**Maintaining the pluripotency of mouse embryonic stem cells on gold nanoparticle layers with nanoscale but not microscale surface roughness**

Zhonglin Lyu, Hongwei Wang, Yanyun Wang, Kaiguo Ding, Huan Liu, Lin Yuan*, Xiujuan Shi, Mengmeng Wang, Yanwei Wang, Hong Chen*

*The Key Lab of Health Chemistry and Molecular Diagnosis of Suzhou, Department of Polymer Science and Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou, P. R. China.

**Statement of purpose:** Efficient control of the self-renewal and pluripotency maintenance of embryonic stem cells (ESCs) is a prerequisite for translating stem cell technologies to clinical applications. It has been demonstrated that physical cues directly influence stem cell behaviors. Of these factors, precisely controlled substrate nanoscale roughness, with features that can mimic the natural extracellular matrix, has been reported to play a crucial role in affecting ESCs fate[1]. However, the influence of surface microscale roughness on the maintenance of ESC pluripotency remains unclear until now. In the present work, we studied the role of surface roughness in affecting the pluripotency of mouse ESCs (mESCs) during long-term culture by preparing gold nanoparticle layers (GNPLs) with nano- and microscale surface roughness via a convenient chemical plating method. The relationship between surface roughness and mESC pluripotency was described. Besides, the likely signaling cascades engaged in the topological sensing of mESCs cultured on various surfaces were investigated.

**Methods:** Gold-coated silicon slides were aminated by mercaptoethylamine in ethanol and then immersed in plating solution (12 mM $HAuCl_4 \cdot 4H_2O$, 0.5M $KHCO_3$, and 25 mM glucose) to prepare GNPLs. By increasing the volume of the plating solution, GNPLs with nano-, submicro- and microscale surface roughnesses were prepared. The surface morphology and roughness were studied by SEM and AFM. The pluripotency of cells cultured on various surfaces were investigated by immunofluorescence staining of Oct-4. The expression of proteins related to E-cadherin mediated cell-cell interactions and integrin-mediated cell-matrix interactions in cells cultured on different surfaces was studied by immunofluorescence staining of E-cadherin and vinculin, respectively.

**Results:** SEM images showed that GNPL has three-dimensional micro- and nanosized structures, which consist of gold nanoparticle aggregates with different sizes. AFM results showed that by adjusting the volume of the gold plating solution, GNPLs with nano-, submicro- and microscale roughnesses were prepared successfully. The surface roughness parameter Rq and Ra reached a plateau before the evaluation length of 20 μm (Figure 1). The results from immunostaining of Oct-4 (Figure 2) showed that nanorough GNPL can provide good support for the long-term maintenance of mESC pluripotency. However, microrough GNPL decreased the cell pluripotency from day 3 and at day 7, the loss of pluripotency was more significant. Figure 3 showed that the expression of E-cadherin and vinculin (a focal adhesion protein) on nanorough GNPL was much stronger than that on microrough GNPL, indicating stronger cell-cell and cell-matrix interactions on nanorough GNPL, which might cause the difference in the cell pluripotency status between nanorough GNPL and microrough GNPL.

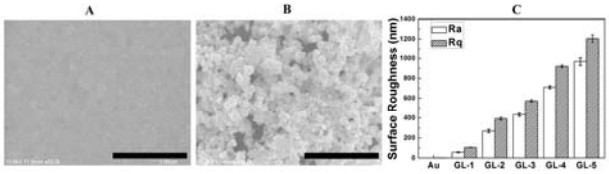


Figure 1. SEM images of an Au surface and a GNPL prepared by chemical gold plating. A: Au, B: GNPL. Bar, 1 μm; C: surface roughness parameters Rq and Ra for Au and GNPLs with the evaluation length of 20 μm.

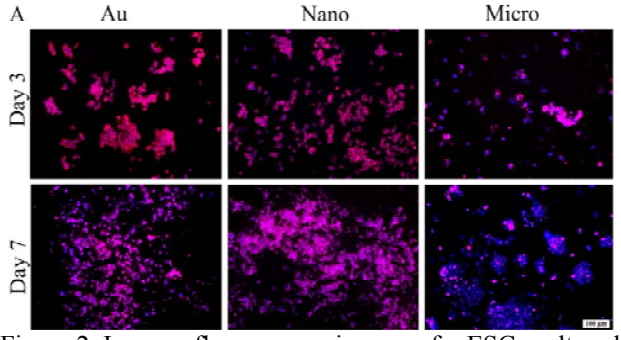


Figure 2. Immunofluorescence images of mESCs cultured for 3 and 7 days on various surfaces. Undifferentiated mESCs were positively immuno-labeled for Oct-4 (red) and were stained red. DAPI (blue) labeled all cells in the population. Bar, 100 μm.

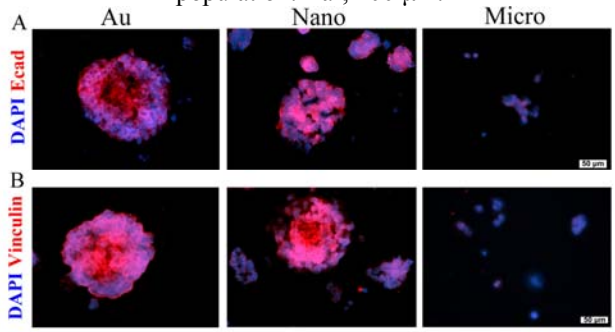


Figure 3. Immunofluorescence images of mESCs grown on Au and GNPLs after culture of 3 days. A: cells were costained for nuclei (DAPI; blue) and E-cadherin (red); B: cells were costained for nuclei (DAPI; blue) and vinculin (red). Bar, 50 μm.

**Conclusions:** The resulting findings are of fundamental interest and have important consequences for designing a synthetic cell microenvironment to control and direct ESC behaviors, which will help us design polymer-modified surface for directed differentiation of ESCs in the future research.

**References:** [1] Chen W. ACS Nano 2012; 6: 4094-4103.