Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Effects of Co-Culture of Graphene Oxide Scaffolds with Different Concentrations and Umbilical Mesenchymal Stem Cells on the Proliferation and Differentiation of Stem Cells

Aifeng Liu
Anhui University of Traditional Chinese Medicine
Shuwei Gong
Anhui University of Traditional Chinese Medicine
Qiang Wei
Anhui University of Traditional Chinese Medicine
Yao Wang
Tianjin University of Traditional Chinese Medicine
Ye Yuan (yuanyewins@hotmail.com)
Hebei University of Technology https://orcid.org/0000-0002-1656-9053

Research article

Keywords: graphene oxide, mesenchymal stem cell, co-culture, scaffolds, knee osteoarthritis

Posted Date: August 24th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-57941/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Background

The main role of the scaffold materials is to enable cells to survive in the scaffold binding as while as to further promote their proliferation and differentiation ability. For mesenchymal stem cell, the scaffold could provide an environment for them to maintain their phenotype, and synthesize all necessary molecules and proteins. Generally, scaffold materials for stem cell need to possess basic characteristics such as high porosity, large surface area, surface rigidity and biodegradability. Thus, the two-dimensional graphene oxide (GO) with oxygen-containing functional groups may be suitable scaffold materials for mesenchymal stem cell culture.

Methods

In this study, the effect of GO on the value-added differentiation activity of mesenchymal stem cell was systematically investigated.

Results

It was found that low concentration of GO and sufficient concentration of umbilical cord mesenchymal stem cells are suitable for the second Co-culture. Furthermore, the addition of hyaluronic acid will make this culture more evenly distributed.

Conclusions

The adsorption of GO on umbilical cord mesenchymal stem cells can also make the two closely linked, which avoids the impact of animal joint activities on cells.

1. Background

Recently, the significant advantages of graphene oxides (GO) used as cell scaffold materials have attracted tremendous interests due to their large surface areas, friendly biological compatibility and good hydrophility.[1–5] As an ideal seed cell in tissue engineering, mesenchymal stem cell (MSC) has been widely used in the field of regenerative medicine.[6–11] MSC has the effect of regulating local immunity and improving the inflammatory environment. At the same time, MSC is low in immunogenicity, which will not bring risks to transplantation.[12–17] Many early studies have confirmed that MSC transplanted in damaged structures can promote the repair effect of damaged areas. Up to now, the possibility of GO combined with MSC as the cell scaffold has also been initially explored.[18–22] However, there are few related studies and bone marrow stromal cells (BMSC) are mostly used as seed cells in the research.

At present, umbilical cord mesenchymal stem cells (UCMSC) has been employed to try to replace bone marrow stromal cells (BMSC).[23–25] On one hand, the human-derived BMSC needs to puncture the human bone marrow, which brings pain, safety risks and ethical problems to the human to some extent. On the other hand, bone marrow-derived MSCs are actually very small in content. The extremely low proportion of MSCs in bone marrow as well as the low-activity have a great impact on the results of research. Although some studies have confirmed that in vitro cultured MSCs and GO are biocompatible, they are also limited to the observation of the effect of GO on MSC survival without further in-depth studies and specific dose-effect relationships.

The dynamic knee joint is one of the most important structures that carries the human body's movements.[26–28] The joint cavity must maintain the stability of mechanical and biochemical microenvironment. MSC inhibits the inflammatory response through paracrine effects. Meanwhile, MSC releases growth factors such as platelet-derived growth factor (PDGF), transforming growth factor (TGF-β), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF). These growth factors could improve the biochemical micro-environment of the knee joint and promote repair of cartilage tissue damage.

The problems of cell flow and low cell survival rate can be effectively solved by using cell scaffolds. The GO scaffold with favorable hydrophility, biocompatibility, ductility and damping properties can increase the electrical activity of cells.[29–31] In addition to being equipped with MSC, GO scaffold can also be combined with sodium hyaluronic acid (HA) to prepare materials with lubricating effect on the joint cavity. Furthermore, GO can also improve the joint as lubricants. Herein, in this experiment, the culture effect of UCMSC with GO was initially explored in order to provide a novel strategy for the treatment of osteoarthritis.

2. Methods

2.1 Materials and instruments

GO is purchased from C6G6Technology Co., Ltd, China. The basic characterization of GO is in the Supporting Information (Figure S1 and Figure S2). The 3rd or 4th generation human umbilical cord mesenchymal stem cells (Boya stem cell technology co. LTD). DMEM/F12 medium and HA injection 2.5 ml/piece (Japan, H20050370). High-throughput multi-sample tissue grinding machine (Nanjing, Xianou Instrument Manufacturing Co., Ltd.). Electronic platform scale (Switzerland, Mettler Toledo). Constant temperature and humidity incubator (SHEL LAB in the United States). OLYMPUS photomicroscope (OLYMPUS in Japan). ADAM automatic cell counter (NanoEnTek, Korea).

2.2 Mixture of GO and HA

GO is placed in a small reagent tube and put it into a tissue grinder. The control frequency is set as 70 Hz and the stop time is 60 s. After the machine stops running, remove the reagent tube, put it into a centrifuge, and centrifuge Operation: According to the needs of the experiment, use an electronic balance

to weigh the masses of graphene oxide required by 4 groups of GO solution, and put them into four small reagent tubes labeled with groups, put them on the ultraviolet operating table, and use 1 ml without The pipette tip of the bacteria tube transfers a part of the HA injection in the unsealed syringe containing 2.5 ml of HA injection into a small reagent tube filled with GO. The GO combined HA solution is configured to two concentrations: 15ug/ml GO + 0.5% HA and 30 ug/ml GO + 0.25% HA. After mixing, use the pipette Transfer all the liquid guns to the large test tube cap, and then transfer the HA injection solution in the remaining syringes to the large test tube caps of the same group. Use the pipette head to mix them thoroughly and turn on the ultraviolet light., Perform UV sterilization for 35 min, and then use a pipette to transfer the mixture in the large test tube cap into the original syringe.

2.3 Isolation, culture and identification of human UCMSC

Take a healthy full-term fetal umbilical cord, rinse it thoroughly with PBS, remove the umbilical arteriovenous vein under sterile conditions, cut the remaining interstitial tissue (Walton's gel) into 1.0–2.0 mm size tissue blocks, and flatten the tissue blocks on In the cell culture flask, add an appropriate amount of DMEM/F12 culture solution containing 10% fetal bovine serum in volume, and place in 37 °C, 5% CO_2 volume incubator for incubation. Replace the culture solution according to the cell growth rate. When the cells have reached the bottom of the culture flask, remove the tissue blocks, pass them down according to the number of cells, observe with an inverted microscope and take a video. Take the 3rd or 4th generation human UCMSC, digest with 0.25% trypsin, centrifuge at 1200 r / min for 5 minutes, count the cells, and use about 2 × 10 cells per tube. Use 0.1% sodium azide and 0.5 Wash the cells twice with PBS, resuspend the cells in PBS, add mouse anti-rat CD34, CD45, CD90, and CD105 primary antibodies (1:50 dilution), leave them at 4 ℃ for 30 min, wash them twice with PBS, add isocyanate Rabbit anti-mouse IgG secondary antibody labeled with fluorescein thiocyanate, placed at 4 ℃ for 30 min, washed twice with PBS, resuspended the cells with PBS without BSA, and detected cell surface markers CD34, CD45, CD90 and CD105 expression.

