Recovery and Maintenance of NESTIN Expression in Umbilical Cord-MSC Using a Novel Culture Medium

Yuncheng Liu
National-Local Associated Engineering Laboratory for Personalized Cellular Therapy
Feidi Xiao
Shenzhen Bieke Biotechnology Co., Ltd.
Xiang Hu
Shenzhen Bieke Biotechnology Co., Ltd.
Zan Tang
Shenzhen Beike Bieke Biotechnology Co., Ltd.
Zeqin Fu
Shenzhen Beike Biotechnology Co., Ltd
Xiao Liang
Shenzhen Beike Biotechnology Co., Ltd
Guifang Zeng
Shenzhen Beike Biotechnology Co., Ltd.
Weijie Zeng
National-Local Associated Engineering Laboratory for Personalized Cellular Therapy
Yan Liao
Shenzhen Beike Biotechnology Co., Ltd.
Yuan Ren
National-Local Associated Engineering Laboratory for Personalized Cellular Therapy
Zhiyu Liu
National-Local Associated Engineering Laboratory for Personalized Cellular Therapy
Hao Peng
National-Local Associated Engineering Laboratory for Personalized Cellular Therapy
Qiuhong Mei
Shenzhen Kenuo Medical Lab
Muyun Liu (liumuyun@nlelpct.com)
National-Local Assoctiated Engineering Laboratory for Personalized Cellular therapy

---

Original article

Keywords: NESTIN, UC-MSC, culture medium, UltraGRO

Posted Date: April 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-20909/v1

Version of Record: A version of this preprint was published on July 28th, 2020. See the published version at https://doi.org/10.1186/s13568-020-01067-7.

Abstract

Mesenchymal stem cells (MSC) are a popular candidate in cellular therapy for many diseases. It is well known by its feature of self-renewal and differentiation potential. NESTIN is a cytoskeletal protein that expresses in MSC and plays an important role in cell proliferation and differentiation. Here, we demonstrated that adding UltraGRO, which is a medium supplement, could maintain and partially recover the expression of NESTIN in human umbilical cord derived MSCs (UC-MSCs). Furthermore, the UC-MSCs cultured with UltraGRO showed a better immunomodulation ability in colitis mouse model compared with that cultured in other mediums. This indicates that the use of novel culture medium benefits to the maintenance of NESTIN expression and NESTIN may be one of the vital factors that regulate the performances of MSC.

Introduction

Since Friedenstein and co-workers first identified the differentiation potential of bone marrow cells in 1987, it has given a rise to numerous studies on stem cell in various fields. It demonstrated “bone marrow fibroblasts” and their descendants originated from non-hematopoietic could differentiate into mesenchymal cells of other tissues (Friedenstein, Chailakhyan, & Gerasimov, 1987). The term “mesenchymal stem cell” (MSC) is widely spread mainly due to its feature of self-renewal and differentiation potential (Uder, Bruckner, Winkler, Tautenhahn, & Christ, 2018). The International Society of Cell Therapy (ISCT) specifically defines human MSC by three criterions. The first is that MSCs have a capacity of adherence to plastic surfaces, which is utilized to easily isolate cells from the tissues under standardized culture condition. Second, MSCs from different sources share a similar combination of surface marker profile, that is, up to 95% of MSC population expresses CD105, CD73, and CD90. However, less than 2% of MSC may express CD45, CD34, CD14or CD11b, CD79 or CD19 and HLA-DR (Dominici et al., 2006). Lastly, MSC must feature a robust differentiation potential, which must be able to differentiate into at least three different lineages in vitro, such as osteoblasts, adipocytes, and chondrocytes (Uder et al., 2018). These criterions have become a golden standard in stem cell therapy industry to confirm MSC during all manufacture processes, from cells isolation to stimulated culture and finally to large-scale expansion.

In spite of MSC was first identified from bone marrow, it was also isolated from many other tissues and organs, such as muscle, thymus, pancreas, adipose, dental pulp, umbilical cords, and placental (Ferrari et al., 1998; B. M. Seo et al., 2004; Zuk et al., 2001). The distribution of MSCs in many different organs may because MSC can generate and migrate via large and small blood vessels. It also suggested that it might be relevant to their residential preference in perivascular niches throughout the post-natal organism (da Silva Meirelles, Chagastelles, & Nardi, 2006). Accordingly, MSC was manufactured by adipose, umbilical cords, and placental, which are commonly treated as clinical wastes, under current Good Manufacture Practice (cGMP) in stem cell therapy industry. However, flow cytometry analyses revealed MSCs between different sources and species have similar but not identical surface markers and functions (Donnenberg & Ulrich, 2013). Thus, investigations in expression of surface markers and morphology were performed to

monitor the association of effects on variability in terms of isolation yield, proliferation rates, and expandability (Kern, Eichler, Stoeve, Kluter, & Bieback, 2006; Oedayrajsingh-Varma et al., 2006; M. J. Seo, Suh, Bae, & Jung, 2005).

NESTIN, known as a neuroepithelial stem cell protein, is also a cytoskeletal protein expressed in stem cells (Lendahl, Zimmerman, & McKay, 1990; Mignone, Kukekov, Chiang, Steindler, & Enikolopov, 2004). It is believed that NESTIN plays a role in the stem cell function of self-renew, proliferation, differentiation, and migration (Bernal & Arranz, 2018; Kulkarni et al., 2017; Mignone et al., 2004). According to the gene sequence and protein structure homology, NESTIN is classified as type VI intermediate filament that contributes to cytoskeleton constitution (Bernal & Arranz, 2018). The constitutive expression of NESTIN by undifferentiated MSC is regarded as a marker of “multi-differentiated” state that cells can retain their neuronal differentiation property (Tondreau et al., 2004). This also well explains the high plasticity of MSC both in vitro and in vivo. Besides, NESTIN expression is developmentally regulated as it was revealed inversely correlated with cell differentiation (Wiese et al., 2004). The down regulation of NESTIN caused by the transition from proliferating neural stem cell to post-mitotic neurons with specific stimulus was observed (Wiese et al., 2004; Zimmerman et al., 1994). Many of the recent studies have indicated that NESTIN may be an important reporter of cell state related to proliferation and differentiation potential of MSC.

