American Journal of Biomedical Science and Engineering
2015; 1(5): 51-57

American Association for Science and Technology

Keywords
Graphene Oxide,
Ultrahigh-Molecular-Weight Polyethylene,
Mechanical,
Biocompatibility

Received: June 30, 2015
Revised: July 13, 2015
Accepted: July 14, 2015

Graphene Oxide/Ultrahigh Molecular Weight Polyethylene Composites: Ball-Milling Preparation Mechanical Performance and Biocompatibility Effects

Fengzhen Liu[1], Yunhua Wang[1], Keyi Li[1], Licheng Jiang[1], Xiumei Wang[2], Xin Shao[3], Bin Zhang[1, *], Fuzhai Cui[2, *]

[1]Liaocheng People’s Hospital, Medical School of Liaocheng University, Liaocheng, P. R. China
[2]School of Materials Science and Engineering, Tsinghua University, Beijing, P. R. China
[3]Non-Ferrous Metal Research Institute, Liaocheng University, Liaocheng, P. R. China

Email address
ldcllfz@163.com (Fuzhai Cui)

Citation
Fengzhen Liu, Yunhua Wang, Keyi Li, Licheng Jiang, Xiumei Wang, Xin Shao, Bin Zhang, Fuzhai Cui. Graphene Oxide/Ultrahigh Molecular Weight Polyethylene Composites: Ball-Milling Preparation Mechanical Performance and Biocompatibility Effects. *American Journal of Biomedical Science and Engineering.* Vol. 1, No. 5, 2015, pp. 51-57.

Abstract

Graphene oxide (GO)/ultrahigh-molecular-weight polyethylene (UHMWPE) composites were successfully fabricated through the mechanical ball milling technology and processed according to the hot pressing method. The fractured microstructure features and mechanical properties of the GO/UHMWPE composites were investigated by Scanning electron microscope (SEM) and universal testing machine, respectively. By immersion process with simulated body fluid (SBF) and then by in vitro cytotoxicity test with MC3T3-E1 osteoblasts, GO/UHMWPE composites exhibited desirable stability and admirable cytocompatibility. Moreover, the attachment and proliferation of the MC3T3-E1 cells on the surfaces of GO/UHMWPE composites were investigated by methyl thiazolyl tetrazolium (MTT) assay, SEM and fluorescence staining observations to evaluate the biocompatibility of GO/UHMWPE composites. The addition of 0.5 wt.% GO increased the hardness of the pure UHMWPE gradually. The MC3T3-E1 cells well attached and grew on the surfaces of the composites, and the adding of GO did not affect the morphology and viability of MC3T3-E1 cells. The GO/UHMWPE composites displayed a remarkable combination of enhanced mechanical properties and good biocompatibility, making the composites attractive for potential candidate as artificial joints in the human body.

1. Introduction

Ultrahigh-molecular-weight polyethylene (UHMWPE), with molar weights exceeding one million, has high impact strength, good biocompatibility and low friction coefficient. So it is widely used as the material for artificial joints in the human body [1-4]. However, owing to the low surface hardness and poor wear resistance of polymer, wear particles lead to osteolysis [5-7], which will cause bone loss, joint loosening, discomfort, and ultimately limit the lifespan of the artificial joints [8]. Therefore, much efforts have been carried out to improve the mechanical and tribological properties of the UHMWPE materials. One way to improve the wear and mechanical properties of UHMWPE is the use of inorganic fillers. Carbon based reinforcing fillers with different shapes, sizes and dimensions have been studied extensively in the field of polymer composites [9-11]. In particular, graphene, a new class of two-dimensional carbon nanostructure, has attracted considerable attentions, owing to its high Young's modulus, hardness, excellent flexibility, low cost, and has potential applications in polymer composites [12-14]. Recently, there are several reports related to graphene/polymer composites. When incorporated, graphene improved significantly the electrical, thermal and mechanical properties of the host polymers at a small loading [15-18]. Graphene oxide (GO) is easily available through the controlled chemical oxidation of graphite. GO contains epoxide and hydroxyl groups are located on the basal planes, carbonyl, and carboxyl groups are found at the edges [19, 20]. The presence of these functional groups makes GO strongly hydrophilic, readily swell and disperse in water. And the most important, the oxygen functionality allows for enhanced interactions with the polar polymer matrices, creates a percolated domain of an "interphase" polymer that affects dramatically the thermal and mechanical properties [21]. These have motivated us to explore the possibility of GO as a reinforcement in UHMWPE matrix for a new kind of artificial joints. More recently, biocompatibility of graphene films was compared with carbon nanotubes using a mouse fibroblast cell line to assess the cytotoxicity, the results suggested that the cells adhered and proliferated on graphene film well than carbon nanotubes, which indicated that the material is biocompatible and has no cytotoxicity [22-24].