2.4 Experimental grouping and processing

The experiment was divided into 4 groups. GO was mixed with HA injection after being treated with a tissue grinder and a centrifuge, and a mixture of 10 ug/ml, 20 ug/ml, 30 ug/ml and 40 ug/ml was used as a culture medium. Mark the cells separately, divide the human UCMSC into four groups of approximately equal amounts, mark the serial numbers, and transfer them to the corresponding medium with a pipette for cultivation.

2.5 Effect of the mixture of GO and HA injection on the proliferation of human UCMSC

The four concentration mixtures were placed in 96-well culture plates, and 100 μL of medium was added to each well. The inoculated 96-well culture plate was placed in a constant temperature incubator (5% CO_2, 100% humidity, 37 ℃ constant temperature) for 24 hours, and observed under continuous microscope.

3. Results And Discussions

3.1 In vitro culture of UCMSC and GO particles

UCMSC and GO were co-cultured for 24 hours. It was observed that UCMSC grew normally and GO did not cause a large number of deaths of UCMSC cells. Meanwhile, GO nanosheets began to aggregate while UCMSC cells tended to aggregate toward GO until they gradually wrapped GO nanosheets (Fig. 1).

In Fig. 2, UCMSC and GO were co-cultured for 72 hours. UCMSC grew normally. GO did not cause a large number of deaths of UCMSC cells. The tendency of UCMSC cells to further aggregate toward GO was observed and climbed toward GO particles to wrap them into clusters. UCMSC began to grow in three dimensions and stimulated proliferation.

UCMSC and GO were co-cultured for 7 days. UCMSC grew normally. GO did not cause a large number of deaths of UCMSC cells. It was observed that almost all UCMSC cells aggregated to GO. The GO particles were tightly wrapped to form a group structure of GO + UCMSC (Fig. 3)

According to the above experimental results, following results can be concluded. Firstly, UCMSC can coexist with GO in vitro. Relatively large GO was chosen to co-culture with MSC. It can be observed that not only a large number of cell death did not occur after one week of culture, but the situation of cell proliferation appeared. This phenomenon indicates that GO at this concentration is not cytotoxic to UCMSC and it can promote the growth of UCMSC to some certain extent. Secondly, GO nanoparticles are attractive to UCMSC. Cells farther away from the GO particles can also be attracted and wrapped by them, forming a three-dimensional approximately spherical structure. This phenomenon indicates MSC and GO can form a good binding without resorting to other material structures, so as not to affect the treatment effect.

3.2 Culture results of UCMSC and GO with different concentrations

In Fig. 4, UCMSC was co-cultured with GO with different concentrations for 48 h. It was observed that the GO concentrations of 30 mg/ml, 10 mg/ml, 300 ug/ml, 200 ug/ml and 100 ug/ml were too high, which is not suitable for co-culture with UCMSC. GO was observed to aggregate into small clumps and was surrounded by cells after co-culturing of UCMSC and GO (15 ug/ml and 30 ug/ml) for 48 h (Fig. 5).

After Co-culturing of GO (30 ug/ml) and UCMSC with different concentrations for 48 h, it was observed that the cell concentration of 4.0×10^4/ml and 2.0×10^4/ml were lower in both groups (Fig. 6).

The above Co-culturing results of GO and UCMSCs with different concentrations confirmed that excessively high concentrations of GO are not conducive to the in vitro culture of cells. This may be ascribed to the high concentration of GO, which compressed the cell survival space. The research results show that concentrations of 30ug/ml and below are suitable for coexistence with UCMSC. However, if the concentration of GO is too low, it may reduce its lubrication and protection effect on articular cartilage

surface. On the other hand, the low concentration of UCMSC cells under the low concentration of GO environment can not reflect the migration and proliferation effect. It is due to a certain distance limitation of GO on the cell forms a separate culture of GO and UCMSC, which cannot form a co-cultivation environment. However, it does not mean that the higher the density of UCMSC is better. Actually, the higher the density of inoculation will also cause cell death. Therefore, GO needs a low concentration environment to ensure its safety. On the contrary, UCMSC must at least ensure a sufficient concentration to form a co-culture system.

3.3 Culture results of UCMSC and GO nanoparticle lubricants

UCMSC was mixed with a granular lubricant with a concentration of 15ug/ml GO + 0.5% HA and 30ug/ml GO + 0.25% HA for 7 days (Fig. 7). It was observed that GO showed a relatively uniform distribution while HA with a low concentration. Also, UCMSC@GO particles are evenly distributed and can promote the growth and proliferation of UCMSC. The 30 ug/ml GO + 0.25% HA group had a slightly stronger promotion effect on UCMSC than the 15ug/ml group (Fig. 8). Meanwhile, low concentration of HA was more conducive to the uniform distribution of UCMSC + GO structure. The 24 h culture results in UCMSC culture dishes reveals that the detectable cell concentration in the saline group was 4.45×10^6 /ml and the cell survival rate was 8%. The detectable cell concentration in the GO particle lubricant group was 3.41×10^6 /ml and the cell survival rate 13% (Fig. 9).

The above results indicate the relationship between the ratio of GO and HA mixed lubricants and the culture of UCMSC. Sodium hyaluronate injection is a macromolecular structure and has a high viscosity. Thus, it will have a certain effect on the migration of MSC and also hinder the aggregation to GO. Therefore, the concentration of HA should not be too high. However, HA also hinders the aggregation of GO, which is beneficial to the uniform distribution of GO. Small size of GO is more safe and less toxic than large size of GO. Therefore, the mixing of HA is conducive to the long-term uniform distribution of UCMSC + GO. Furthermore, low concentration of HA is more suitable for co-cultivation of the two.

4 Discussion

4.1 Serum NO

The difference between GO + MSC group and blank group in group A was statistically significant ($P < 0.01$), and there was no significant difference between MSC group, GO group and blank group ($P > 0.05$). The difference was not statistically significant ($P > 0.05$). The difference between GO + MSC group and blank group in group B was statistically significant ($P < 0.01$), the difference between MSC group, GO group and blank group was statistically significant ($P < 0.05$). The difference between GO + MSC group and MSC group was It has statistical significance ($P < 0.05$). Compared between groups, the difference between GO + MSC group, MSC group and GO group was not statistically significant ($P > 0.05$), and the difference between blank group was statistically significant ($P < 0.05$) (Table 1).

Table 1
Serum NO results after treatment(±s,ng/ml)

Group	Blank	GO	MSC	GO + MSC
Group A	22.097 ± 0.352	21.436 ± 0.031[1)]	21.020 ± 0.026[2)]	17.624 ± 0.127[3)]
Group B	23.662 ± 0.056	20.544 ± 0.085[4)]	19.424 ± 0.046[5)]	17.799 ± 0.049[6)]
P value	0.029	0.430	0.254	0.725

4.2 Serum COL-II

Within group comparison, the difference between GO + MSC group, MSC group and blank group in group A was statistically significant (P < 0.01), and the difference between GO group and blank group was not statistically significant (P > 0.05); GO + MSC The difference between the group and the MSC group was statistically significant (P < 0.01). In group B, the difference between GO + MSC group and blank group was statistically significant (P < 0.01), the difference between MSC group, GO group and blank group was not statistically significant (P > 0.05); GO + MSC group and MSC group. The difference was statistically significant (P < 0.01).

Compared between groups, the difference between GO + MSC group and MSC group was statistically significant (P < 0.01), and the difference between GO group and blank group was not statistically significant (P > 0.05) (Table 2).

