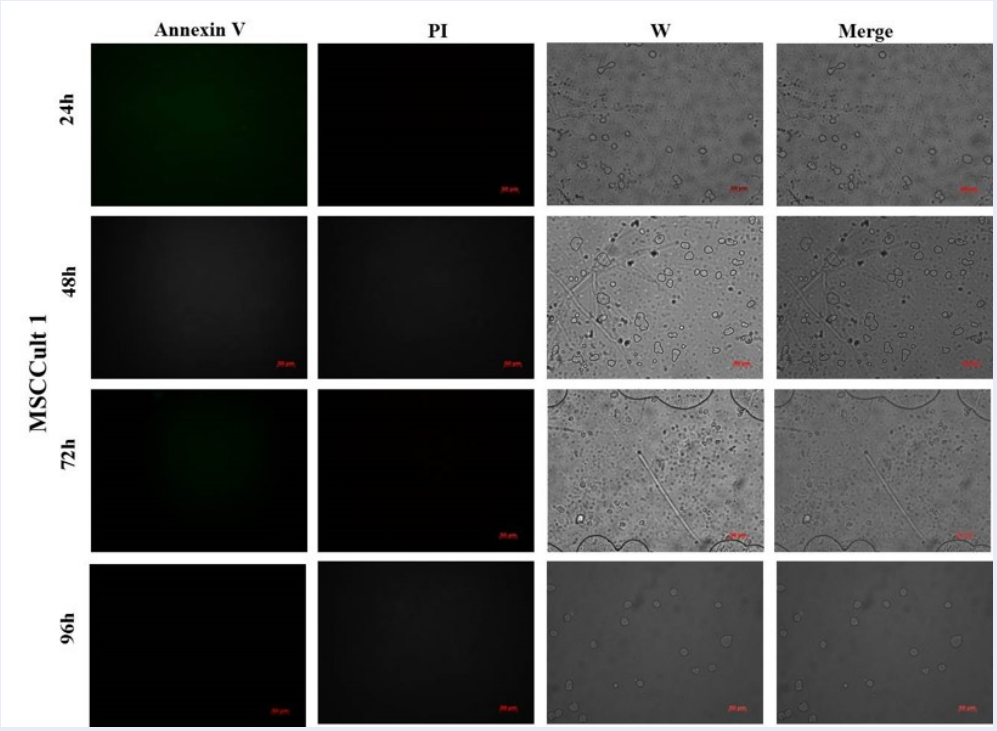

Figure 4: **Representative images of Annexin V/PI staining of hUCMSCs cultured in MSCCult I for 24 h, 48 h, 72 h, and 96 h.**

ABs recorded after 48 h of culture was particularly low (only 0.12% confirmed and 1.49% AB-potential bodies). According to previous studies, cells produce many elements, such as particles, proteins, organelles, and cell debris, during their developmental progress. These are subsequently released into their surroundings when the cells undergo apoptosis. ABs make up a very small proportion of the collected population with sizes ranging from 1 to 6 μm (**Figure 5** B). This result confirmed that the induction of apoptosis in MSCCult1 in vitro cultures was trivial. Instead, MSCCult 1 is suitable for the proliferation of hUCMSCs. We have not found a study evaluating the presence of ABs in such culture conditions. Studies on the ABs of MSCs have previously been evaluated after induction by physical and chemical agents [12,21].

All samples of the ABs obtained from the two treatments showed fluorescence signals (**Figures 6 and 7**). The increase in late apoptotic cells was associated with an increase in the green fluorescent signals of annexin V. The results also showed that ABs were produced in late apoptosis [2,6]. Fluorescence signals from the apoptotic cells in the MSCCult I medium were not detected or were only detectable at a very low rate. This may be because the ratio between the number of apoptotic cells and normal cells is too low. Moreover, the number of hUCMSC-derived ABs in MSCCult I showed a strong decrease after 96 h of culture. Research has shown that ABs last for a short period in the culture medium [2,18]. It can be assumed that because the number of newly born ABs is much less than that of the dead ABs, there is a sharp decrease.