However, NESTIN is now not a common selective marker for MSC in both ISCT criterion and stem cell therapy industry. MSC is prominent for its self-renew potential which allows the propagation of MSC in vitro under specific isolation and cultivation procedures (Uder et al., 2018). Since MSC application for clinical use has a strict requirement in sufficient cell number and consistent cell quality all along the whole cultivation process, the expansion in vitro of MSC was extensively investigated. It revealed that the isolation, culture, and expansion procedures are possible factors that would result in the inconsistency (Lodie et al., 2002; Uder et al., 2018). The conventional MSC culture protocol as it was described in numerous studies is using Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS). Since frequent iterated passaging for MSC would lead to cell aging and loss of stemness, human MSC culture was confined beneath the 6th passage in order to maintain the unrestricted differentiation capacities (Bonab et al., 2006; Halfon, Abramov, Grinblat, & Ginis, 2011). In this study, we monitored the NESTIN expression in umbilical cords MSCs (UC-MSCs) in vitro for up to 10 passages using three different culture mediums. Moreover, we further verified the immunomodulatory ability of UC-MSCs in colitis mice models.

Materials And Methods

Human UC-MSCs isolation and culture

Institutional review board approval was obtained for all procedures. Fresh umbilical cords (UC) were collected for scientific study from healthy donors who were informed and consented. Mesenchymal tissue was scraped off from Wharton’s jelly after blood vessels were removed (Devito et al., 2019). After

cutting into pieces, the tissue was centrifuged at 600 g for 10 min at room temperature. Tissue was then washed with 0.9% saline and cultured in 37 °C and 5% CO2 with serum-free Dulbecco's modified Eagle's medium (DMEM). The primary UC-MSCs (P0) was obtained after 10-days culture. A sufficient number of MSCs from the identical source was equally divided into three groups in order to ensure the same starting condition. During the 10-passage culture, UC-MSCs were plated at the density of 5×10^3 cells/cm^2into flask for each group and cultured at 37 °C and 5% CO2.Three culture medium supplements were applied for comparison including fetal bovine serum (FBS), fetal bovine serum substitute (FS) (TBD, China), and UltraGRO-Advanced (Helios Bioscience, UK), while the base culture mediums are identical which is DMEM/F-12 (Gibco, US). The first culture ingredient consists of DMEM and 10% FBS, which is the conventional culture medium. The second one is DMEM with 2% TBD FS and 1% GlutaMax, the 3rd one is DMEM with 5% UltraGRO-advanced and 1% GlutaMax.

Induction Assay For Uc-msc Differentiation

Based on the published procedure, the UC-MSCs was induced to differentiate into osteogenic, adipogenic and chondrogenic in vitro (Lei et al., 2013).

Uc-msc Assay Proliferation

To investigate the effects of different culture systems, 10th passage UC-MSCs was used to perform the following experiments. The 10th passage UC-MSCs of the three groups were passaged and the cell count was recorded at the 6th, 12th, 24th, 48th hours. The expansion ratio was calculated by dividing the total cell count with the initiate planted cell number. Moreover, 10th passage UC-MSCs of the three groups were also treated with 200 ng/ml IFN-γ for 4 hours. IDO and PGE2weredetected in the cultural supernatant by ELISA from BioLegend (San Diego, CA).

Flow Cytometry Analyses

Flow cytometry analyses were performed by the cytometer of FACSariaTM III (BD Bioscience, San Jose, CA) and the data were analyzed with the FlowJo7.5 (Treestar, Ashland, OR) software packages. In order to identify UC-MSCs, Antihuman CD29-PE (MAR4), CD73-PE-CyTM7 (AD2), CD90-FITC (5E10), CD105-APC (266), CD34-PE (563), CD45-FITC (HI30), CD14-FITC (M5E2), CD79α-APC (HM47) and HLA-DR-PreCP (G46-6) antibodies, along with the corresponding isotype control antibodies purchased from BD Pharmingen. For monitoring the NESTIN expression, antihuman NESTIN-APC(IC1259A) antibody along with the corresponding isotype control antibody was also purchased from R&D Pharmingen. As NESTIN is a protein of cytoskeleton, cell fixation and permeabilization was performed before cell staining for flow cytometry. Sufficient MSCs from each group at each passage was harvested for flow cytometry analyses.

Quantitative Pcr Assay

UC-MSC samples were collected at each time point and stored at -80 °C. RNA extraction was then performed using the RNeasy Mini Kit from QIAGEN. The forward and reverse primers for qPCR were designed for NESTIN detection (F: CTCCAAGAATGGAGGCTGTAGGAA, R: CCTATGAGATGGAGCAGGCAAGA). QuantiFast®SYBR® Green PCR Kit from QIAGEN was used to perform qPCR.

Experimental Colitis Induced By Tnbs

All of the animal procedures were reviewed and approved by the Beike Animal Care and Use Committee. For inducing colitis in 9-weeks-old male BALB/c mice, we referred to the previously published protocol (Wirtz, Neufert, Weigmann, & Neurath, 2007). On day 1, mice were smeared with 200 μl of pre-sensitization solution (TNBS; Sigma) on their backs. On day 8, mice were divided into ten groups (8 mice/group) and fasted (but allowed to drink ad libitum) for 24 hours. On day 9, mice were weighed and treated intrarectally with 150 μl 3% TNBS in saline (eight groups) and no solution (blank control, two groups). At 10 hours post TNBS injection, animals were transplanted i.p. with 500 μl saline, or 4×10^6MSCs from the three groups that were suspended in 500 μl saline. Colons were collected from caecum to the anus of day − 1, day 0, day 2, day 4, day 6 and day10 after TNBS injection.