Table 2
Serum COL-II results after treatment(±s,ng/ml)

Group	Blank	GO	MSC	GO + MSC
Group A	13.475 ± 0.342	14.127 ± 0.102[1)]	15.589 ± 0.063[2)]	19.372 ± 0.063[3)]
Group B	12.253 ± 0.147	13.644 ± 0.028[4)]	14.429 ± 0.092[5)]	16.257 ± 0.416[6)]
P value	0.315	0.249	0.009	0.000

4.3 Serum GAG

In group A, the difference between GO + MSC group and blank group was statistically significant (P < 0.01), the difference between MSC group and blank group was statistically significant (P < 0.05), and the difference between GO group and blank group was not statistically significant. Significance (P > 0.05). The difference between GO + MSC group and MSC group was statistically significant (P < 0.01). In group B, the difference between GO + MSC group, MSC group and blank group was statistically significant (P < 0.01), the difference between GO group and blank group was statistically significant (P < 0.05); comparison between GO + MSC group and MSC group. The difference was statistically significant (P < 0.05).

Compared between groups, the difference between the blank group and the GO + MSC group was statistically significant (P < 0.01), and the difference between the MSC group and the GO group was not statistically significant (P > 0.05) (Table 3).

Table 3
Serum GAG results after treatment(±s,ng/ml)

Group	Blank	GO	MSC	GO + MSC
Group A	23.832 ± 0.891	26.342 ± 1.042[1)]	29.022 ± 0.973[2)]	37.439 ± 2.155[3)]
Group B	18.709 ± 0.552	22.689 ± 0.641[4)]	24.028 ± 0.675[5)]	26.554 ± 0.450[6)]
P Value	0.002	0.096	0.051	0.000

4.4 Serum IL-6.

In group A, the difference between GO + MSC group, MSC group, GO group and blank group was statistically significant (P < 0.01); the difference between GO + MSC group and MSC group was statistically significant (P < 0.01). In group B, the difference between GO + MSC group, MSC group, GO group and blank group was statistically significant (P < 0.01); the difference between GO + MSC group and MSC group was statistically significant (P < 0.05).

Compared between groups, the difference between GO + MSC group was statistically significant (P < 0.01), the difference between blank group was statistically significant (P < 0.05), the difference between MSC group and GO group was not statistically significant (P > 0.05) (Table 4).

Table 4
Result of serum IL-6 after treatment(±s,ng/ml)

Group	Blank	GO	MSC	GO + MSC
Group A	16.082 ± 0.323	10.957 ± 0.343[1)]	9.668 ± 0.194[2)]	7.426 ± 0.294[3)]
Group B	18.367 ± 0.861	10.002 ± 0.191[4)]	9.506 ± 0.123[5)]	8.680 ± 0.242[6)]
P Value	0.024	0.169	0.565	0.009

4.5 Serum TNF-α

In group A, the difference between GO + MSC group, MSC group, GO group and blank group was statistically significant (P < 0.01); the difference between GO + MSC group and MSC group was statistically significant (P < 0.01). In group B, the difference between GO + MSC group, MSC group, GO group and blank group was statistically significant (P < 0.01); the difference between GO + MSC group and MSC group was statistically significant (P < 0.05).

Compared between groups, the difference between GO + MSC group was statistically significant (P < 0.01), the difference between blank group was statistically significant (P < 0.05), the difference between

MSC group and GO group was not statistically significant (P > 0.05) (Table 5).

Table 5
Result of serum TNF-α after treatment(±s,ng/ml)

Group	Blank	GO	MSC	GO + MSC
Group A	9.466 ± 0.177	8.447 ± 0.113[1)]	6.109 ± 0.044[2)]	5.139 ± 0.183[3)]
Group B	10.013 ± 0.197	8.891 ± 0.188[4)]	6.856 ± 0.160[5)]	6.210 ± 0.058[6)]
*P*Value	0.037	0.087	0.146	0.006

5. Conclusions

In summary, we have confirmed that GO can coexist with UCMSC *in vitro* and play the role of adsorption and promotion. Cell survival and growth under different concentrations of environment were preliminary discussed. It was found that low concentration of GO and sufficient concentration of UCMSC are suitable for the second Co-culture. Furthermore, the addition of HA will make this culture more evenly distributed. The adsorption of GO on UCMSC can also make the two closely linked, which avoids the impact of animal joint activities on cells. Meanwhile, two KOA animal models were selected in this experiment. The modified Hulth + cartilage defect model in group A is the main sports injury model. For the current multiple ligament tears and meniscus destruction, this injury will cause the intra-articular inflammation to gradually develop into KOA The results show that the role of the three-dimensional mesh scaffold of this kind of GO particles is more important, which can make UCMSC better adhere to the cartilage defect area, so as to grow and proliferate. Can make UCMSC better adhere to cartilage defect areas. The model of group B is a model of cartilage degeneration induced by chemical factors. It can be seen that cartilage has different degrees of necrosis, which is relatively close to the pathogenesis of degenerative osteoarthritis in clinic. GAG, IL-6, and TNF-α have a statistically significant difference compared with the blank group, which also shows that the use of GO particle lubricants to carry UCMSC has a better therapeutic effect. UCMSC loaded with graphene oxide can promote the chondrocyte secretion of two knee osteoarthritis animal models, reduce the level of inflammation in the joints, and play a role in cartilage repair. Our findings may provide some help for the efficacy of subsequent knee osteoarthritis (KOA) animal models exploration.

Declarations

Ethics approval and consent to participate

Not aplicable.

Consent for publication

Not aplicable.

Availability of data and materials

The data that supports the findings of this study are available within the manuscript and its supplementary material.

Competing interests

All the authors declare no conflicts on financial interests of the manuscript.

Funding

This work was supported by the Natural Science Foundation of China (81873316 and 81673994), the Foundation Strengthening Program (2019-JCJQ-142-00) and Higher Education Science and Technology Research Project of Hebei Province (ZD2019087).

Authors' contributions

AL came up the basic ideas and analyzed the data of this manuscript. SG and YW conducted this experiment. QW and YY were major contributors in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Corresponding authors: Qiang Wei and Ye Yuan

References

1. Holt BD, Wright ZM, Arnold AM, Sydlik SA. Graphene oxide as a scaffold for bone regeneration. WIREs Nanomedicine Nanobiotechnology. 2017;9(3):e1437.
2. Shuai C, Zeng Z, Yang Y, Qi F, Peng S, Yang W, He C, Wang G, Qian G. Graphene oxide assists polyvinylidene fluoride scaffold to reconstruct electrical microenvironment of bone tissue. Materials Design. 2020;190:108564.
3. Serrano MC, Patiño J, García-Rama C, Ferrer ML, Fierro JLG, Tamayo A, Collazos-Castro JE, del Monte F. M.C. Gutiérrez, 3D free-standing porous scaffolds made of graphene oxide as substrates for neural cell growth. Journal of Materials Chemistry B. 2014;2(34):5698–706.
4. Rostami F, Tamjid E, Behmanesh M. Drug-eluting PCL/graphene oxide nanocomposite scaffolds for enhanced osteogenic differentiation of mesenchymal stem cells, Materials Science and Engineering: C (2020) 111102.
5. Guo W, Qiu J, Liu J, Liu H. Graphene microfiber as a scaffold for regulation of neural stem cells differentiation. Sci Rep. 2017;7(1):5678.
6. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DWJWR, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells.

Science. 1999;284(5411):143–7.