The development of new routes for fabrication of GO/UHMWPE composites is still highly desired. In this study, we reported on the synthesis of GO/UHMWPE composites using a simple combination of ball-milling dispersion followed by hot-pressing, which is a facile route operated under ambient conditions with the advantages of low cost and simplicity. As the prepared GO/UHMWPE composites showed remarkably enhanced hardness and slightly improved yield strength compared with pure UHMWPE. The addition of small amounts of GO did not affect the attachment and proliferation of the MC3T3-E1 cells cultured on GO/UHMWPE composites surfaces, indicating its excellent biocompatibility. We expect such GO/UHMWPE composites with good mechanical properties and biocompatibility may find important applications in artificial joints.

2. Experimental Sections

All of the chemical reagents were of analytic-grade and used without further purification (purchased from Shanghai Chemical Reagent Co., Ltd., Shanghai, China), UHMWPE was purchased from Hercules, the compound had a number average molecular weight, Mn, of 50,000 and a mass average molecular weight, Mw, of 190,000.

2.1. Synthesis of GO and GO/UHMWPE Composites

Natural graphite powder was utilized as the raw material to prepare graphite oxide by suspension through a modified Hummers' method [25, 26]. In a typical synthesis, synthesized GO was mixed with UHMWPE and six different types of GO/UHMWPE composites, containing 0, 0.1, 0.3, 0.5, 0.7 and 1.0 wt.%. The nomenclature of these six composites was GO-00/UHMWPE, GO-01/UHMWPE, GO-03/UHMWPE, GO-05/UHMWPE, GO-07/UHMWPE, and GO-10/UHMWPE. The GO/UHMWPE composites were prepared using the follow method. First, GO were dispersed in ethylalcohol solution (50 mL) under vigorous stirring to form a stable suspension A. Then stoichiometric amount of UHMWPE (50 g) was added to ethylalcohol solution (450 mL) under vigorous stirring to form solution B. A and B were mixed together, ultrasonicated for several times at room temperature using an ultrasonic generator with a frequency and nominal power of 28 kHz and 600 W, respectively. The GO/UHMWPE composites were washed several times with ethanol, and were dried in a vacuum oven for 72 h at 60 ℃. Finally, the GO/UHMWPE composites were ball-milled 2.5 h with a frequency of 300 R/min to get the obtained products.

2.2. Characterizations

The morphology characterization and microstructure of GO/UHMWPE hybrid materials analysis were carried out using Scanning electron microscope (SEM) measurement on a JEOL-6380LV and GO was observed by Transmission electron microscope (TEM) measurement on a JEOL-2100. The structure of GO and GO/UHMWPE was examined by Fourier transform infrared spectroscopy (FT-IR, VERTEX 80v, Bruker Optics, Germany). Differential scanning calorimetry (DSC, Perkin-Elmer 7) was carried out in dry nitrogen gas at a flow rate of 10 mL/min. The DSC was calibrated using indium as the standard, and the sample weight was 7.0 ± 0.1 mg. The thermal history of the products was removed by scanning them from 30 to 220 °C at a heating rate of 10 °C /min followed by cooling to 25°C at a scan rate of 10 °C /min. The tensile properties were tested using an Instron 4665 ultimate tensile testing machine (UTM) at 20 °C and a humidity of 30%. The dumb-bell specimens were made according to the ASTM D 638 standard for tensile testing. The cross-head speed was set to 50 mm/min for both the dumb-bell samples. The mean value of each product was determined as the average value of five test specimens.

2.3. Biocompatibility Test

2.3.1. MSC Cells Culture and Seeding

MC3T3-E1 cells were cultured in Revolutions-Per-minute Indicator (RPMI 1640) containing 10% FBS, 50 U/mL penicillin and 50 U/mL streptomycin. The medium was refreshed every 3 days and the cells were incubated in a tissue culture incubator at 37 °C with 5% CO_2. After reaching about 80% confluence, the cells were detached by 0.25% trypsin. The sample size for cell culture and seeding was 10×10 mm^2. A density of 104 cells/mL was seeded in 24-well plates for methyl thiazolyl tetrazolium (MTT) assay and SEM observation after the composite samples were sterilized by 75% ethyl alcohol for 2 h, and a density of 104 cells/mL was seeded for fluorescence staining observation.