Cytokine Assays

The culture supernatant of 10th passage UC-MSCs was used to detect the cytokine production of IDO (DY6030-05) and PGE2(KGE004B) by ELISA from R&D system Inc. The serum of mouse was used to detect the cytokines. IL-6 (431304), IL-10 (431414), TNF-α (430904) was analyzed by ELISA from BioLegend (San Diego, CA) and SOD (MAB3419-SP) by ELISA from R&D system Inc.

Statistical analysis

Statistical comparisons were performed using the two-tailed Student's t-test (between two groups) or a one-way analysis of variance (ANOVA). $P < 0.05$ was considered to represent a significant difference.

Results

The three different culture conditions used to obtain mesenchymal stem cells (MSCs) for the comparisons were: DMEM-F12 with 10% fetal bovine serum, DMEM-F12 with 10% fetal bovine serum substitute, and DMEM-F12 with 5% UltraGRO-Advanced. UC-MSCs were harvested at various passages throughout the culture period, and the immunophenotype of the MSCs was investigated via quantitative flow cytometry (Uder et al., 2018). Flow cytometric analyses revealed that the UC-MSCs at the 10th passage in all the three culture conditions express representative surface markers, including CD29, CD73,

CD90, CD105, but not CD34, CD45, CD14, CD79, or HLA-DR (Fig. 1A), and the second passage to 9th passage also have the same surface marker repertoire (data not shown). To compare the differentiation potential, the UC-MSCs in different culture conditions were induced to differentiate into adipogenic, osteogenic, or chondrogenic lineage. It was verified that the UC-MSCs in all three culture conditions at the 10th passage could be induced to differentiate into adipogenic, chondrogenic, and osteogenic cells (Fig. 1B), and the UC-MSCs from first passage to 9th passage also have the same differentiation ability. The results showed above suggest that all the three culture conditions could sustain the surface marker repertoire and differentiation potential of the UC-MSCs from first passage to 10th passage.

We next examined the percentage of $NESTIN^+$ UC-MSCs by flow cytometry analysis (Fig. 2A and B) and NESTIN expression level in UC-MSCs by quantitative reverse transcription PCR (RT-qPCR) in three different culture conditions in each passage. The ratio of $NESTIN^+$ UC-MSCs could be stably maintained before 6th passage in all three culture conditions. However, the percentage of $NESTIN^+$ UC-MSCs significantly decreased from 7th passage in the other two culture conditions compared with UltroGRO-medium (Fig. 2C). Consistently, NESTIN in UC-MSCs in three different culture conditions had a similar trend with the exception of expression level (Fig. 2D). NESTIN in UltroGRO-medium expresses relatively higher level than the others. In spite of the use of UltroGRO-medium could sustain the percentage of $NESTIN^+$ UC-MSCs, we further investigated whether the shift of UltroGRO-medium could recover the expression of NESTIN in UC-MSCs in other two culture conditions. The UC-MSCs were initially cultured in FBS-medium or TBD-FS-medium, and then changed into UltroGRO-medium at 4th passage. We found that the percentage of $NESTIN^+$ UC-MSCs increased after changing medium compared with the UC-MSCs cultured in the one consistent culture condition. More interestingly, if we replaced the culture medium back to FBS-medium or TBD-FS-medium at 6th passage, the percentage of $NESTIN^+$ UC-MSCs declined significantly and it rose up significantly when the medium change back into UltroGRO-medium at 8th passage (Fig. 2E and 2G). The expression level of NESTIN is also consistent with this trend (Fig. 2F and 2H). These results indicated that UltroGRO-medium could not only maintain but also recover the expression of NESTIN in UC-MSCs.

We then evaluated the proliferation capacity of UC-MSCs in different culture conditions. The expansion ratio of the UC-MSCs cultured in three different culture conditions was calculated between the starting cell density ($5 \times 10^3/cm^2$ in 175 cm^2 flask) and the cell density from every two other days. UC-MSCs cultured in UltroGRO-medium have sustained their proliferation capacity with the expansion ratio above 10 in each passage. However, the expansion ratio of UC-MSCs cultured in the FBS-medium or TBD-FS-medium gradually decreased (Fig. 3). This result suggested UltroGRO-medium could sustain a greater proliferation capacity of UC-MSCs than the medium with the other two culture supplements.

To evaluate the immunomodulation ability of UC-MSCs that highly express NESTIN, we treated the 10th passage US-MSC in all the three media with IFN-γ, and measured the IDO and PEG2 secretion by ELISA. The IDO and PEG2 were significantly increased after IFN-γ treatment. Furthermore, IDO and PEG2 level was significantly higher in the UC-MSCs cultured with UltroGRO than that with the other two supplements

(Fig. 4A and B). In order to analysis the immunomodulation ability in vivo, we intraperitoneally injected UC-MSCs into a trinitrobenzene sulfonic acid (TNBS)-induced colitis mouse model, and measured the proinflammatory cytokines and anti-inflammatory cytokines by ELISA. The pro-inflammatory cytokines (IL-6 and IL-1β) were down-regulated after UC-MSC injection, and UC-MSCs cultured in UltroGRO-medium have better inhibition effect than that in other two mediums. (Fig. 4C and D). The anti-inflammatory cytokines (IDO and PEG2) were up-regulated simultaneously (Fig. 4E and F). This indicated that UC-MSCs may contribute to the immunomodulation against colitis and the UltraGRO group presents the best performance in the immunomodulation among the three different culture conditions.