7. Dominici M, Blanc KL, Mueller I, Slapercortenbach I, Marini FC, Krause DS, Deans R, Keating A, Prockop DJ, Horwitz EM, Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, Cytotherapy 8(4) (2006) 315–317.
8. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso Z, Fraser JK, Benhaim P, Hedrick MH. Human Adipose Tissue Is a Source of Multipotent Stem Cells. Mol Biol Cell. 2002;13(12):4279–95.
9. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortizgonzalez XR, Reyes M, Lenvik T, Lund TC, Blackstad M. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002;418(6893):41–9.
10. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks MW, Reinhard F, Zhang CC, Shipitsin M. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008;133(4):704–15.
11. Prockop DJ. Marrow Stromal Cells as Stem Cells for Nonhematopoietic Tissues. Science. 1997;276(5309):71–4.
12. Caplan AI. Mesenchymal stem cells. J Orthop Res. 1991;9(5):641–50.
13. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005;105(4):1815–22.
14. Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells. 2006;24(5):1294–301.
15. Blanc KL, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, Ringden O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. The Lancet. 2004;363(9419):1439–41.
16. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L. A Perivascular Origin for Mesenchymal Stem Cells in Multiple Human Organs. Cell Stem Cell. 2008;3(3):301–13.
17. Sato T, Vries RGJ, Snippert HJ, De Wetering MV, Barker N, Stange DE, Van Es JH, Abo A, Kujala P, Peters PJ. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009;459(7244):262–5.
18. Lee WC, Lim CHYX, Shi H, Tang LAL, Wang Y, Lim CT, Loh KP. Origin of Enhanced Stem Cell Growth and Differentiation on Graphene and Graphene Oxide. ACS Nano. 2011;5(9):7334–41.
19. Tatavarty R, Ding H, Lu G, Taylor R, Bi X. Synergistic acceleration in the osteogenesis of human mesenchymal stem cells by graphene oxide–calcium phosphate nanocomposites. Chem Commun. 2014;50(62):8484–7.
20. Kim J, Kim HD, Park J, Lee E, Kim E, Lee S, Yang J, Lee Y, Hwang NS. Enhanced osteogenic commitment of murine mesenchymal stem cells on graphene oxide substrate. Biomaterials Research. 2018;22(1):1–9.

21. Wang Z, Shen H, Song S, Zhang L, Chen W, Dai J, Zhang Z. Graphene Oxide Incorporated PLGA Nanofibrous Scaffold for Solid Phase Gene Delivery into Mesenchymal Stem Cells. J Nanosci Nanotechnol. 2018;18(4):2286–93.
22. Yang J, Hsieh KY, Kumar PV, Cheng S, Lin Y, Shen Y, Chen G. Enhanced Osteogenic Differentiation of Stem Cells on Phase-Engineered Graphene Oxide. ACS Appl Mater Interfaces. 2018;10(15):12497–503.
23. Kim S, Han H, Chae G, Lee S, Bo S, Yoon J, Lee YS, Lee K, Park H, Kang K. Successful Stem Cell Therapy Using Umbilical Cord Blood-Derived Multipotent Stem Cells for Buerger's Disease and Ischemic Limb Disease Animal Model. Stem Cells. 2006;24(6):1620–6.
24. Nicola MD, Carlostella C, Magni M, Milanesi M, Longoni P, Matteucci P, Grisanti S, Gianni AM. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood. 2002;99(10):3838–43.
25. Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, Kikutani H, Kishimoto T. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature. 1996;382(6592):635–8.
26. Ewald FC. The Knee Society total knee arthroplasty roentgenographic evaluation and scoring system. Clin Orthop Relat Res. 1989;248(248):9–12.
27. Girgis FG, Marshall JL, Monajem A. The Cruciate Ligaments of the Knee Joint: Anatomical. Functional and Experimental Analysis. Clin Orthop Relat Res. 1975;106(106):216–31.
28. Insall JN, Salvati EA. Patella position in the normal knee joint. Radiology. 1971;101(1):101–4.
29. Hernandez Y, Nicolosi V, Lotya M, Blighe FM, Sun Z, De S, Mcgovern IT, Holland B, Byrne MT, Gunko YK. High-yield production of graphene by liquid-phase exfoliation of graphite. Nat Nanotechnol. 2008;3(9):563–8.
30. Elias DC, Nair R, Mohiuddin T, Morozov SV, Blake P, Halsall MP, Ferrari A, Boukhvalov DW, Katsnelson MI, Geim AK. Control of Graphene's Properties by Reversible Hydrogenation: Evidence for Graphane. Science. 2009;323(5914):610–3.
31. Kudin KN, Ozbas B, Schniepp HC, Prudhomme RK, Aksay IA, Car R. Raman spectra of graphite oxide and functionalized graphene sheets. Nano Lett. 2008;8(1):36–41.

Figures

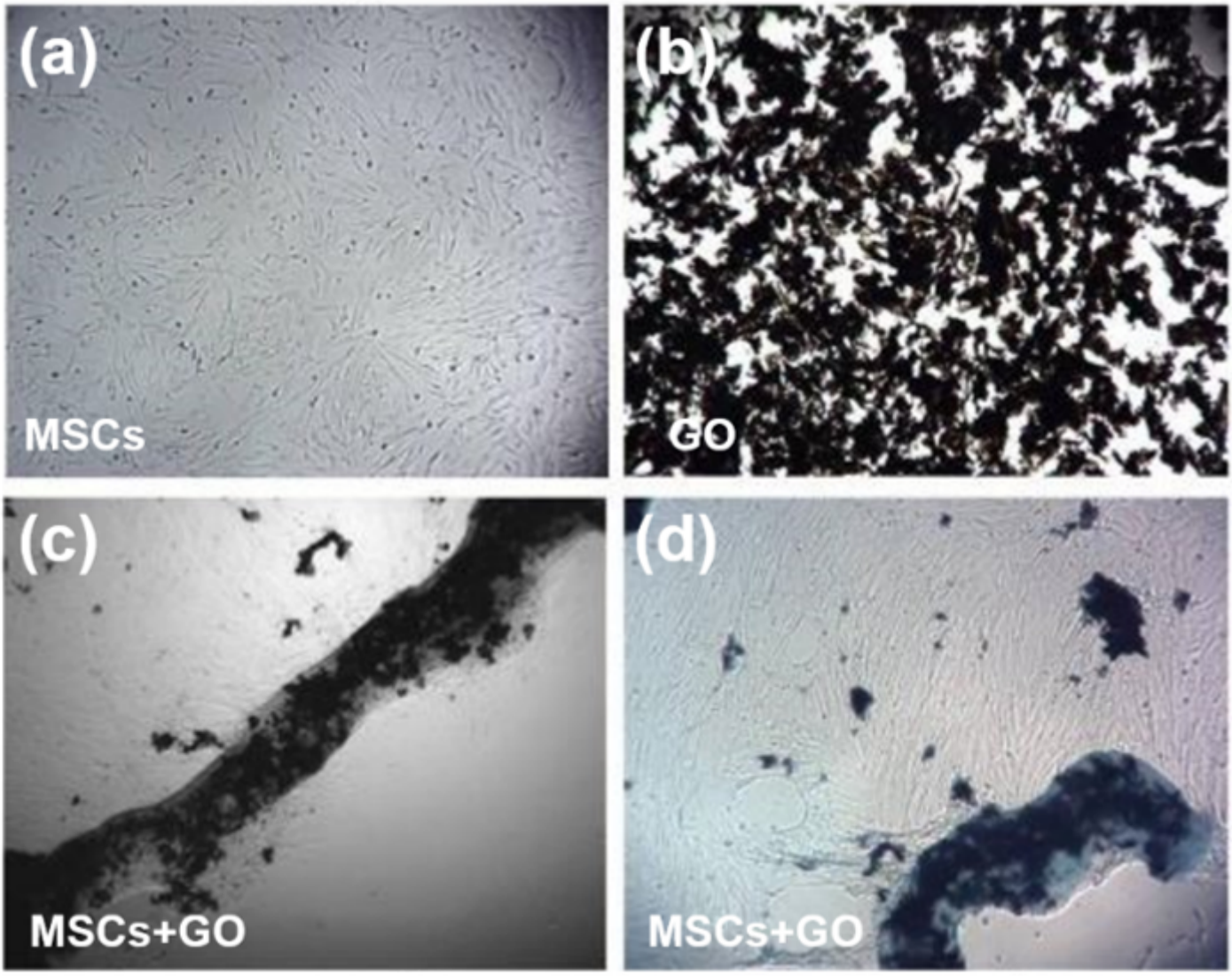


Figure 1

UCMSC co-cultured with GO for 24h (40× magnification).