2.3.2. MTT Assay

The MTT assay is one of the chromatic assays that have been used to test the cytotoxicity and cell viability [27]. MC3T3-E1 Cells were seeded as previously described and the cell viability was evaluated after 1, 2 and 4 days by MTT assay, which was indicated by the reduction of MTT into a acridine orange dye by living cells. MTT solution (100 μL) at 5 mg/mL in phosphate buffered saline (PBS) was added to each well and incubated for 4 h under the same conditions described. After removal of the medium, the converted dye was dissolved in 750 μL/well dimethyl sulfoxide (DMSO). Solution (150 μL) of each sample was transferred to a 96-well plate. Absorbance of converted dye was measured at a wavelength of 490 nm using an ELISA plate reader.

2.3.3. SEM and Fluorescence Staining Observations

After 1, 2 and 4 days of culture, samples were rinsed twice with PBS to remove non-adherent cells and subsequently fixed with 3% glutaraldehyde at 4 °C for 2 h. Thereafter, the samples were dehydrated through a series of graded ethanol solutions and air-dried overnight. Dry cellular constructs were sputtered with gold and observed under SEM to examine morphology of the cells. In the same way, cells were dehydrated through absolute ethyl alcohol, and the samples were stained with acridine orange, which was cleaved to yield a green fluorescent product by metabolically active cells. The density of the cells which adhered on each sample was measured from randomly selected views of observed at 100-fold magnification with a microscopy.

3. Results and Discussion

3.1. Morphology and Crystalline Structure

Fig. 1 showed the TEM image and XPS spectrum of GO sheets. It was evident that the GO exhibited a typically wrinkled, thin, sheet-like structure and a two dimensional sheet morphology, which had a thickness and mean lateral size (Fig. 1(a)). Furthermore, the XPS peak of C1s was decomposed into four Gaussian peaks ranging from 282.0 to 292.0 eV, which centered at the binding energies of 289.3, 287.5, 286.3, and 284.8 eV were assigned to the C=O, C-O-C, C-OH and C=C, respectively (Fig. 1(b)). It indicates the considerable degree of the oxidation existing in GO material, which results in the hydrophilic nature of GO.

The DSC curves and crystallinity of GO/UHMWPE composites were shown in Fig. 2. The recrystallization temperatures of the GO/UHMWPE composites increased gradually with increasing GO content. This suggests that GO acts as a nucleation agent for crystallization of the UHMWPE matrix by providing a very large surface area for adsorption of the UHMWPE chain, resulting in easier nucleation. The peak temperature of crystallization melting occurred at about 141°C for all the GO/UHMWPE composites, indicating that the addition of GO has little effect on the melting point of the composites.

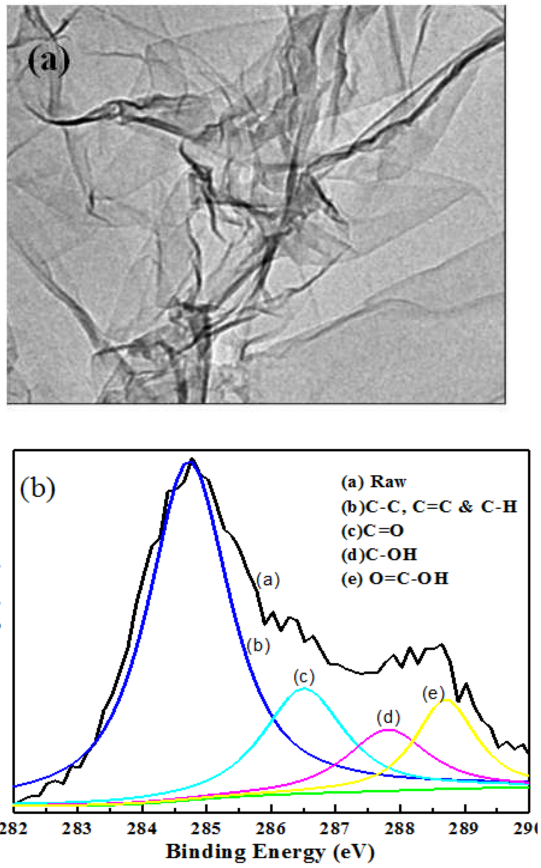

Fig. 1. Characterizations of GO sheets: (a) TEM image and (b) C1s XPS spectrum.