Discussion

It was reported that the differentiation and proliferation capacity of UC-MSC weakens as the frequent iterated passaging increases especially after 40–50 population doubling (Halfon et al., 2011). Furthermore, the loss of stem cell features after the 6th passage was showed coincident with the decline of the mean telomere length from 9.19 kb to 8.7 kb at the 9th passage (Bonab et al., 2006). The previous studies demonstrated the expression of NESTIN plays an important role in the cellular differentiation and regulation so that it is acknowledged as a maker of progenitor cells (Wiese et al., 2004; Wong, Ghassemi, & Yellowley, 2014). However, there is no study tracking the connection between the expression of NESTIN and passaging.

In this study, we first observed a significant change in the expression of NESTIN in UC-MSCs and the percentage of $NESTIN^+$ cells as passaging increased when cell was cultured in the conventional medium (DMEM/F12 with 10% FBS). Flow cytometry analyses for 10th passages revealed that the NESTIN ratio in either FBS or TBD FS Group experienced a dramatic decline after passage 6. UltraGRO was innovatively applied in the UC-MSC culture and the $NESTIN^+$ UC-MSCs in UltraGRO group was maintained at a high percentage (> 90%). NESTIN mRNA level was also in keeping with this finding. Furthermore, the UltroGRO could maintain the proliferation capacity of UC-MSCs for up to 10th passages. The typical proliferative property of stem cells basically relies on asymmetric cell division, which results in asymmetric inheritance of cellular components for each daughter cell(Bernal & Arranz, 2018; Gomez-Lopez, Lerner, & Petritsch, 2014). This subsequently leads to two paths of cell development: one is the maintenance of stem cell population that retains self-renew capacity and differentiation potential, whereas the other contributes to the generation of functional differentiated cells(Gomez-Lopez et al., 2014). Asymmetric cell division requires a series of profound change in morphology including cytoskeletal arrangements that regulates cell polarity. NESTIN, as a cytoskeletal protein, may utilize protein phosphorylation to regulate the assembly and disassembly of other intermediate filaments such as vimentin during mitosis (Chou, Khuon, Herrmann, & Goldman, 2003). Therefore, this suggests the expression of NESTIN may be a contributing factor that is associated with the self-renew and proliferation property of UC-MSC, which may explain the findings in this study.

The attractive use of UC-MSCs in clinical therapy is their anti-inflammatory and immunomodulatory capability in a large range of diseases. UC-MSC has been proved to be a good candidate in clinical cellar

therapy treating both acute and chronic inflammatory tissue deteriorations in humans and animals. It was reported that UC-MSC have immunosuppressive effects on treating Crohn's disease (CD) which is one of two major types of inflammatory bowel disease(Liao et al., 2016). Interestingly, the UC-MSC from UltraGRO group showed a better anti-inflammatory performance than the others. This suggests that the high expression of NESTIN in UC-MSC could have a better immunomodulatory performance. Further investigation will be required to understand the mechanism how NESTIN regulate the immunomodulation ability of UC-MSC.

In this study, we monitored the expression of surface markers and NESTIN for up to 10 passages under three different culture conditions. Unsurprisingly, the expression of NESTIN does experience a dramatic decline in late passages using the conventional medium (FBS). By comparison, the use of FBS substitute and UltraGRO can retain the expression of NESTIN which barely drops in UltraGRO group for 10 passages. Furthermore, UC-MSC in UltraGRO group also displays a better immunomodulatory performance. However, the role that NESTIN plays in UC-MSC immunomodulation requires further investigation. It is believed that NESTIN could be a reporter of the status of UC-MSC during the large-scale manufacture process, which may be used to evaluate the UC-MSC quality in the stem cell therapy.

Abbreviations

MSC
Mesenchymal stem cell
ISCT
International Society of Cell Therapy
cGMP
current Good Manufacture Practice
UC-MSCs
umbilical cords - Mesenchymal stem cell
DMEM
Dulbecco's modified Eagle's medium
FBS
fetal bovine serum
TNBS
trinitrobenzene sulfonic acid
CD
Crohn's disease

Declarations

Ethics approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All of the animal procedures were reviewed and approved by the Beike Animal Care and Use Committee.

Consent for publication

Not applicable.

Availability of data and material

All relevant data are within the manuscript.

Competing interests

The author declares that they have no competing interests.

Funding

This work was funded by Shenzhen Beike Biotechnology Co. Ltd..

Authors' contributions

YL and FX conceived and designed research. XH, ML and XL provided research idea and sufficient resources for research. YL, FX and ZL conducted experiments. FX, ZF and HP analyzed data. GZ, QM and WZ contributed new reagents or analytical tools. FX wrote the manuscript. ZT, YL and YR reviewed and revised manuscript. All authors read and approved the manuscript.

Acknowledgements

Not applicable.

Authors' information (optional)

Yuncheng Liu and Feidi Xiao equally contributed to this work.

YL and FX are R&D engineers from Shenzhen Beike Biotechnology Co. Ltd.. Both of them have extensive experiences in the manufacture of stem cells and immune cells. Beike is a China's leading biotech company specializes in the research, clinical translation, and technological advancement of adult stem cells and their associated applications.