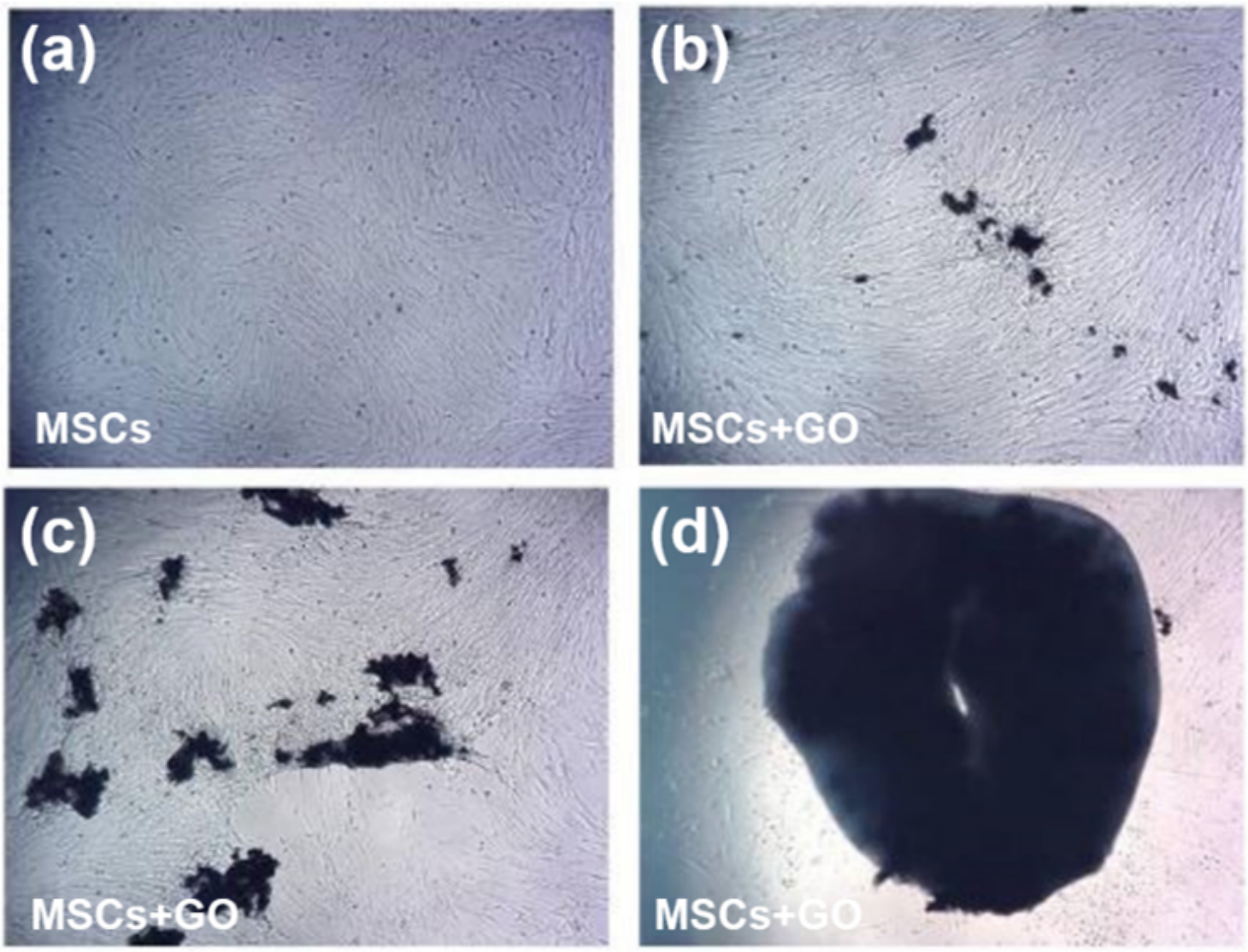


Figure 2

UCNSC and GO co-culture for 72h (40× magnification).