CONCLUSIONS

The study confirmed the presence of ABs in the hUCMSCs when cultured in commercial MSCCult I medium. The study also proposed a centrifugation procedure to obtain a suitable size of apoptotic bodies from the hUCMSCs. This research is a basis for further studies looking into apoptosis and ABs in the hUCMSCs. We proposed that studies on commercial culture media can quantify apoptotic bodies as a criterion for further optimization and development.

ABBREVIATIONS

ABs: apoptotic bodies
ADSCs: Adipose tissue-derived stem cells
CD: cluster of differentiation
hUCMSC: human umbilical cord mesenchymal stem cell
MHC-II: major histocompatibility complex class II

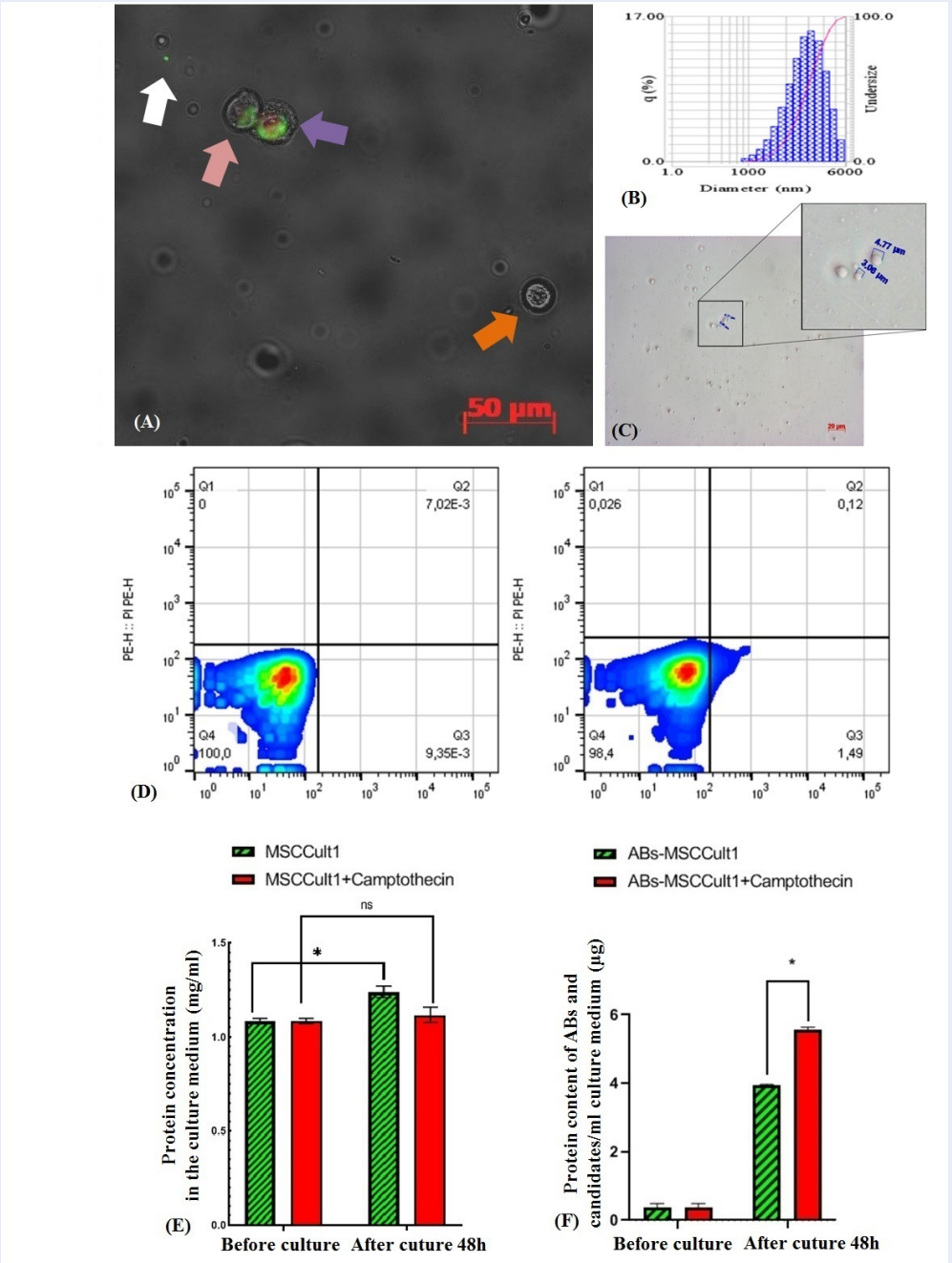