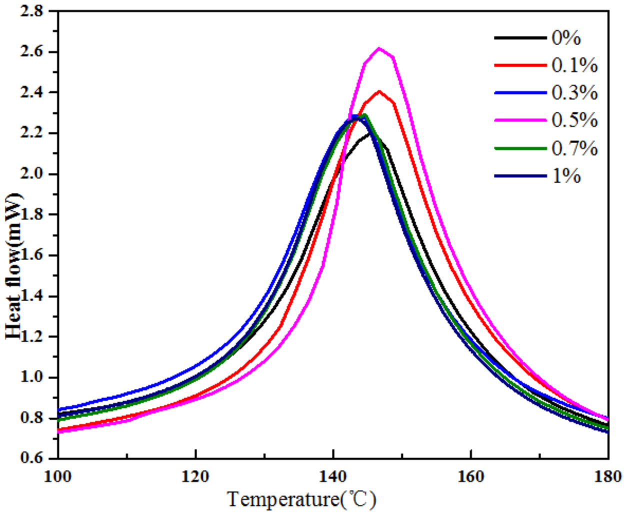

Fig. 2. DSC curves of GO/UHMWPE composites with different amounts of GO.

Fig. 3 showed the SEM images of the fractured surfaces of GO/UHMWPE composites with different amounts of GO. It could be seen that the fracture surface of unfilled UHMWPE was relatively flat. When the GO content was 0.1 to 0.3 wt.%, the corresponding fracture surface was uneven and the GO sheets were randomly distributed within the polymer matrix. Furthermore, as the content of GO increased to 0.5 to 0.7 wt.%, the morphology of the fracture surface was totally different. The image showed GO sheets were embedded into the polymer so that GO and polymer could combine tightly to each other to form a layered structure. As the content of GO further increased to 1.0 wt.%, the layers stacked in a more compact manner. As a result, the extension of the polymer chain was hindered by the closely compacted GO sheets. Our fabrication processing was presented to explain the combination of GO and polymer matrix. After they were mixed by ultrasonication dispersion and high speed ball-milling, UHMWPE powders were homogenously covered by GO sheets. To some extent, ultrasonication dispersion and high speed ball-milling were an effective way to disperse GO sheets into UHMWPE. Subsequently, these GO/UHMWPE powders were hot-compressed at a high temperature to stick together tightly and form a continuous mixed phase.

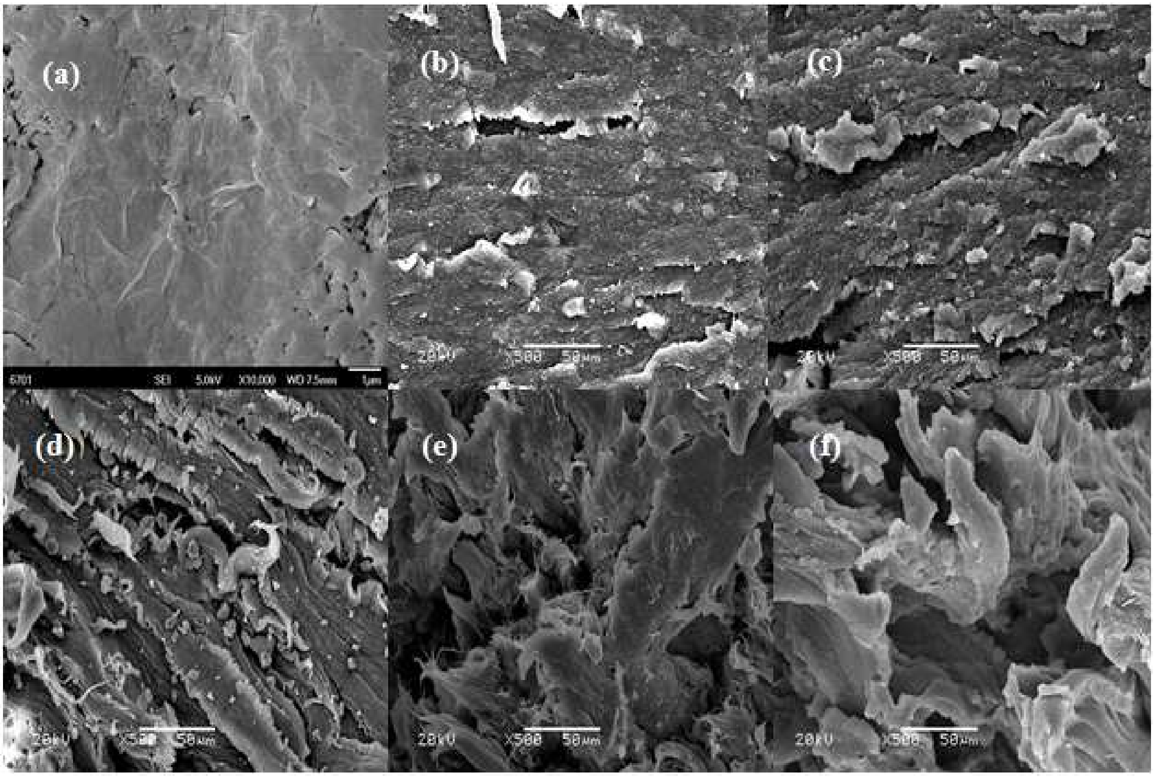

Fig. 3. SEM images of the fractured surfaces of the GO/UHMWPE composites with different amounts of GO: (a) 0 wt.%, (b) 0.1 wt.%, (c) 0.3 wt.%, (d) 0.5 wt.%, (e) 0.7 wt.% and (f) 1 wt.%.