References

Bernal A, Arranz L (2018) Nestin-expressing progenitor cells: function, identity and therapeutic implications. Cell Mol Life Sci, 75(12), 2177-2195. doi:10.1007/s00018-018-2794-z

Bonab M M, Alimoghaddam K, Talebian F, Ghaffari S H, Ghavamzadeh A, Nikbin B (2006) Aging of mesenchymal stem cell in vitro. BMC Cell Biol, 7, 14. doi:10.1186/1471-2121-7-14

Chou Y H, Khuon S, Herrmann H, Goldman R D (2003) Nestin promotes the phosphorylation-dependent disassembly of vimentin intermediate filaments during mitosis. Mol Biol Cell, 14(4), 1468-1478. doi:10.1091/mbc.e02-08-0545

da Silva Meirelles L, Chagastelles P C, Nardi N B (2006) Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci, 119(Pt 11), 2204-2213. doi:10.1242/jcs.02932

Devito L, Klontzas M E, Cvoro A, Galleu A, Simon M, Hobbs C, Dazzi F, Mantalaris A, Khalaf Y, Ilic D (2019) Comparison of human isogeneic Wharton's jelly MSCs and iPSC-derived MSCs reveals differentiation-dependent metabolic responses to IFNG stimulation. Cell Death Dis, 10(4), 277. doi:10.1038/s41419-019-1498-0

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy, 8(4), 315-317. doi:10.1080/14653240600855905

Donnenberg V S, Ulrich H (2013) Mesenchymal stem cells, therapy, and cytometry. Cytometry A, 83(1), 8-10. doi:10.1002/cyto.a.22238

Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. Science, 279(5356), 1528-1530. doi:10.1126/science.279.5356.1528

Friedenstein A J, Chailakhyan R K, Gerasimov U V (1987) Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. Cell Tissue Kinet, 20(3), 263-272.

Gomez-Lopez S, Lerner R G, Petritsch C (2014) Asymmetric cell division of stem and progenitor cells during homeostasis and cancer. Cell Mol Life Sci, 71(4), 575-597. doi:10.1007/s00018-013-1386-1

Halfon S, Abramov N, Grinblat B, Ginis I (2011) Markers distinguishing mesenchymal stem cells from fibroblasts are downregulated with passaging. Stem Cells Dev, 20(1), 53-66. doi:10.1089/scd.2010.0040

Kern S, Eichler H, Stoeve J, Kluter H, Bieback K (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells, 24(5), 1294-1301. doi:10.1634/stemcells.2005-0342

Kulkarni S, Micci M A, Leser J, Shin C, Tang S C, Fu Y Y, Liu L, Li Q, Saha M, Li C, Enikolopov G, Becker L, Rakhilin N, Anderson M, Shen X, Dong X, Butte M J, Song H, Smith E M S, Kapur R P, Bogunovic M, Pasricha P J (2017) Adult enteric nervous system in health is maintained by a dynamic balance between neuronal apoptosis and neurogenesis. Proc Natl Acad Sci U S A, 114(18), E3709-E3718. doi:10.1073/pnas.1619406114

Lei J, Hui D, Huang W, Liao Y, Yang L, Liu L, Zhang Q, Qi G, Song W, Zhang Y, Xiang A P, Zhou Q (2013) Heterogeneity of the biological properties and gene expression profiles of murine bone marrow stromal cells. Int J Biochem Cell Biol, 45(11), 2431-2443. doi:10.1016/j.biocel.2013.07.015

Lendahl U, Zimmerman L B, McKay R D (1990) CNS stem cells express a new class of intermediate filament protein. Cell, 60(4), 585-595. doi:10.1016/0092-8674(90)90662-x

Liao Y, Lei J, Liu M, Lin W, Hong D, Tuo Y, Jiang M H, Xia Huimin, Wang M, Huang W, Xiang A P (2016) Mesenchymal Stromal Cells Mitigate Experimental Colitis via Insulin-like Growth Factor Binding Protein 7-mediated Immunosuppression. Mol Ther, 24(10), 1860-1872. doi:10.1038/mt.2016.140

Lodie T A, Blickarz C E, Devarakonda T J, He C, Dash A B, Clarke J, Gleneck K, Shihabuddin L, Tubo R (2002) Systematic analysis of reportedly distinct populations of multipotent bone marrow-derived stem cells reveals a lack of distinction. Tissue Eng, 8(5), 739-751. doi:10.1089/10763270260424105

Mignone J L, Kukekov V, Chiang A S, Steindler D, Enikolopov G (2004) Neural stem and progenitor cells in nestin-GFP transgenic mice. J Comp Neurol, 469(3), 311-324. doi:10.1002/cne.10964

Oedayrajsingh-Varma M J, van Ham S M, Knippenberg M, Helder M N, Klein-Nulend J, Schouten T E, Ritt M J P F, van Milligen F J (2006) Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure. Cytotherapy, 8(2), 166-177. doi:10.1080/14653240600621125

Seo, B M, Miura M, Gronthos S, Bartold P M, Batouli S, Brahim J, Yong M, Robey P G, Wang C Y, Shi S. (2004). Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet, 364(9429), 149-155. doi:10.1016/S0140-6736(04)16627-0

Seo M J, Suh S Y, Bae Y C, Jung J S (2005) Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. Biochem. Biophys. Res. Commun., 328(1), 258-264. doi:10.1016/j.bbrc.2004.12.158

Tondreau T, Lagneaux L, Dejeneffe M, Massy M, Mortier C, Delforge A, Bron D (2004) Bone marrow-derived mesenchymal stem cells already express specific neural proteins before any differentiation. Differentiation, 72(7), 319-326. doi:10.1111/j.1432-0436.2004.07207003.x

Uder C, Bruckner S, Winkler S, Tautenhahn H M, Christ B (2018) Mammalian MSC from selected species: Features and applications. Cytometry A, 93(1), 32-49. doi:10.1002/cyto.a.23239

Wiese C, Rolletschek A, Kania G, Blyszczuk P, Tarasov K V, Tarasova Y, Wersto R P, Boheler K R, Wobus A M (2004) Nestin expression–a property of multi-lineage progenitor cells? Cell Mol Life Sci, 61(19-20), 2510-2522. doi:10.1007/s00018-004-4144-6