Figure 5: **The ABs of hUCMSCs**. (**A**) Annexin V /PI staining detected early apoptosis (pink arrow), late stage of apoptosis (violet arrow), normal cell (orange arrow), and apoptotic body (white arrow); (**B**) The population size of ABs was determined by Particle Size Analyzer (LB550, Horiba); (**C**) The size of ABs was measured by inverted microscope (40x). (**D**) Flow cytometry analysis of ABs in MSCCult1 at 48h. (E, F) Protein concentration of culture medium and ABs at pre-culture and after 48h in MSCCult I and MSCCult I + camptothecin, respectively.

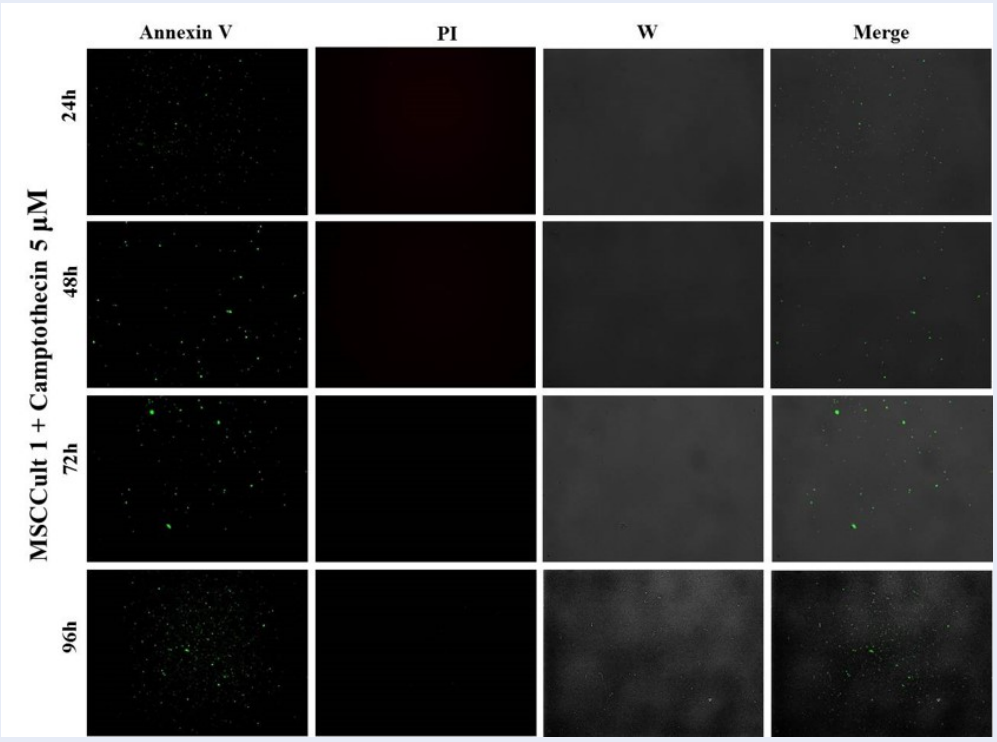

Figure 6: **The ABs were harvested from MSCCult I + 5μM camptothecinafter staining Annexin V /PI.**