3.2. Microhardness

Fig. 4 showed the variation of microhardness values with the increase of GO into UHMWPE. It could be noticed that the microhardness of the GO/UHMWPE composites increased gradually with the increase content of GO. The addition of 1.0 wt.% GO increased the microhardness of unfilled UHMWPE from 5.18 to 6.87, corresponding to an increasing amount of about 33.33%. It was indicated that low additions of GO sheets could obviously enhance the hardness of UHMWPE. This was due to the excellent mechanical properties of GO sheets, which could bear partial load and be very essential for load transfer. According to Archard's prediction [28], the increase of hardness would reduce the plastic contact areas for UHMWPE or its composite to metal surface. Therefore, we believe the GO/UHMWPE composites would have improved friction performance with coordination metal material compared with unfilled UHMWPE materials.

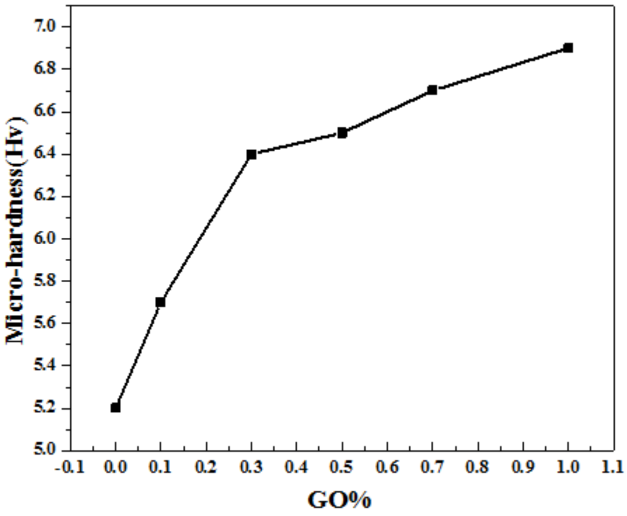

Fig. 4. The microhardness of the GO/UHMWPE composites with different amounts of GO.

3.3. Biocompatibility of GO/UHMWPE Composites

Biocompatibility is an essential factor for joint prosteheses applications. Measuring the biocompatibility depends on the techniques used for synthesis and the selection of the biological model system for study [29]. MTT assay is a cytotoxicity test method by evaluating the number of living cells and the strength to living cells metabolism. In order to evaluate the biocompatibility of as-prepared GO/UHMWPE, the cytotoxic effect of GO/UHMWPE and UHMWPE against cells was investigated. Fig. 5 showed the viability of cells which were incubated with GO/UHMWPE and UHMWPE. The observed results revealed that all samples were suitable for the proliferation of the MSC cells in a fast rate. And this result indicated that GO/UHMWPE was significantly biocompatible and cell viability was not compromised. There was no obvious change in the adsorption on the GO/UHMWPE composites compared with that on the pure UHMWPE, indicating that the addition of GO sheets into UHMWPE had no negative effect on the cell growth. Taken together, these results suggested that GO/UHMWPE is more compatible than UHMWPE which is due to the functionalization of GO. Therefore, it was considered that the surface chemistry was the primary contributor to the difference of toxicity between GO/UHMWPE and UHMWPE. It can be definitely attributed to the excellent intrinsic biocompatibility and the hydrophilic nature of GO material.

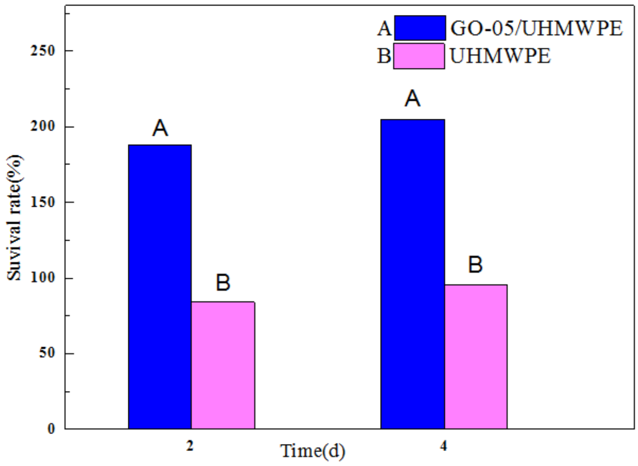

Fig. 5. *Viability of the MC3T3-E1 cells on the GO/UHMWPE and UHMWPE composites at different incubation times.*

