Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Method

- **Evaluation of the effect of UV irradiation on BBR function**

The cells were seeded (5×10^3 cells/well) and cultured (24 h) in a 96-well plate. The cells were then exposed to the UV-irradiated and filtered BBR (21 µg/ml). After incubation for 1, 3, and 7 days, MTT assay was done based on section 2.11 of the paper.

- **Cell density and distribution**

To further analysis the death of osteosarcoma cells, phalloidin staining was used which makes the live cells green. First, 5×10^3 MG63 cells were cultured with the complete culture medium (24h), then the cells were cultured with the extract medium of BBR nano-hydroxyapatite/gelatin scaffolds for 72h. After that, the medium was discarded and the cells were fixed with paraformaldehyde (4%) for 15 min at 4°C. Then, paraformaldehyde was removed, and the wells were thoroughly washed with PBS. To make the cells permeable, 0.1% Triton was used for 15 minutes. In the next step, the cells were exposed to 2% bovine serum albumin (BSA) for 60 minutes. At the end, 1.3 µM phalloidin (Sigma-Aldrich, Germany) was added to the cells, and they were incubated for 30 minutes. After PBS washing, images were taken by a fluorescent microscopy.

Results

- **Effect of UV irradiation on BBR function**

The results of MTT assay are shown in Figure 1. The statistical analyses indicated no significant differences between the UV-irradiated (BBR-U) and filtered (BBR-F) groups for both cell lines (MG63 and MC3T3-E1). The groups without a shared-letter are significantly different.

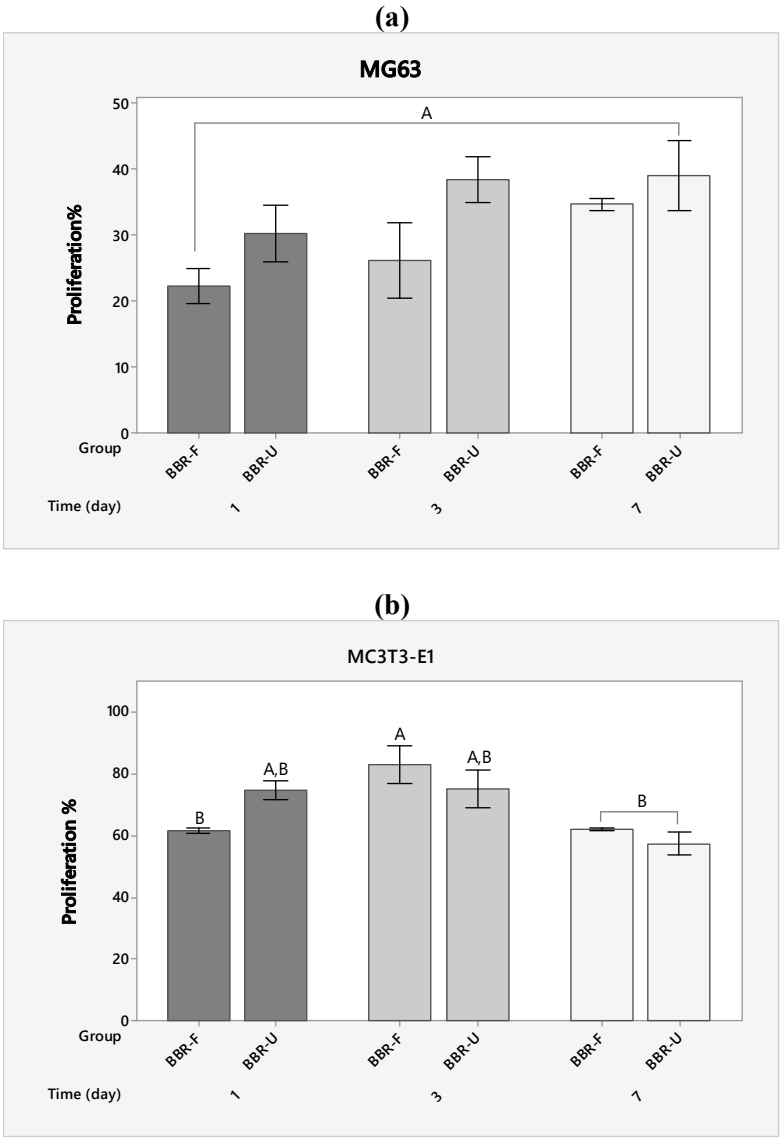

Figure 1: The results of MTT assay for UV-irradiated and filtered BBR for (a) MG63, and (b) MC3T3-E1

- **Cell density and distribution**

In order to further evaluate the viability of MG63 cells, phalloidin-fluorescein isothiocyanate (FITC) solution was used. The live cells were stained into green in fluorescence images. As it can be seen in Figure 2, the number of MG63 cells stained was quite small in SB20, SB40, SB60, and SB80. However, the live cells in SB0 are moderately high. This indicates the berberine could inhibit cell growth and cause cell death.

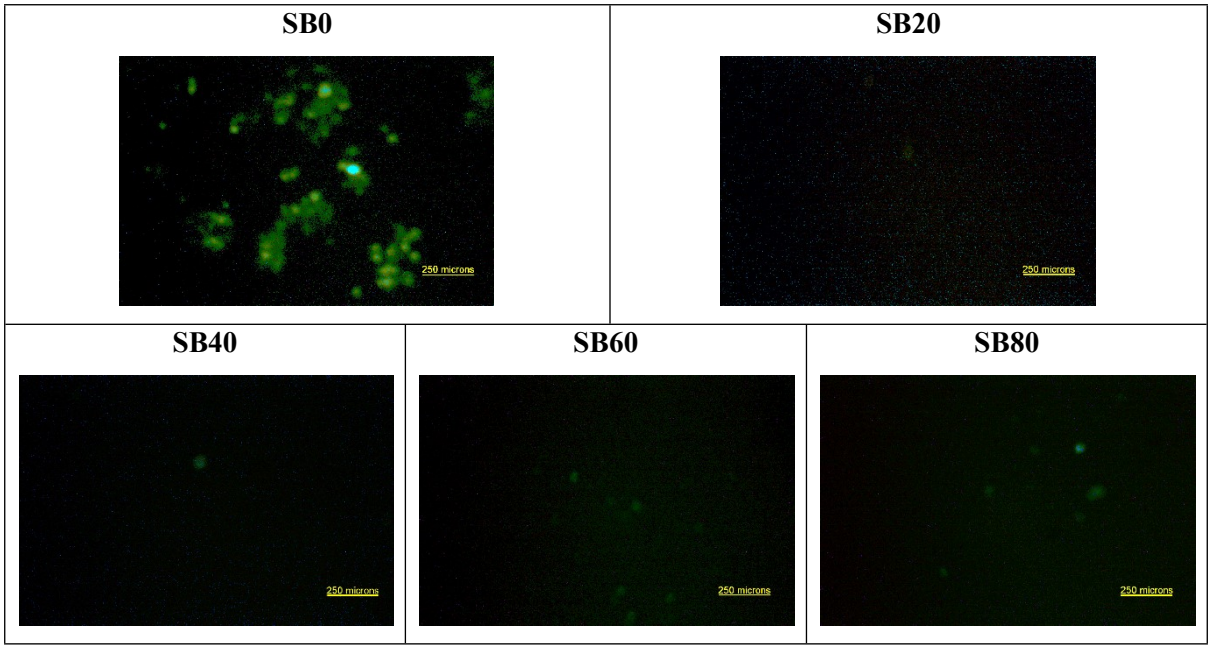

Figure 2. Phalloidin staining of MG63 cells cultured with extract medium of different scaffolds for 72h