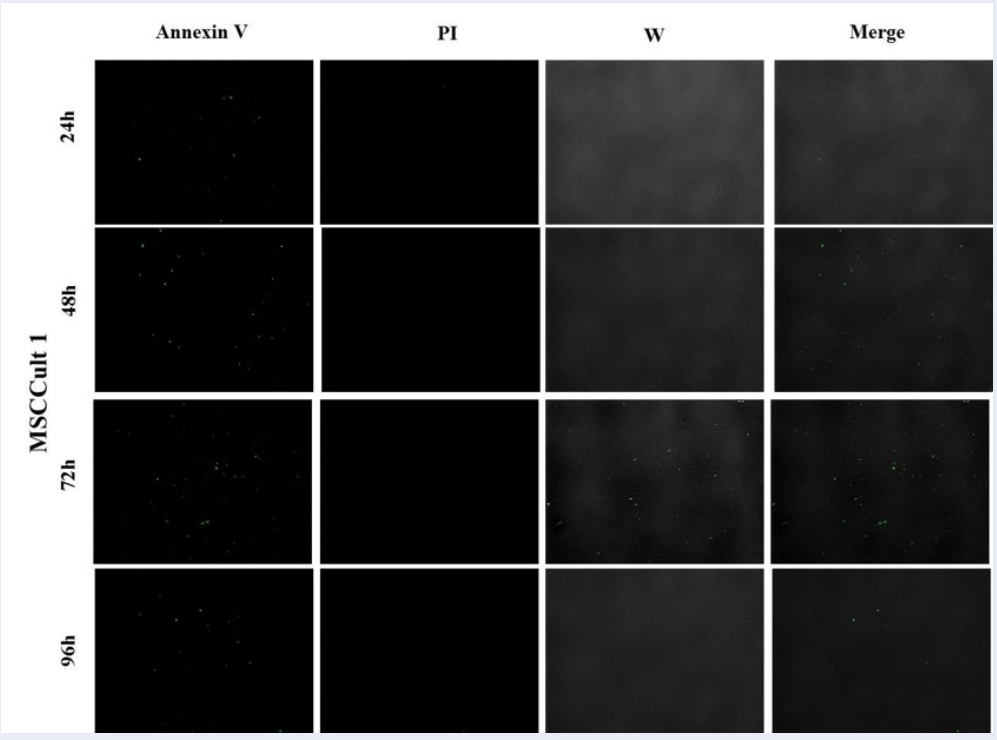

Figure 7: **The ABs were harvested from MSCCult I after staining with Annexin V/PI.**

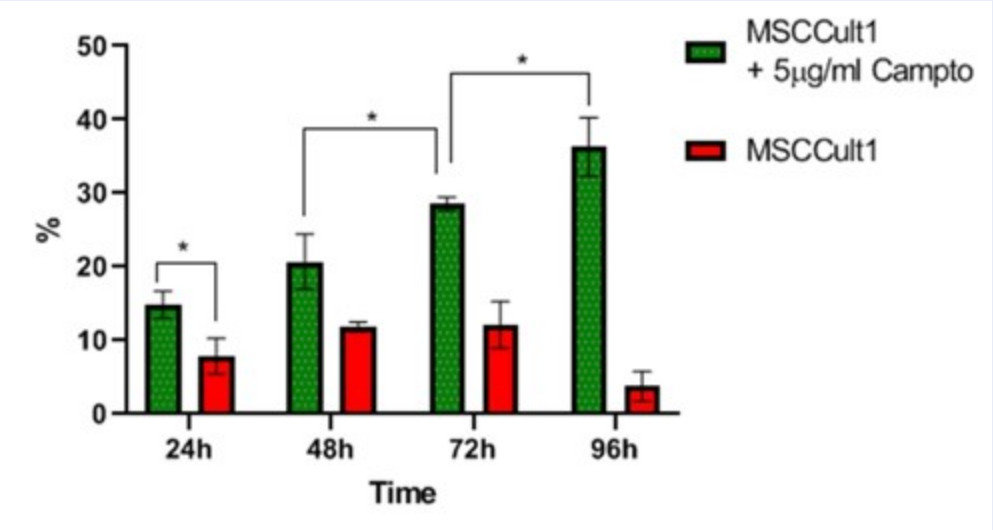

Figure 8: Fluorescence analysis of the ABs between MSCCult I and MSCCult I combined with 5 μM camptothecin using ImageJ software.

ACKNOWLEDGMENTS

None.

AUTHOR'S CONTRIBUTIONS

All authors equally contributed to this work. All authors read and approved the final version of the manuscript for submission.