Fig. 6 showed the cell attachment on the GO/UHMWPE composites with 0.5 wt.% GO at the incubation periods of 12, 24 and 48 h, respectively. It was clear that MC3T3-E1 cells well adhered and proliferated on the GO/UHMWPE scaffold with the increase of the culture time. After 48 h incubation, the sample surface was almost covered with cells. Based on MTT results analysis, it could be concluded that the GO/UHMWPE composites promoted the proliferation of osteoblasts after cocultivation for 2 and 4 days, and that the cell viability was hardly restrained by the composites.

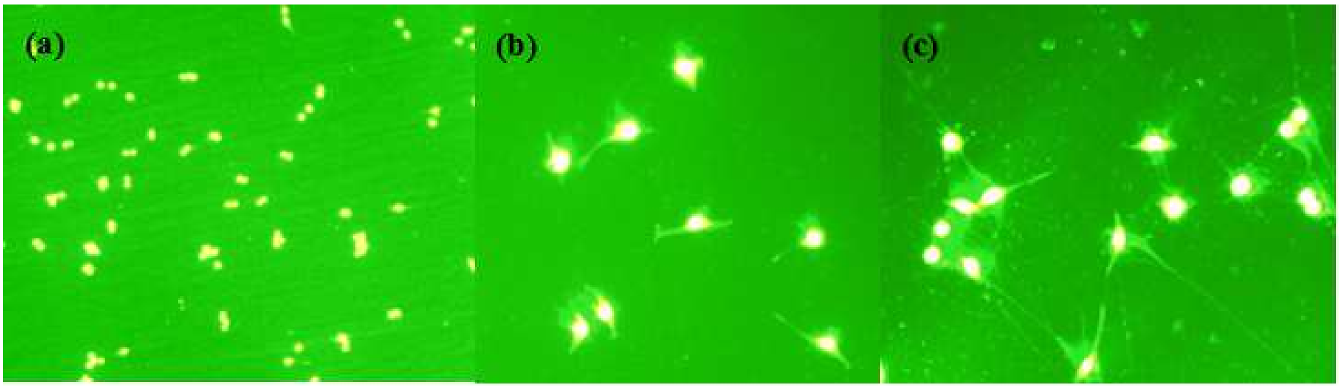

Fig. 6. *Fluorescent microscopy images of the MC3T3-E1 cells on the GO/UHMWPE composite with 0.5 wt.% GO for (a)12 h, (b) 24 h, and (c) 48 h.*

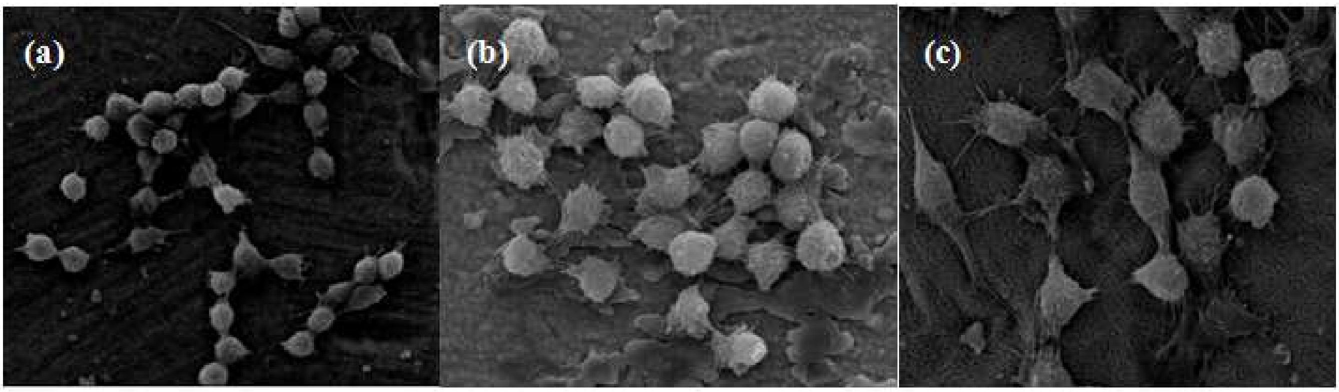

Fig. 7. *SEM images of the MC3T3-E1 cells on the GO/UHMWPE composite with 0.5 wt.% GO for (a)12 h, (b) 24 h, and (c) 48 h.*

