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Supplementary information

Antibacterial property, angiogenic and osteogenic activity of Cu-incorporated TiO_2 coating

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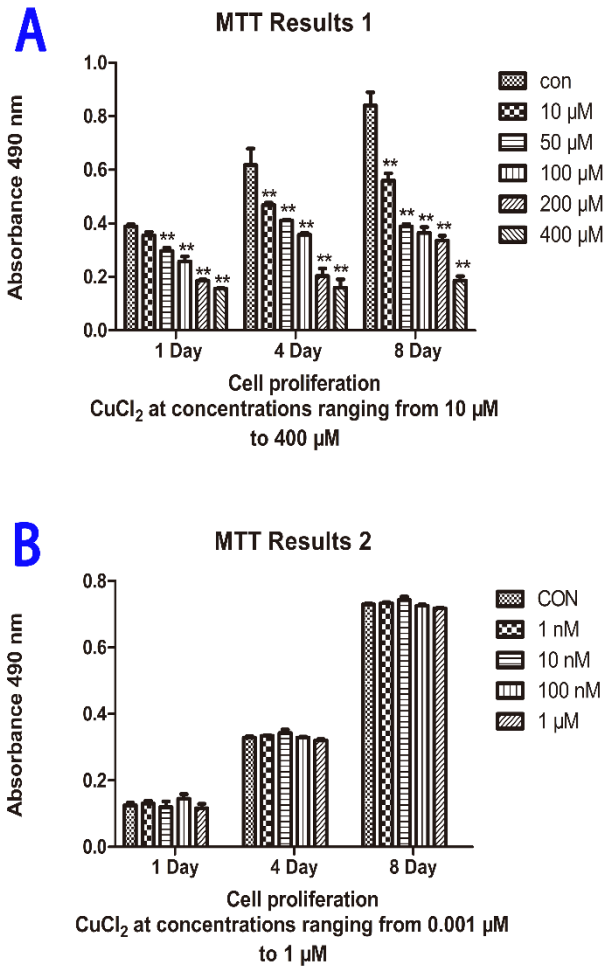
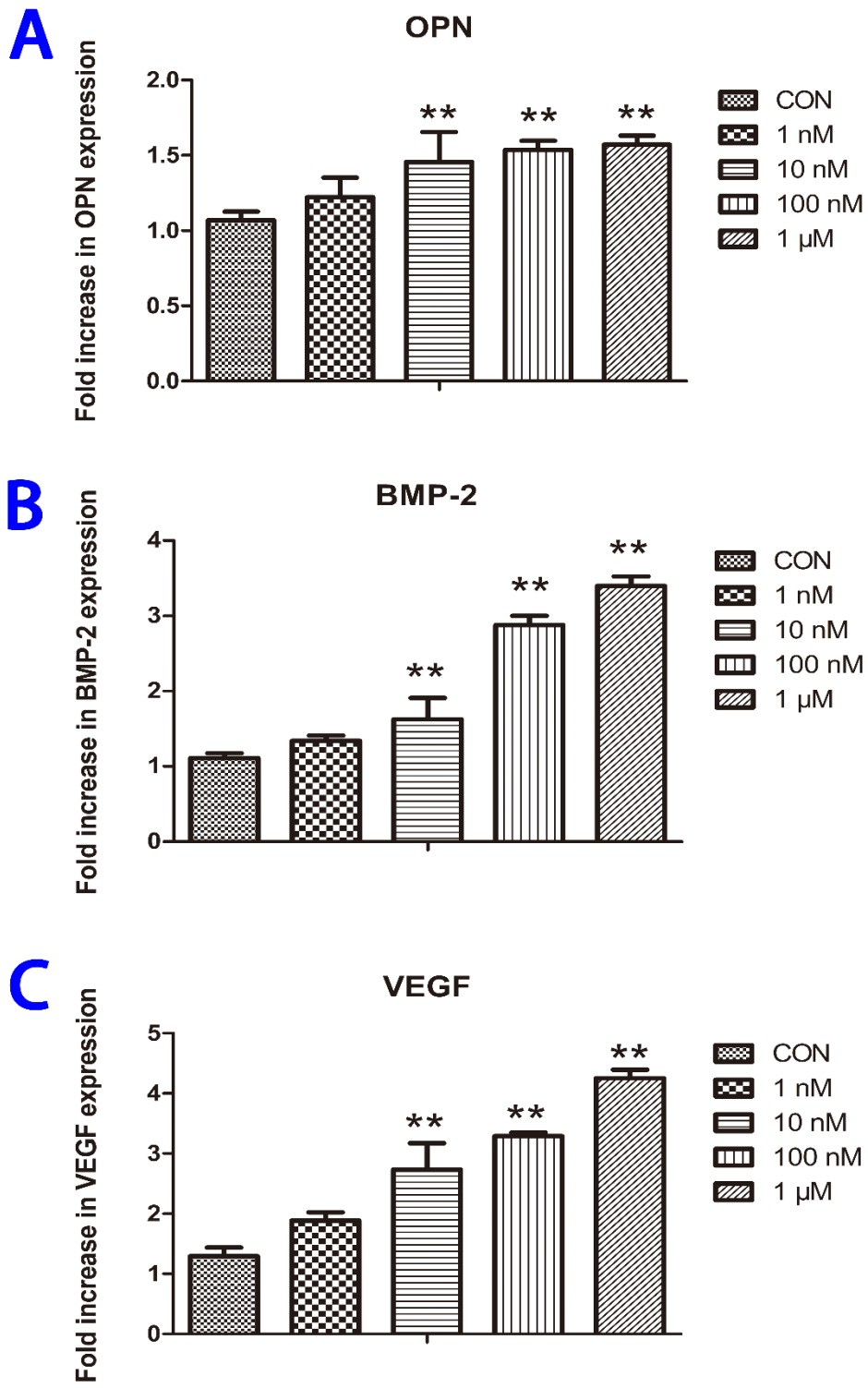
The biological responses of stem cells to copper ions (Cu^{2+}) are considered to show a concentration dependence due to the potential cytotoxicity of copper ions. As a consequence, it is quite essential and rational to investigate the function law of copper ions at a series of concentrations when culturing stem cells together. In order to optimize the experimental design and explore the appropriate concentrations of copper ions to provide guidance for the subsequent incorporation into TiO_2 coating by hydrothermal method, a primary research on the role of copper ions with different concentrations for the biological activities of BMSCs derived from rats was performed in the present work.



