Supporting Information

Gr/Si Promoted Osteogenic Differentiation of BMSCs through Light Illumination

Xiaojun Long,[†] Xiaozhao Wang,[#] Lili Yao,[†] Suya Lin,[†] Jiamin Zhang,[†] Wenjian Weng,[†] Kui Cheng, [*,†] Huiming Wang,[‡,§] and Jun Lin[§]

[†] School of Materials Science and Engineering, State Key Laboratory of Silicon Materials, Cyrus Tang Center for Sensor Materials and Applications, Zhejiang University, Hangzhou 310027, China

Zhejiang University-University of Edinburgh Institute & School of Basic Medicine, Zhejiang University School of Medicine, Hangzhou 314400 , China

‡ The Affiliated Stomatologic Hospital of Medical College, Zhejiang University, Hangzhou 310003, China

§ The First Affiliated Hospital of Medical College, Zhejiang University, Hangzhou 310003, China

Corresponding Author

*E-mail: chengkui@zju.edu.cn.

Cell Morphology

The cell morphology on Gr was observed by immunofluorescence staining. BMSCs were cultured on the various samples for 24 h (5000 cells/cm^2) with and without light illumination, and fixated with 4% paraformaldehyde for 15 min, then permeablized with 0.4% Triton X-100 in phosphate buffered saline **(**PBS) for 15 min, next, blocked 2% BSA/PBS solution. The fluorescent dye of rhodamine phalloidin (Phalloidin-iFluor™ 594 Conjugate, AAT Bioquest, Inc. USA) and 4', 6-diamidino-2-phenylindole (DAPI, ENZ-52404, Enzo Life Sciences, Switzerland) were used for cytoskeleton and nucleus staining, respectively. Finally, the stained cells were visualized by confocal laser scanning microscopy (Zeiss LSM 780, Germany). Cell area, perimeter and width of the cytoplasm was quantified by using the software of Image-J 1.45 system.

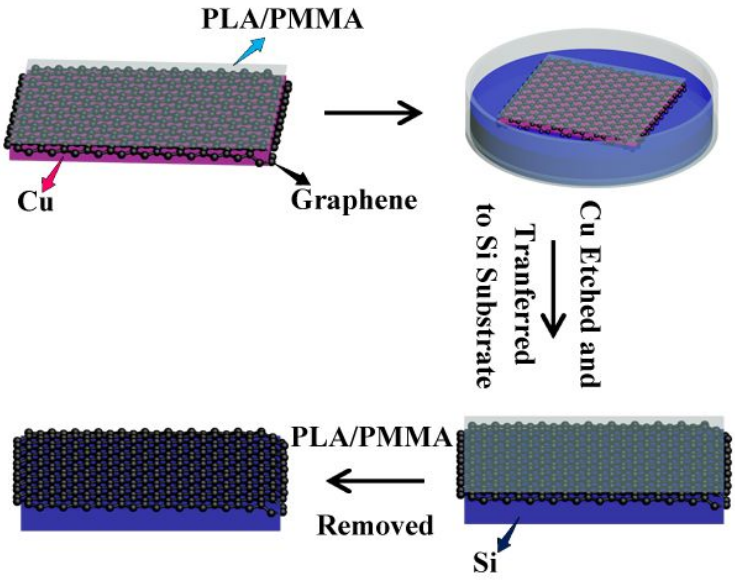

Figure S1. Schematic illustration of the PLA/PMMA-Etching-Free transfer procedures.

First, monolayer Gr was spin coated with PLA solution or PMMA solution at 4000 rpm. The spin-coated polymer film dries immediately as the solvent evaporates. Then, the copper on the uncoated side was etched away by ammonium persulfate aqueous solution, and the etching time was within 24 h. After the copper sheet was completely etched, the residual polymer/Gr film was rinsed with deionized water for 2~4 times and transferred to a substrate. Finally, Gr was fully bonded to the Si substrate in the air atmosphere for 24 h, the substrate with Gr was submerged into methylene dichloride bath (about 30 ℃) for about 10 min to remove the PLA, or in hot acetone bath (about 80 ℃) for about 10 min to remove PMMA. The obtained Gr/Si substrates rinsed with deionized water and keep in the dark atmosphere.