The surface properties of implant materials play an important role in bone cells activities at interfaces, which refers to surface chemistry, topography, roughness and energy [30-32]. In vitro experimental evidences [33-35] presented that submicron roughness surface was beneficial to osteoblastic adhesion, and the corresponding results were obtained in this study. The cell adhesion on various specimens was illustrated in Fig. 7, after the cells were seeded directly on the GO/UHMWPE composites scaffold and incubated for 24 h, the scaffold surface appeared to be adhered with some cells. The cells maintained the normal morphology with some pseudopods around them and multiple microvilli, indicating strong adhesion to the substrate and active cell migration, which were probably produced from the cells. With the increase of culture time, the cells secreted extracellular matrices (ECM), where cells were able to adhere, grow and spread on the scaffold surface. The osteoblasts attached to UHMWPE surface also presented with normal morphology and typical spreading with spindly filopodia. The observation from SEM images confirmed that the GO/UHMWPE composites had no adverse influence on the morphology of osteoblastic cells. Furthermore, the results from MTT test proved that the film was not cytotoxic, or did not inhibit cell growth.

4. Conclusions

A simple ball-milling mixing followed by hot-pressing was used to prepare GO/UHMWPE composites, where GO sheets were well dispersed into polymer matrix. The addition of GO with small amounts could obviously increase the microhardness and the GO/UHMWPE composites exhibited good biocompatibility. More importantly, this robust and durable GO/UHMWPE composites are non-cytotoxic and have potential applications in the biomedical field to deposit it on various substrates. Therefore, the combined advantages of the GO/UHMWPE composites make them promising materials for artificial joints applications.

Acknowledgment

We gratefully acknowledge financial support from the National Natural Science Foundation of China (No. 51172102).

References

[1] Turos A, Abdul-Kader A M, Grambole D, Jagielski J, Piat-kowska A, Madi N K, Al-Maadeed M. The effects of ion bombardment of ultra-high molecular weight polyethylene. Methods Phys. Res. Sect. B 249 (2006) 660-664.

[2] Gomez-Barrena E, Puertolas J A, Munuera L. Update on UHMWPE research from the bench to the bedside. Acta Orthop. 79 (2008) 832-840.

[3] Suñer S, Emami N. Investigation of graphene oxide as reinforcement for orthopaedic applications. Trib. Mater. Surf. Interface 8 (2014) 1-6.

[4] Puértolas J A, Kurtz S M. Evaluation of carbon nanotubes and graphene as reinforcements for UHMWPE-based composites in orthoplastic applications: A review. JMBBM 39 (2014) 129-145.

[5] Satyanarayana N, Sinha S K, Ong B H. Tribology of a novel UHMWPE/PFPE dual film coated onto Si surface. Sens. Actuat. A Phys. 128 (2006) 98-108.

[6] Myo M, Sinha S K. DLC and UHMWPE as hard/soft composite film on Si for improved tribological performance. Surf. Coat. Technol. 202 (2008) 3698-3708.

[7] Samad M A, Sinha S K. Dry sliding and boundary lubrication performance of UHMWPE/CNTs nanocomposite coating on steel substrates at elevated temperatures. Wear 270 (2011) 395-402.

[8] Z. X. Tai, Y. F. Chen, Y. F. An, X. B. Yan, Q. J. Xue. Tribological behavior of UHMWPE reinforced with grapheme oxide nanosheets. Tribol. Lett. 46 (2012) 55-63.

[9] Kang M, Myung S J, Jin H J. Nylon 610 and carbon nanotube composite by in situ interfacial polymerization. Polymer 47(11) (2006) 3961-3966.

[10] Aoki N, Akasaka T, Watari F, Yokoyama A. Carbon nanotubes as scaffolds for cell culture and effect on cellular functions. Dent Mater J 2(26) (2007) 178-185.

[11] Virendra Singh, Daeha Joung, Lei Zhai, Soumen Das, Saiful I. Khondaker, Sudipta Seal. Graphene based materials: past, present and future. Progress in Materials Science 56(8) (2011) 1178-1271.

[12] Mao H Y, Laurent S, Chen W, Akhavan O, Imani M, Ashkarran A A, Mahmoudi M. Challenges in graphene: promises, facts, opportunities, and nanomedicine. Chem Rev 113(5) (2013) 3407-3424.

[13] Shao Y, Wang J, Wu H, Liu J, Aksay I A, Lin Y. Graphene based electrochemical sensors and biosensors. Electroanalysis 22(10) (2010) 1027-1036.

[14] Akhavan O, Ghaderi E, Rahighi R. Toward single-DNA electrochemical biosensing by graphene nanowalls. ACS Nano 6(4) (2012) 2904-2916.