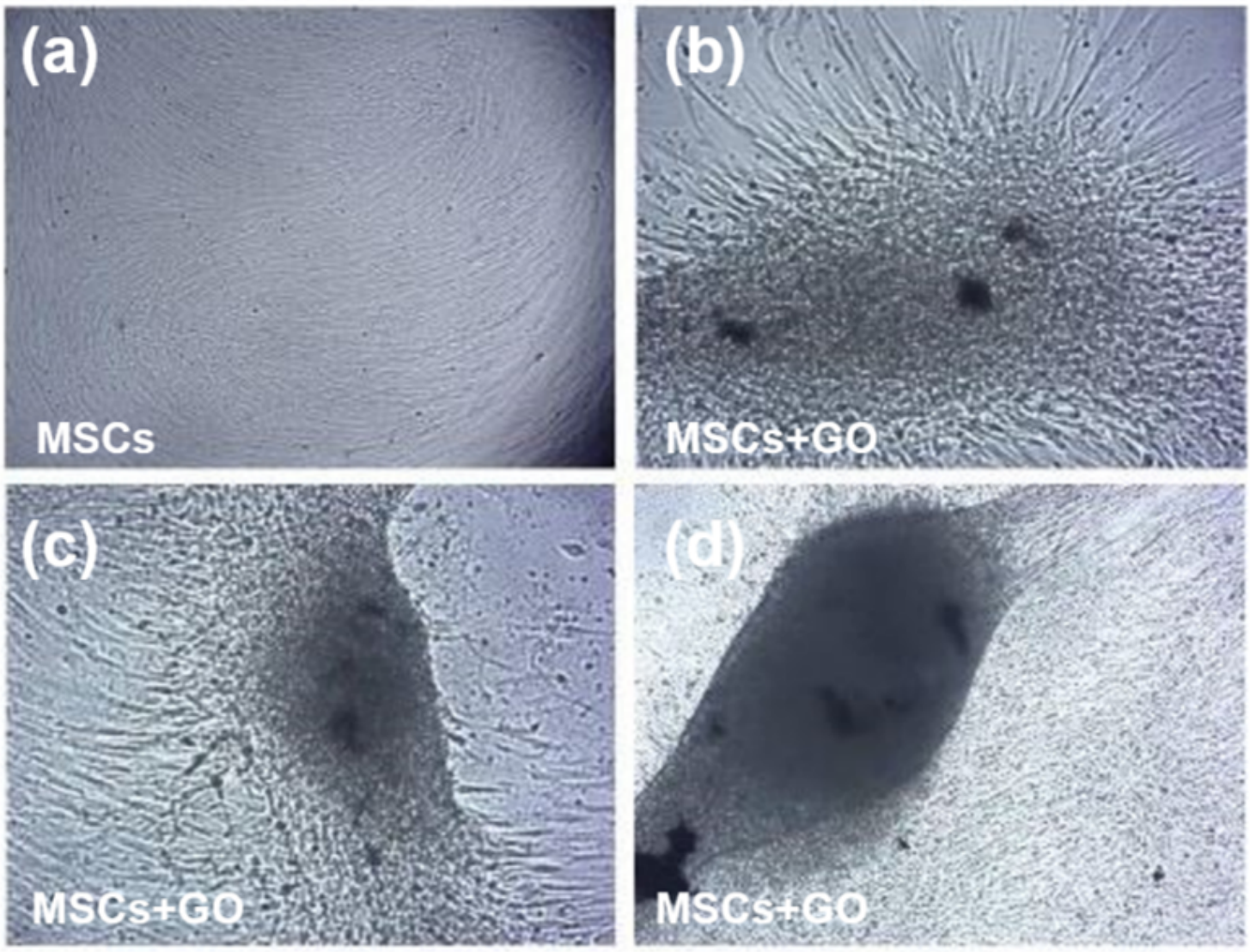


Figure 3

UCMSC and GO co-culture on the 7th day (UCMSC group magnified 40 times, UCMSC+ GO group magnified 100 times).

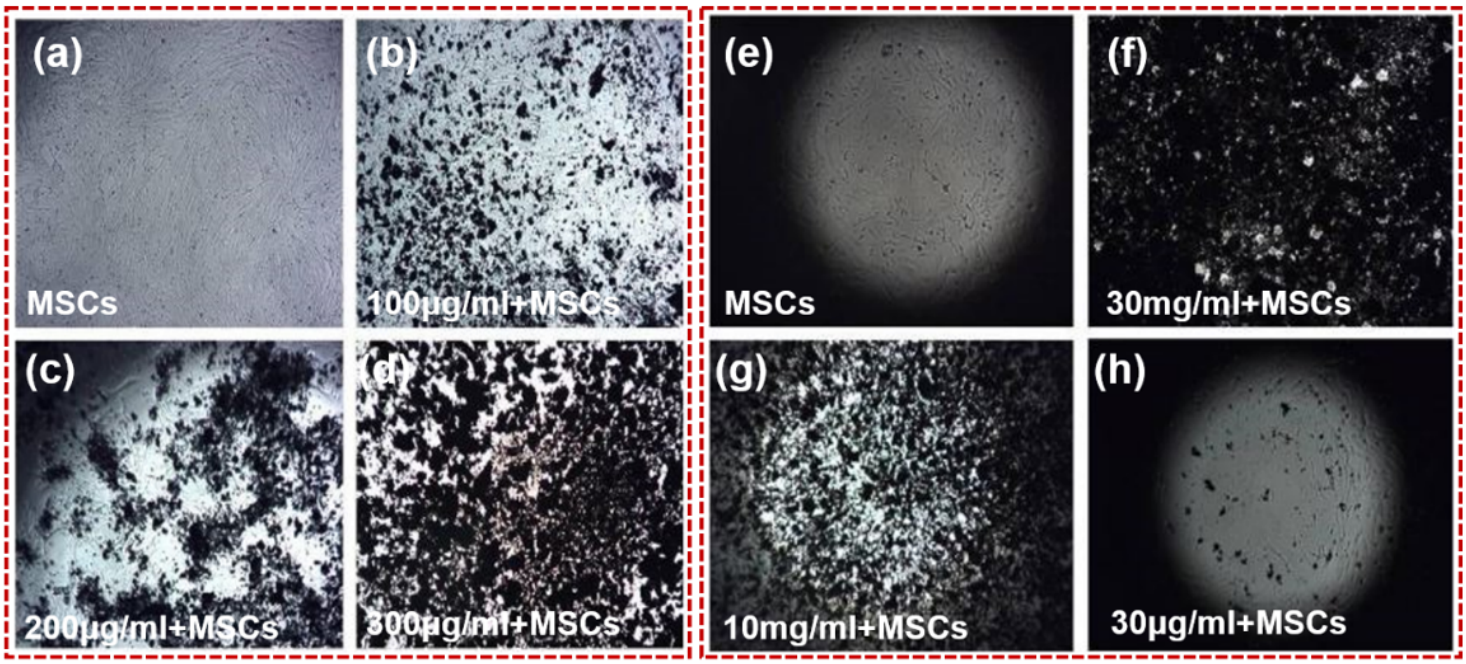


Figure 4

UCMSC with different concentrations of GO and co-culture for 48h (40× magnification).

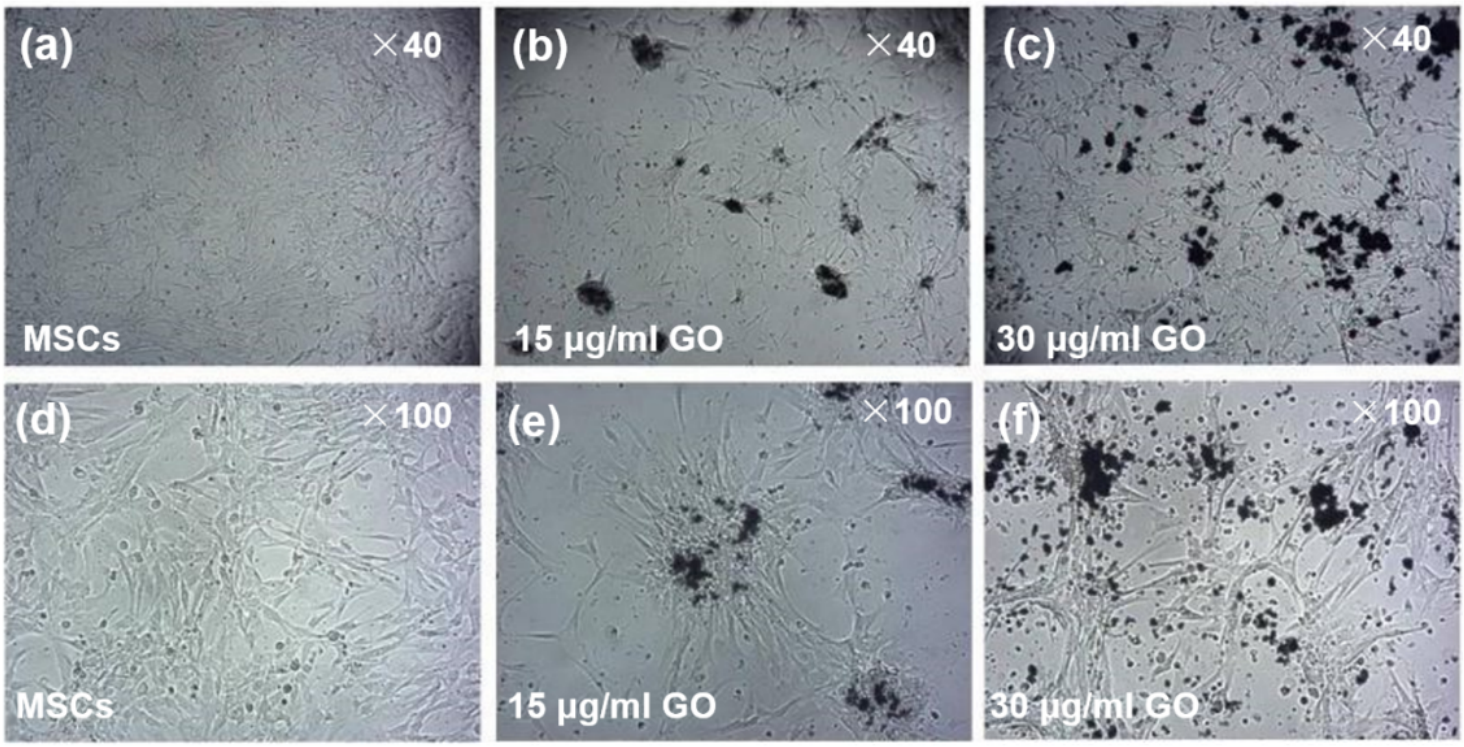


Figure 5

UCMSC co-cultured with 15ug/ml GO, 30ug/ml GO for 48h (The picture above is magnified 40 times and the picture below is magnified 100 times).