FUNDING

This research is funded by University of Science, VNU-HCM under grant number T2020-29.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Chang CL, Leu S, Sung HC, Zhen YY, Cho CL, Chen A. Impact of apoptotic adipose-derived mesenchymal stem cells on attenuating organ damage and reducing mortality in rat sepsis syndrome induced by cecal puncture and ligation. Journal of Translational Medicine. 2012;10(1):244. PMID: 23217183. Available from: 10.1186/1479-5876-10-244.
2. Caruso S, Poon IK. Apoptotic Cell-Derived Extracellular Vesicles: More Than Just Debris. Frontiers in Immunology. 2018;9:1486. PMID: 30002658. Available from: 10.3389/fimmu.2018.01486.
3. Caruso S, Atkin-Smith GK, Baxter AA, Tixeira R, et al. Defining the role of cytoskeletal components in the formation of apoptopodia and apoptotic bodies during apoptosis. Apoptosis. 2019;24(11-12):862–877. PMID: 31489517. Available from: 10.1007/s10495-019-01565-5.
4. Galleu A, Riffo-Vasquez Y, Trento C, Lomas C, Dolcetti L, Cheung TS. Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. Science Translational Medicine. 2017;9(416):416. PMID: 29141887. Available from: 10.1126/scitranslmed.aam7828.
5. Li B, Zhang H, Zeng M, He W, Li M, Huang X. Bone marrow mesenchymal stem cells protect alveolar macrophages from lipopolysaccharide-induced apoptosis partially by inhibiting the Wnt/β-catenin pathway. Cell Biology International. 2015;39(2):192–200. PMID: 25229877. Available from: 10.1002/cbin.10359.
6. Sung PH, Chang CL, Tsai TH, Chang LT, Leu S, Chen YL, et al. Apoptotic adipose-derived mesenchymal stem cell therapy protects against lung and kidney injury in sepsis syndrome caused by cecal ligation puncture in rats. Stem Cell Research & Therapy. 2013;4(6):155. PMID: 24451364. Available from: 10.1186/scrt385.
7. Wall ME, Wani MC, Cook C, Palmer KH, McPhail AT, Sim GA. Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from camptotheca acuminata1, 2. Journal of the American Chemical Society. 1966;88(16):3888–90. Available from: 10.1021/ja00968a057.
8. Horwitz SB, Horwitz MS. Effects of camptothecin on the breakage and repair of DNA during the cell cycle. Cancer Research. 1973;33(11):2834–6. PMID: 4748440.
9. Goldwasser F, Shimizu T, Jackman J, Hoki Y, O'Connor PM, Kohn KW. Correlations between S and G2 arrest and the cytotoxicity of camptothecin in human colon carcinoma cells. Cancer Research. 1996;56(19):4430–7. PMID: 8813137.