[15] Kim H, Abdala A A, Macosko C W. Graphene/polymer nanocomposites. Macromolecules 43(16) (2010) 6515-6530.

[16] Vadukumpully S, Paul J, Mahanta N, Valiyaveettil S. Flexible conductive graphene/poly(vinyl chloride) composite thin films with high mechanical strength and thermal stability. Carbon 49(1) (2011) 198-205.

[17] Veca L M, Meziani M J, Wang W, Wang X, Lu F, Zhang P. Carbon nanosheets for polymeric nanocomposites with high thermal conductivity. Adv Mater 21(20) (2009) 2088-2092.

[18] Rafiee M A, Rafiee J, Wang Z, Song H, Yu Z-Z, Koratkar N. Enhanced mechanical properties of nanocomposites at low graphene content. ACS Nano 3(12) (2009) 3884-3890.

[19] Qiu J J, Wang S. Enhancing polymer performance through graphene sheets. J. App. Poly. Sci. 119 (2011) 3670-3674.

[20] Boukhvalov D W, Katsnelson M I. Modeling of graphite oxide. J Am Chem Soc 130(32) (2008) 10697-10701.

[21] Ramanathan T, Abdala A A, Stankovich S, Dikin D A, HerreraAlonso M, Piner R. D. Functional ized graphene sheets for polymer nanocomposites. Nature Nanotech 3(6) (2008) 327-331.

[22] Chen H, Müller M B, Gilmore K J, Wallace G G, Li D. Mechanically strong, electrically conductive, and biocompatible graphene paper. Adv Mater 2008, 20(18): 3557-3561.

[23] Wang Y, Shi Z X, Fang J H, Xu H J, Yin J. Graphene oxide/polybenzimidazole composites fabricated by a solventexchange method. Carbon 49 (2011) 1199-1207.

[24] Yan X B, Chen J T, Yang J, Xue Q J, Miele P. Fabrication of free-standing, electrochemically active, and biocompatible graphene oxide-polyaniline and graphene-polyaniline hybrid papers. ACS Appl. Mater. Interfaces 2 (2010) 2521-2529.

[25] Hummer W S, Offeman R E. Preparation of graphitic oxide. J. Am. Chem. Soc. 80 (1958) 1339-1339.

[26] J F Shen, Y Z Hu, C Li, C Qin and M X Ye. Synthesis of amphiphilic graphene nanoplatelets. Small 5 (2009) 82-85.

[27] Qiu J J, Wang S. Enhancing polymer performance through graphene sheets. J. App. Poly. Sci. 119 (2011) 3670-3674.

[28] Sachin Kumar, Shammy Raj, Elayaraja Kolanthai, A.K. Sood, S. Sampath, and Kaushik Chatterjee. Chemical functionalization of graphene To augment stem cell osteogenesis and inhibit biofilm formation on polymer composites for orthopedic applications. ACS Appl. Mater. Interfaces DOI: 10.1021/am5079732, 2015.

[29] S. Gurunathan, J. W. Han, V. Eppakayala, A. A. Dayem, D. N. Kwon, J. H. Kim. Biocompatibility effects of biologically synthesized graphene in primary mouse embryonic fibroblast cells. Nanoscale Research Letters 8 (2013) 393-397.

[30] Agarwal S, Zhou X, Ye F, He Q, Chen GCK, Soo J, Boey F, Zhang H, Chen P. Interfacing live cells with nanocarbon substrates. Langmuir 26(4) (2010) 2244-2247.

[31] Heo C, Yoo J, Lee S, Jo A, Jung S, Yoo H, Lee YH, Suh M. The control of neural cell-to-cell interactions through non-contact electrical field stimulation using graphene electrodes. Biomaterials 32(1) (2011) 19-27.

[32] Nayak T R, Jian L, Phua L C, Ho H K, Ren Y P, Pastorin G. Thin films of functionalized multiwalled carbon nanotubes as suitable scaffold materials for stem cells proliferation and bone formation. ACS Nano 4(12) (2010) 7717-7725.

[33] Lee W C, Lim C H, Shi H, Tang L A, Wang Y, Lim C T, Loh K P. Origin of enhanced stem cell growth and differentiation on graphene and graphene oxide. ACS Nano 5(9) (2011) 7334-7341.

[34] Chen G Y, Pang D W, Hwang S M, Tuan H Y, Hu Y C. A graphene-based platform for induced pluripotent stem cells culture and differentiation. Biomaterials 33(2) (2012) 418-427.

[35] Gurunathan S, Han J W, Eppakayala V, Kim J H. Biocompatibility of microbially reduced graphene oxide in primary mouse embryonic fibroblast cells. Colloids Surf B: Biointerfaces 105 (2013) 58-66.