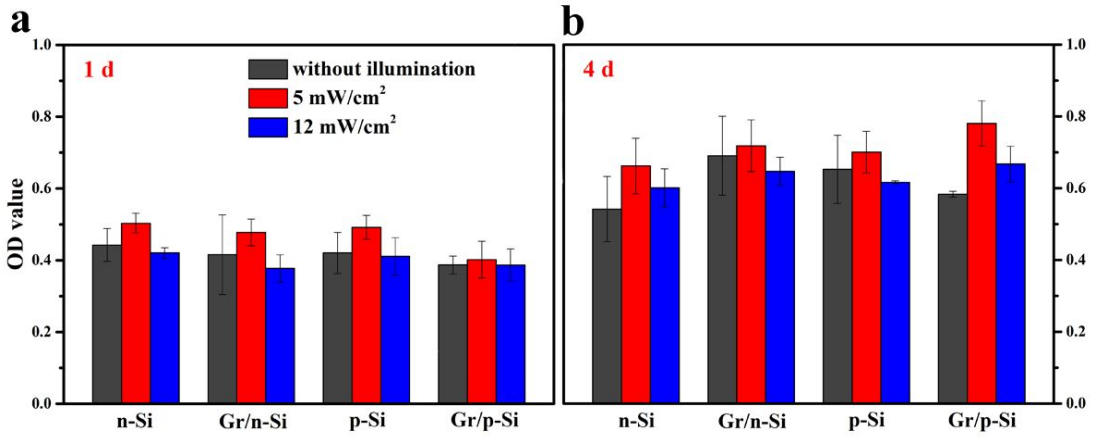

Figure S2. Effect of 450 nm light illumination to the behavior of (a) adhesion and (b) proliferation.

The OD value of cells with light exposure, shows no significant difference, compared with cells without light illumination. Demonstrating that light illumination display no harmful to cells activity, and has little effect on cell adhesion and proliferation behavior.