Wirtz S, Neufert C, Weigmann B, Neurath M F (2007) Chemically induced mouse models of intestinal inflammation. Nat Protoc, 2(3), 541-546. doi:10.1038/nprot.2007.41

Wong A, Ghassemi E, Yellowley C E (2014) Nestin expression in mesenchymal stromal cells: regulation by hypoxia and osteogenesis. BMC Vet Res, 10, 173. doi:10.1186/s12917-014-0173-z

Zimmerman L, Parr B, Lendahl U, Cunningham M, McKay R, Gavin B, Mann J, Vassileva G, McMahon A (1994) Independent regulatory elements in the nestin gene direct transgene expression to neural stem cells or muscle precursors. Neuron, 12(1), 11-24. doi:10.1016/0896-6273(94)90148-1

Zuk P A, Zhu M, Mizuno H, Huang J, Futrell J W, Katz A J, Benhaim P, Lorenz H P, Hedrick M H (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng, *7*(2), 211-228. doi:10.1089/107632701300062859

Figures

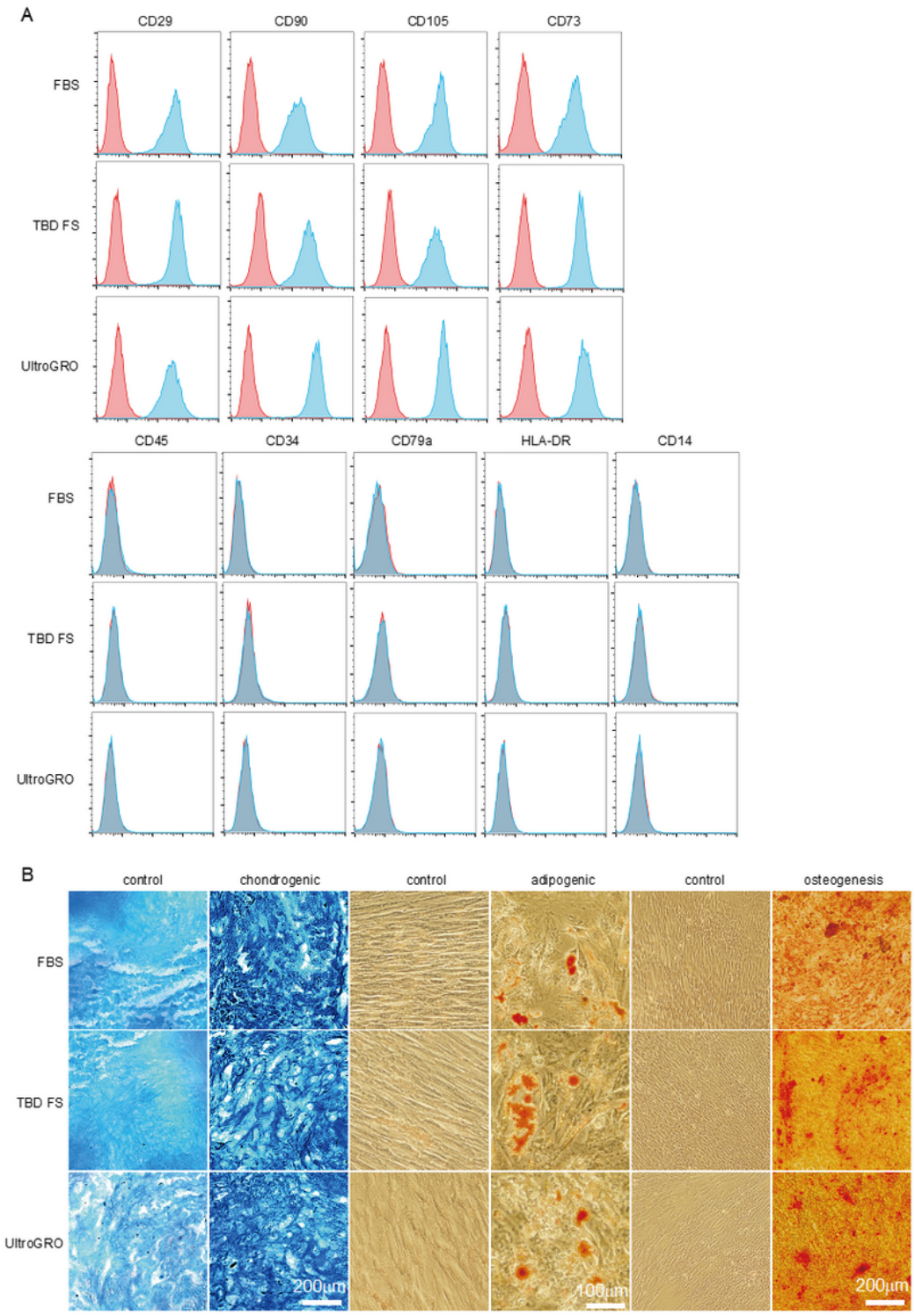

Figure 1

The characterization of UC-MSCs in three different culture conditions. (A) The UC-MSCs at 10th passage in three culture conditions were positive for CD29, CD90, CD105, and CD73, and were negative for CD45, CD34, CD79a, HLA-DR, and CD14. (B) The UC-MSCs at 10th passage in three culture conditions were stained by alcian blue, oil red O, and Alizarin Red S, respectively.