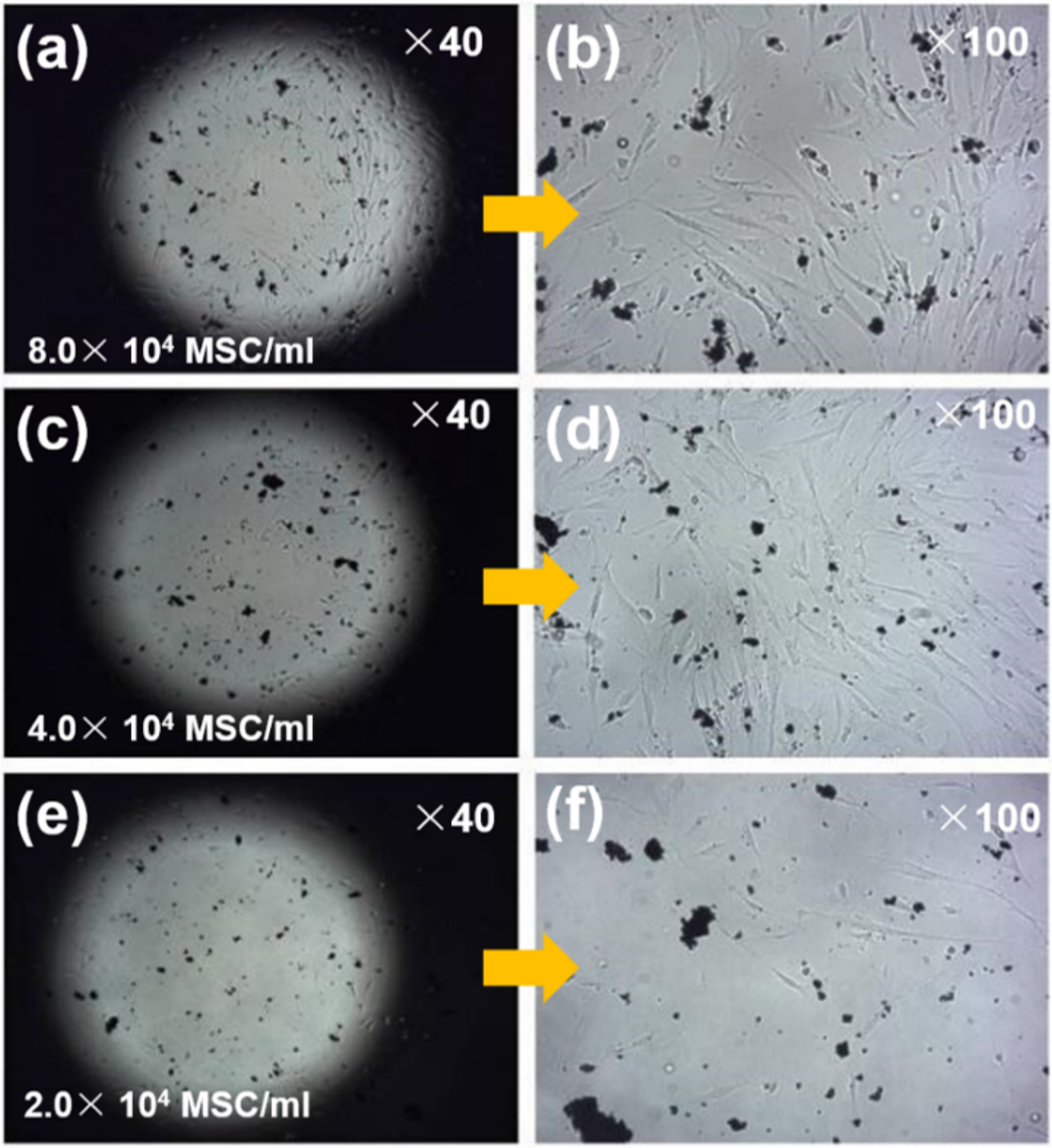


Figure 6

Different concentrations of UCMSCs co-cultured with 30 ug/ml GO for 48h (The picture on the left is magnified 40 times, and the picture on the right is magnified 100 times).