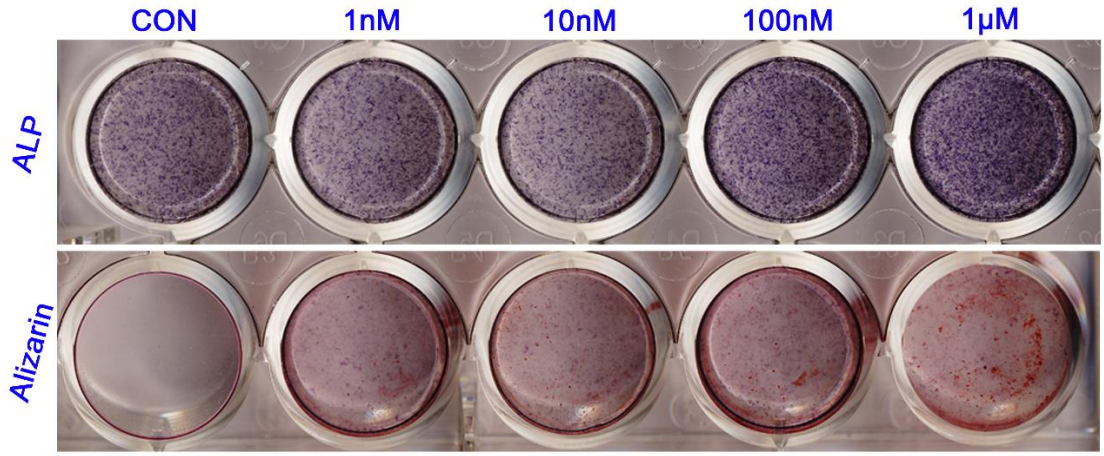
Supplementary Figure S1: MTT results showing the proliferation and viability of BMSCs cultured with adding different concentrations of copper ions. **Notes:** **$p < 0.01$ versus control group.

BMSCs were incubated in Dulbecco's modified Eagle's medium supplemented with copper chloride ($CuCl_2$) at concentrations ranging from 10 μM to 400 μM that have already been used for other types of cells culture in previous literatures[1-3] and the in vitro cell proliferation was determined by MTT assay. However, as shown in **Figure S1A**, the cell proliferation results suggested that these concentrations had a significant

adverse effect on the viability of BMSCs. As a result, we narrowed down the concentrations range from 1 nM to 1 μM, and no significant cytotoxic effect was observed, as confirmed by the proliferation results in **Figure S1B**, which provided promising concentrations that can be employed in the subsequent study.