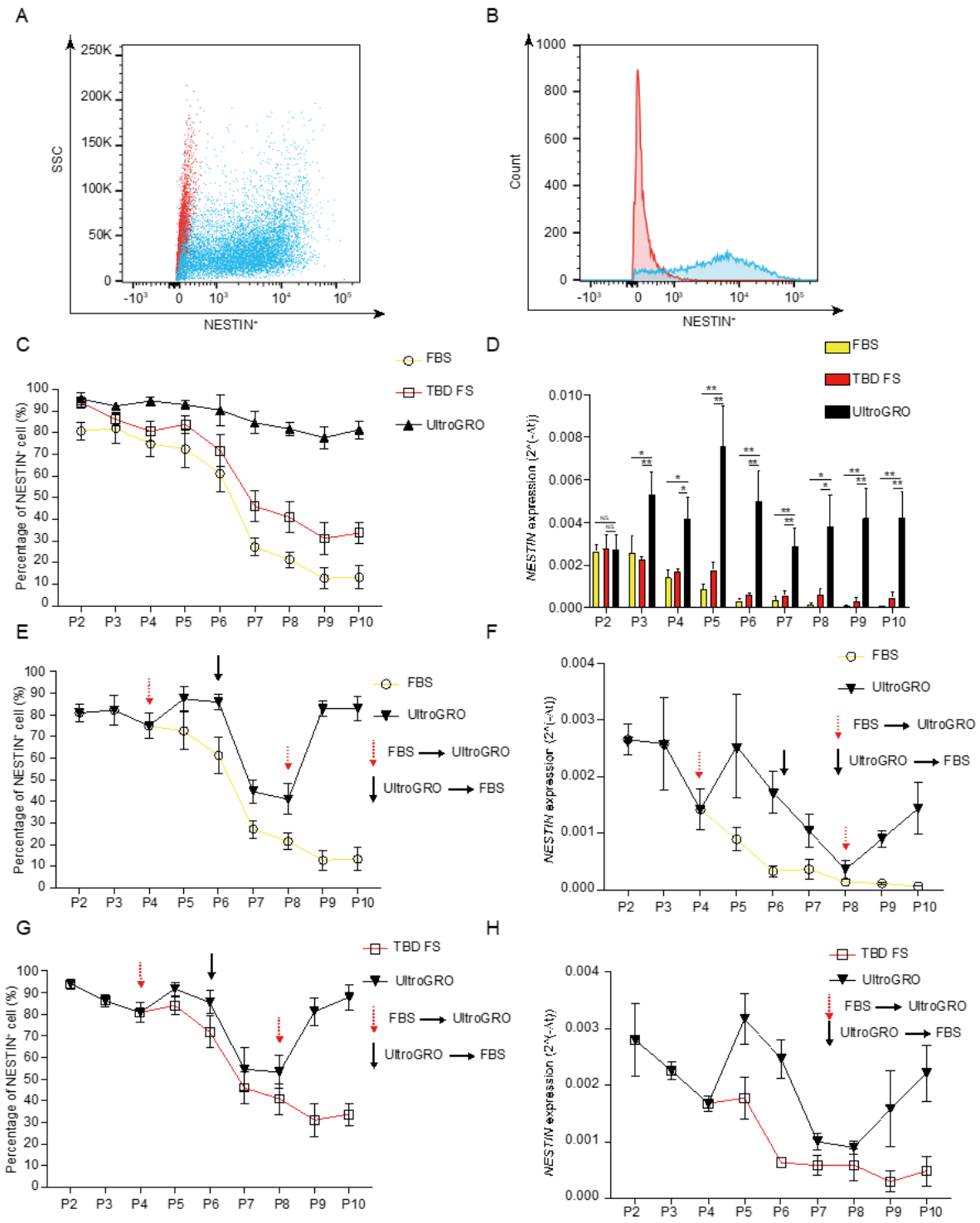

Figure 2

The expression of NESTIN in three different culture conditions. (A, B)The flow cytometry analysis of NESTIN in UC-MSCs. (C) The percentage of NESTIN+ cell in three culture conditions. (D) The expression level of NESTIN in three culture conditions. (E-H) The percentage of NESTIN+ cell and expression level of NESTIN after changing medium. **p< 0.01, * p< 0.05, Student's t test.