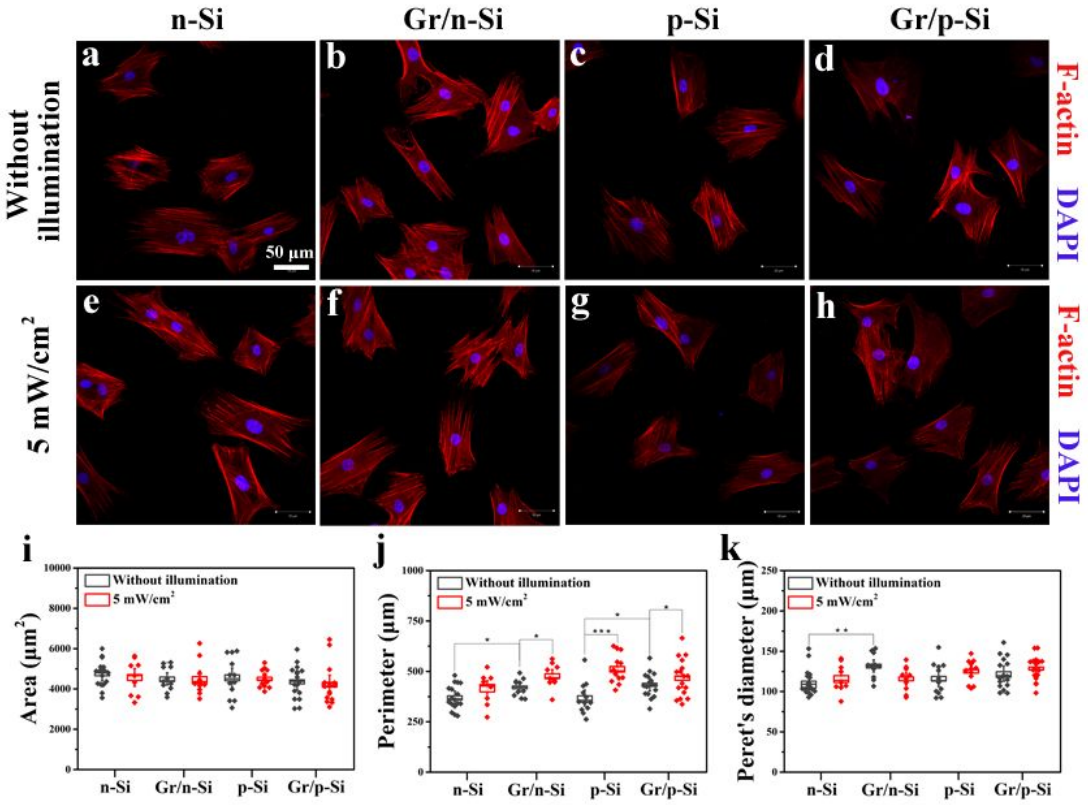

Figure S3. Typical cytoskeleton immunofluorescence evolution of cells with 24 h cultured on (a) n-Si, (b) Gr/n-Si, (c) p-Si, (d) Gr/p-Si without light illumination, and cultured on (e) n-Si, (f) Gr/n-Si, (g) p-Si, (h) Gr/p-Si with light illumination (5 mW/cm^2, 30 min/day). Cells were stained for the actin cytoskeleton (red) and cellular nuclei (blue). Quantitative analysis of the average nuclear area (**i**), perimeter (**j**) and diameter (k) according to immunofluorescence staining, the cell nucleus and F-actin fibers of BMSCs were stained.

Fluorescent images of BMSCs showed that BMSCs homogenously dispersed on all the surfaces (Figure S3a-d). Compared to the results of on the surface of Si substrates, quantitative analysis showed that cells on the surface of Gr achieved a similar area, but a larger perimeter and feret's diameter (Figure S3i-k). Moreover, under light illumination, no significant changes in cell spreading area was obtained, but a larger perimeter and feret's diameter (Figure S3a-k), compared with the results without light illumination. The results indicating that BMSCs exhibit a spindle-shape morphology on the surface of Gr and with light illumination. Such cell morphology is demonstrated to favor osteogenesis[1].

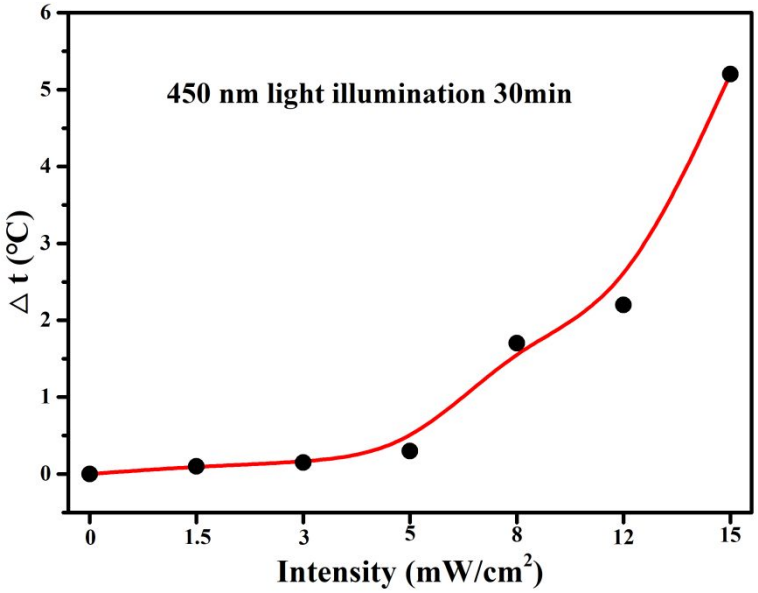

Figure S4. Temperature changes for culture medium under light illumination with different intensities. The culture medium exposed under 450 nm LED flashlight with different intensity for 30 min, when the intensity of light lower than 5 mW/cm^2.

The temperature of culture medium remains stable, then gradually rise up with the increase of light intensity.

REFERENCES

(1) McBeath, R.; Pirone, D. M.; Nelson, C. M.; Bhadriraju, K.; Chen, C. S. Cell Shape, Cytoskeletal Tension, and RhoA Regulate Stem Cell Lineage Commitment. *Dev. Cell* **2004,** *6*, 483–495.