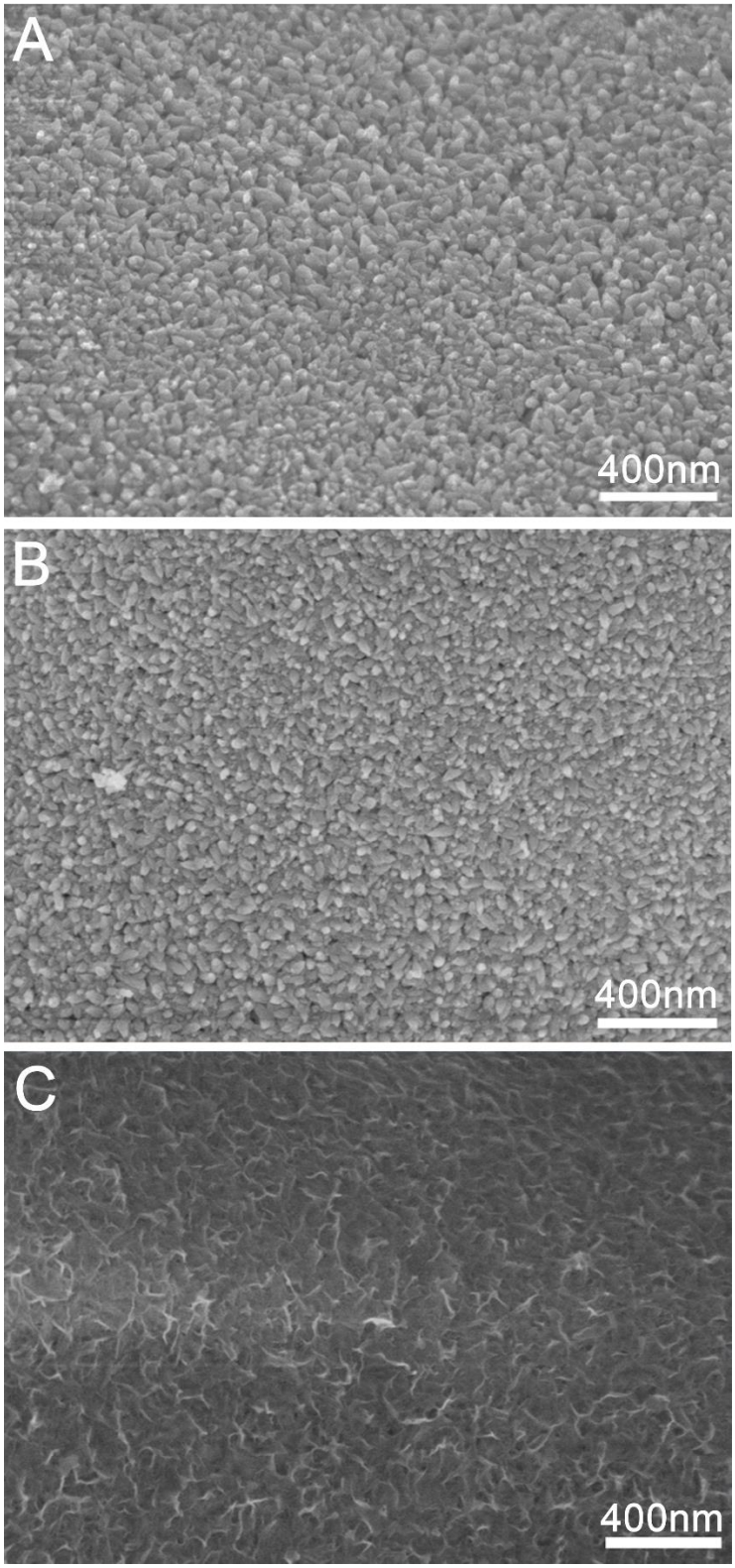
Supplementary Figure S2: Expressions results of osteogenesis and angiogenesis related genes evaluated by RT-PCR technique. **Notes:** **$p < 0.01$ versus control group.



Supplementary Figure S3: Results of alkaline phosphatase staining (**top panel**) and alizarin red staining (**bottom panel**).

The expressions of osteogenic and angiogenic genes were evaluated by quantitative real-time PCR, and alkaline phosphatase (ALP) staining and alizarin red (AR) staining were also performed.

From the acquired experimental data, the concentrations of 10 nM, 100 nM, 1μM can significantly up-regulate the expressions of osteogenic and angiogenic genes than the control group (**Figure S2**), with the order of 1μM > 100 nM > 10 nM > control group. At the same time, more extensive positive staining areas (including ALP staining and AR staining) were observed for those concentrations (**Figure S3**) than that of the control group which agreed well with the RT-PCR results. On the basis of these experimental results, the concentrations of 10 nM, 100 nM and 1μM was chosen to modify the TiO_2 coating on titanium surface via hydrothermal method.



Supplementary Figure S4: Surface topographies of 10nM-Cu (**A**), 100nM-Cu (**B**) and 1μM-Cu (**C**) groups examined by SEM after the hydrothermal treatments in $CuCl_2$ aqueous solutions.

After the hydrothermal reactions in $CuCl_2$ solution with concentrations of 10 nM, 100 nM, 1μM at 200 ℃ for 1 h, we found that the surface topography of 1μM-Cu group (**Figure S4C**) was apparently altered when compared to those of 10nM-Cu and 100nM-Cu groups, as shown in **Figure S4A-B**. Both copper loading and different surface morphologies can induce the different cell responses, and are complicated to be contrastively studied. As a consequence, in the present study, we tried to adopt 10nM-Cu and 100nM-Cu groups with similar hierarchical topography as the test groups to evaluate their biological performance with the micro-arc oxidized TiO_2 coating serving as the control group.

Notes and references

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