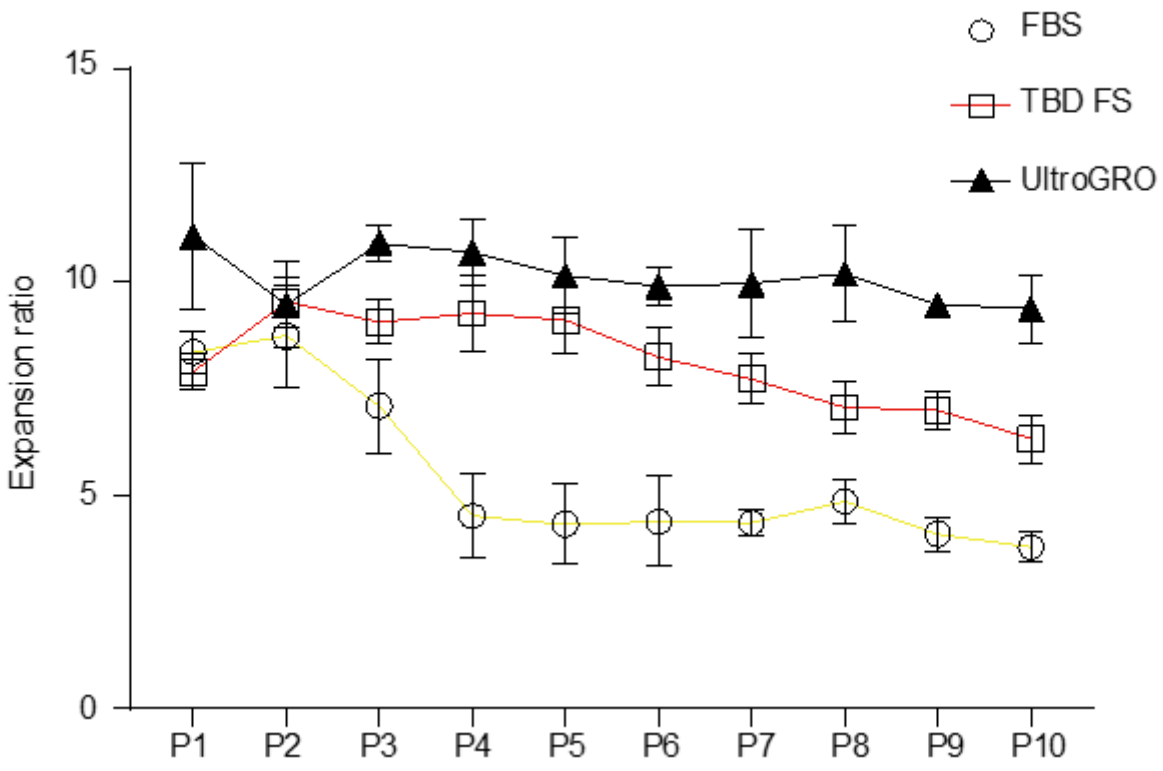

Figure 3

The proliferation capacity of MSCs in the three groups. The proliferation ability of UC-MSCs in FBS, TBS FS, and UltraGRO group was measured by cell count.

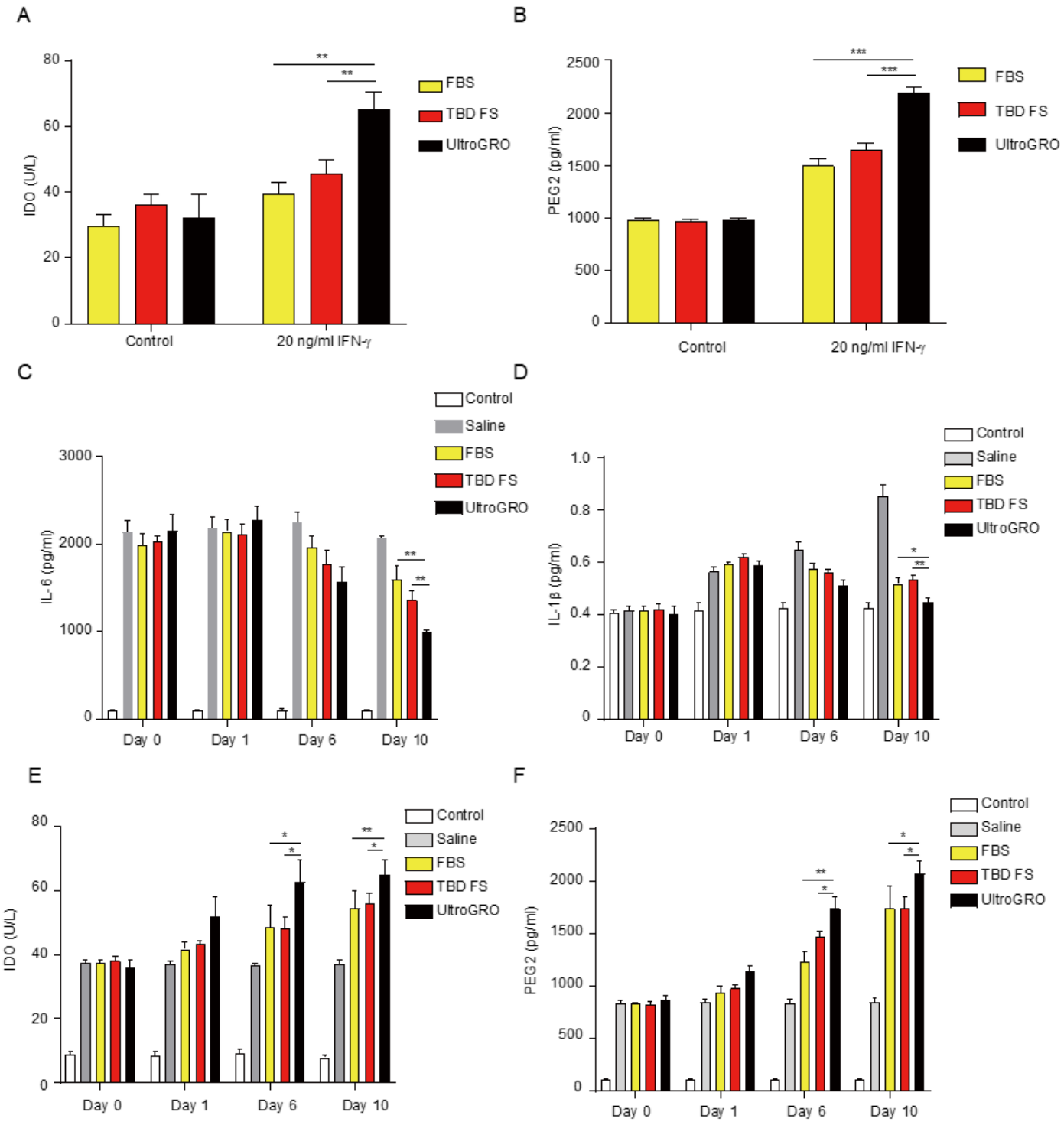

Figure 4

The measurement of UC-MSCs secreted cytokines in vitro and in vivo. (A, B)The anti-inflammatory cytokines IDO and PEG2of secreted by UC-MSCs were measured by ELISA after treated with 200 ng/ml IFN-γ. (C, D) The IL-6 and IL-1β were measured by ELISA in TNBS-induced colitis mouse model. (E, F) The IDO and PEG2 were measured by ELISA in TNBS-induced colitis mouse model. **p< 0.01, * p< 0.05, Student's t test.