10. Tsao YP, D'Arpa P, Liu LF. The involvement of active DNA synthesis in camptothecin-induced G2 arrest: altered regulation of p34cdc2/cyclin B. Cancer Research. 1992;52(7):1823–9. PMID: 1312900.
11. Albihn A, Mo H, Yang Y, Henriksson M. Camptothecin-induced apoptosis is enhanced by Myc and involves PKCdelta signaling. International Journal of Cancer. 2007;121(8):1821–9. PMID: 17565738. Available from: 10.1002/ijc.22866.
12. Liu J, Qiu X, Lv Y, Zheng C, et al. Apoptotic bodies derived from mesenchymal stem cells promote cutaneous wound healing via regulating the functions of macrophages. Stem Cell Res Ther. 2020;11(1):507. Available from: 10.1186/s13287-020-02014-w.
13. Liu D, Kou X, Chen C, Liu S, Liu Y, Yu W. Circulating apoptotic bodies maintain mesenchymal stem cell homeostasis and ameliorate osteopenia via transferring multiple cellular factors. Cell Research. 2018;28(9):918–33. PMID: 30030518. Available from: 10.1038/s41422-018-0070-2.
14. Meckes DG, Raab-Traub N. Microvesicles and viral infection. Journal of Virology. 2011;85(24):12844–54. PMID: 21976651. Available from: 10.1128/JVI.05853-11.
15. Dominici M, Blanc KL, Mueller I, Slaper-Cortenbach I, Marini F, Krause D. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315–7. PMID: 16923606. Available from: 10.1080/14653240600855905.
16. Pham PV, Truong NC, Le PT, Tran TD, Vu NB, Bui KH. Isolation and proliferation of umbilical cord tissue derived mesenchymal stem cells for clinical applications. Cell and Tissue Banking. 2016;17(2):289–302. PMID: 26679929. Available from: 10.1007/s10561-015-9541-6.
17. Jayasooriya RGPT, Dilshara MG, Molagoda IMN, Park C, Park SR, Lee S. Camptothecin induces G2/M phase arrest through the ATM-Chk2-Cdc25C axis as a result of autophagy-induced cytoprotection: implications of reactive oxygen species. Oncotarget. 2018;9(31):21744–57. PMID: 29774099. Available from: 10.18632/oncotarget.24934.
18. Elmore S. Apoptosis: a review of programmed cell death. Toxicologic Pathology. 2007;35(4):495–516. PMID: 17562483. Available from: 10.1080/01926230701320337.
19. Hoang DH, Nguyen TD, Nguyen HP, Nguyen XH, Do PT, Dang VD. Differential Wound Healing Capacity of Mesenchymal Stem Cell-Derived Exosomes Originated From Bone Marrow, Adipose Tissue and Umbilical Cord Under Serum- and Xeno-Free Condition. Frontiers in Molecular Biosciences. 2020;7:119. PMID: 32671095. Available from: 10.3389/fmolb.2020.00119.
20. He XD, Cao Y, Wang H, Liu S, Liao L, Jin Y, et al. MSC-Derived Exosome Promotes M2 Polarization and Enhances Cutaneous Wound Healing. Stem Cells Int. 2019;2019:7132708. Available from: 10.1155/2019/7132708.
21. Xu X, Lai Y, Hua ZC. Apoptosis and apoptotic body: disease message and therapeutic target potentials. Biosci Rep. 2019;39(1):BSR20180992. PMID: 30530866. Available from: 10.1042/BSR20180992.

Tạp chí Phát triển Khoa học và Công nghệ
Đại học Quốc gia Tp. Hồ Chí Minh

Tạp chí Phát triển Khoa học và Công nghệ
ISSN: 1859-0128
Hình thức xuất bản: In và trực tuyến
Hình thức truy cập: Truy cập mở (Open Access)
Ngôn ngữ bài báo: Tiếng Anh
Tỉ lệ chấp nhận đăng 2021: 72%
Phí xuất bản: Miễn phí
Thời gian phản biện: 43 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Tự nhiên
ISSN: 2588-106X
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 75%
Phí xuất bản: Miễn phí
Thời gian phản biện: 30-45 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Kĩ thuật và Công nghệ
ISSN: 2615-9872
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 61%
Phí xuất bản: Miễn phí
Thời gian phản biện: 50 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Kinh tế-Luật và Quản lý
ISSN: 2588-1051
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 65%
Phí xuất bản: Miễn phí
Thời gian phản biện: 45 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Xã hội và Nhân văn
ISSN: 2588-1043
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 62%
Phí xuất bản: Miễn thu phí đối với tác giả là CBVC của ĐHKHXHNV, ĐHQG-HCM; Tác giả khác: 500.000 VNĐ/bài
Thời gian phản biện: 75 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Trái đất và Môi trường
ISSN: **2588-1078**
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt và tiếng Anh
Tỉ lệ chấp nhận đăng 2021: 87%
Phí xuất bản: liên hệ tòa soạn
Thời gian phản biện: 45 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Sức khỏe
ISSN: 2734-9446
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 70%
Phí xuất bản: Miễn phí
Thời gian phản biện: 30 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

SCAN ME

Tạp chí Phát triển Khoa học và Công nghệ, Đại học Quốc gia Tp.HCM
25 năm xuất bản học thuật (1997-2022)
Tòa soạn: Nhà điều hành Đại học Quốc gia Tp.HCM, P. Linh Trung, TP. Thủ Đức, TP. HCM
Email: stdj@vnuhcm.edu.vn; tcptkhcn@vnuhcm.edu.vn; Website: *http://www.scienceandtechnology.com.vn*