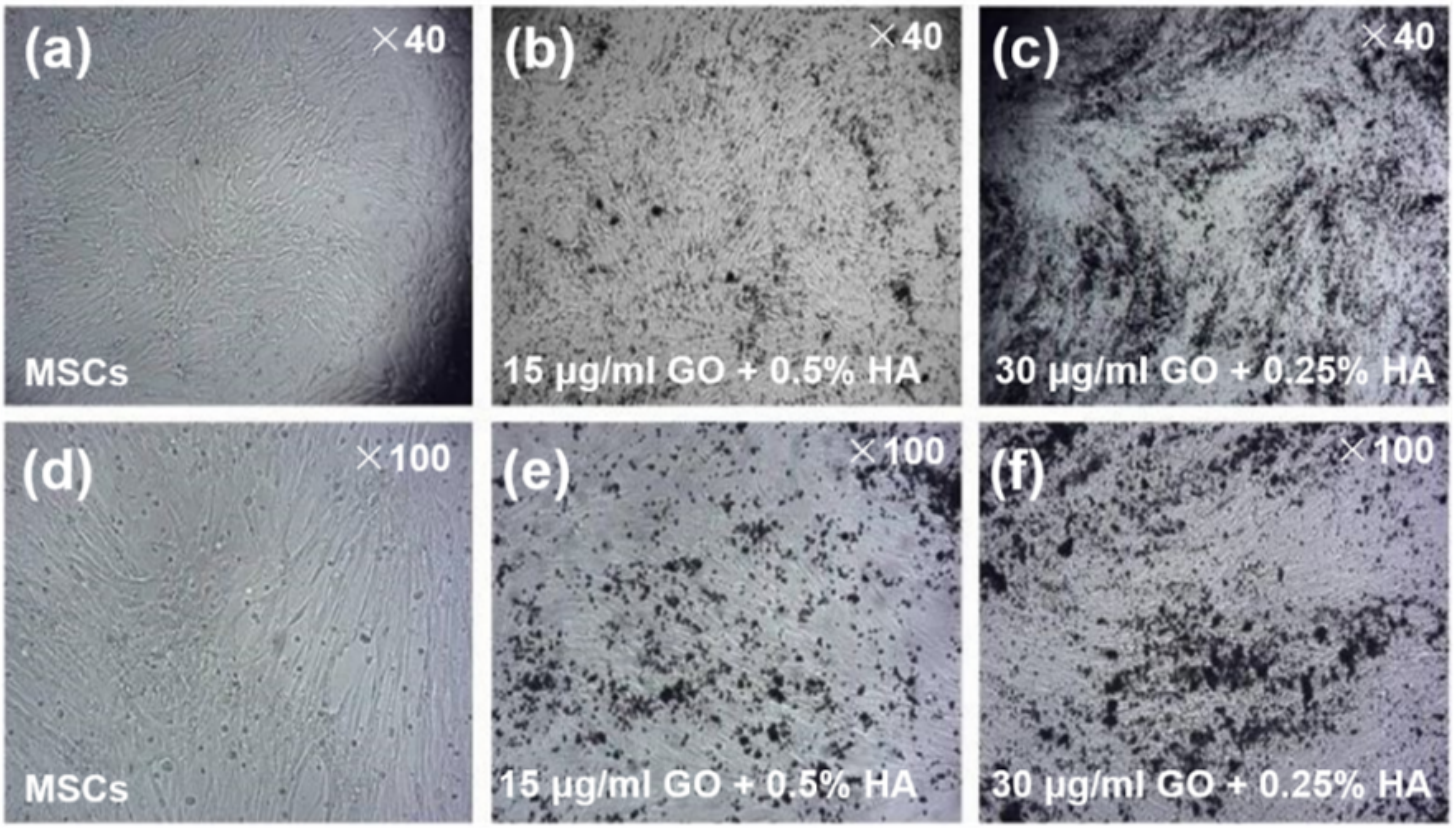


Figure 7

UCMSC co-cultured with 15 ug/ml+0.5%HA and 30 ug/mlGO+0.25%HA for 24h (The picture above is magnified 40 times and the picture below is magnified 100 times).

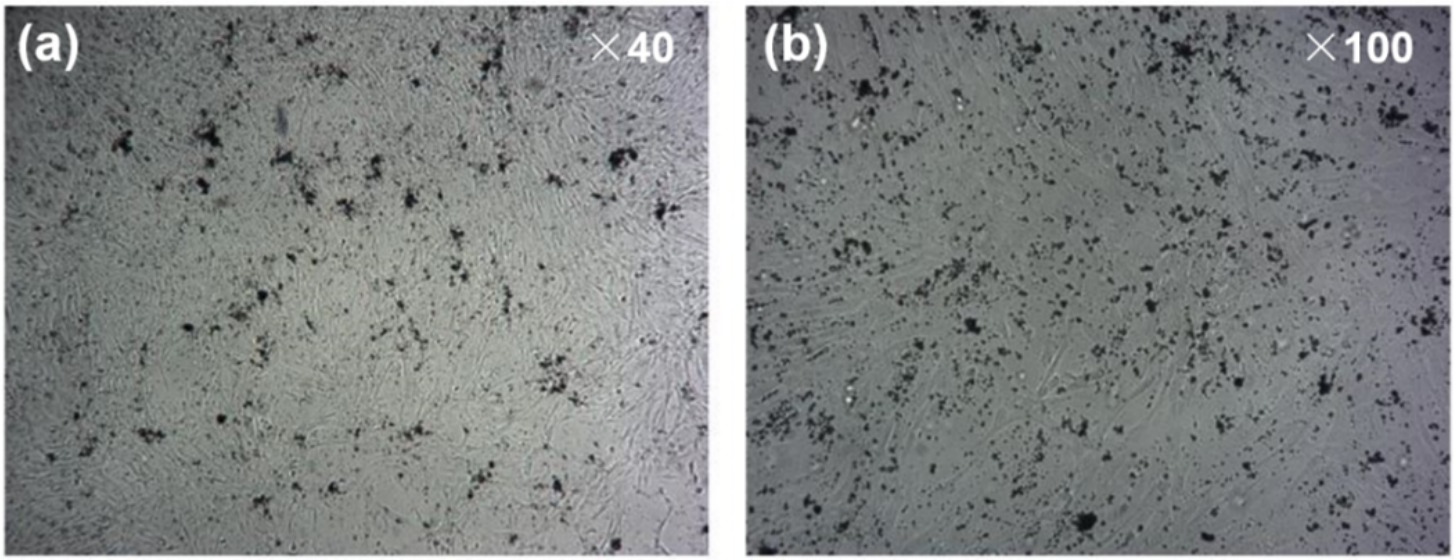


Figure 8

UCMSC co-cultured with 15ug/mlGO+0.5%HA for 7d (The picture on the left is magnified 40 times, and the picture on the right is magnified 100 times).

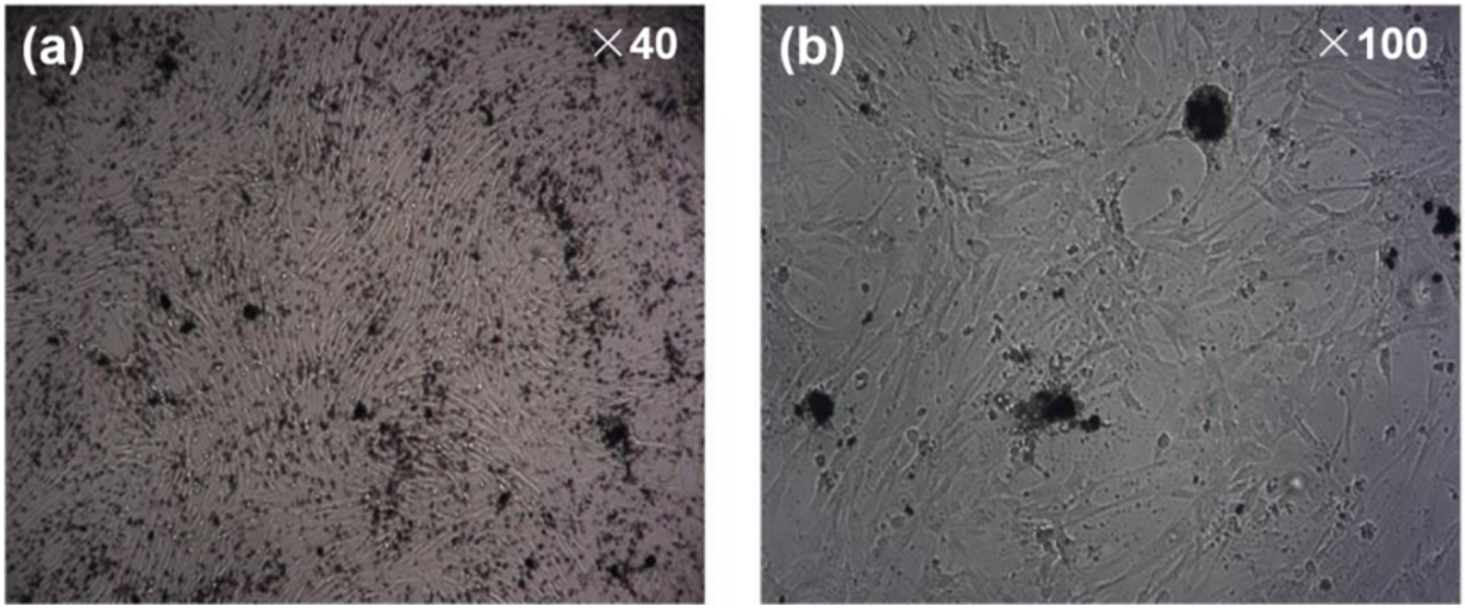


Figure 9

UCMSC co-cultured with 30ug/mlGO+0.25% HA for 7d (The picture on the left is magnified 40 times, and the picture on the right is magnified 100 times).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SI.docx