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Supplementary Materials

Multifunctional Nanocrystalline Cu–Ti Thin Films Enhance Survival and Induce Proliferation of Mouse Fibroblasts In Vitro

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Statistical analysis for the clonogenic test has been developed in order to optimize the research method and concerned the incubation time of the colonies with the $Cu_{25}Ti_{75}$ extracts

Figure 4 shows that the results of mitochondrial activity of L929 cells (MTT test) in contact with $Cu_{25}Ti_{75}$ extracts are similar regardless of the concentration of the extract. All the values obtained, indicative of cell survival in relation to the controls, are between 90% and 110%.

The most important difference between the highest and lowest concentrations can be seen in the case of 24-h exposure. Such uniform results may be due to the lack of significant influence of the amount of copper on the material surface on the survival of L929 cells. Slight differences are confirmed in a detailed data analysis, where the pairwise comparison of the obtained values for individual concentrations is statistically insignificant.

In practice, this means that the values for the pairs: 12.5%–25%; 25%–50%; 25%-control; 12.5%-control are identical from the of statistical analysis point of view.

In order to verify the effect of the exposure time and the concentration of the $Cu_{25}Ti_{75}$ extract, an analysis of variance with repeated measurement was performed in a mixed schedule in plan 3 (24, 48, 72 h) × 4 (control; 12.5%, 25%, 50%, 100%). The factor measured inside the samples was the incubation time, while the factor between samples was the type of concentration.

The analysis of variance was performed in the univariate model. The following statistically significant effects were obtained:

- main effect of incubation time: F (2.54) = 2218.77; $p < 0.001$; eta2 = 0.988;
- main effect of concentration type: F (4.55) = 57.404; $p < 0.001$; eta2 = 0.807;
- effect of interaction of incubation time and type of incubation: F (8,110) = 10,489; $p < 0.001$; eta2 = 0.433.

Since the main effect of incubation time turned out to be significant, post hoc comparisons were made using the Bonferroni test. These comparisons showed statistically significant differences between incubation times: 24 h (M = 0.519; SD = 0.055); 48 h (M = 0.986; SD = 0.111); 72 h (M = 1.075; SD = 0.127).

In order to understand the meaning of the main effect: the type of concentration, post hoc comparisons were made using the Bonferroni test. They showed statistically significant differences between the types of concentration: control (M = 0.934; S = 0.01)—25% (M = 0.874; S = 0.01); control—50% (M = 0.828; S = 0.01); control—100% (M = 0.745; S = 0.01); 12.5% (M = 0.918; S = 0.01)—25% (M = 0.874; S = 0.01); 12.5% (M = 0.918; S = 0.01)—50% (M = 0.828; S = 0.01); 12.5% (M = 0.918; S = 0.01)—100% (M = 0.745; S = 0.01);

25% (M = 0.874; S = 0.01)—50% (M = 0.828; S = 0.01); 25% (M = 0.874; S = 0.01)—100% (M = 0.745; S = 0.01); 50% (M = 0.828; S = 0.01)—100% (M = 0.745; S = 0.01).

Multiple comparisons using the Bonferroni test were performed to interpret the effect of the interaction of incubation time and concentration type.

These comparisons showed that:

- in the control group there were significant differences between the incubation times: 24 h (M = 0.486; S = 0.016)—48 h (M = 1.098; S = 0.016); 24 h—72 h (M = 1.217; S = 0.017); 48 h (M = 1.098; S = 0.016)—72 h (M = 1.217; S = 0.017).
- in the concentration group of 12.5%, there were significant differences between the incubation times: 24 h (M = 0.528; S = 0.016)—48 h (M = 1.066; S = 0.016); 24 h—72 h (M = 1.16 S = 0.017); 48 h (M = 1.066; S = 0.016)—72 h (M = 1.16, S = 0.017).
- in the concentration group of 25%, there were significant differences between the incubation times: 24 h (M = 0.533; S = 0.016)—48 h (M = 1.014; S = 0.016); 24 h—72 h (M = 1.076; S = 0.017); 48 h (M = 1.014; S = 0.016)—72 h (M = 1.076; S = 0.017).
- in the concentration group of 50%, there were significant differences between the incubation times: 24 h (M = 0.536; S = 0.016)—48 h (M = 0.917; S = 0.016); 24 h—72 h (M = 1.031 S = 0.017); 48 h (M = 0.917; S = 0.016)—72 h (M = 1.031, S = 0.017).
- in the concentration group of 100%, there were significant differences between the incubation times: 24 h (M = 0.511; S = 0.016)—48 h (M = 0.835; S = 0.016); 24 h—72 h (M = 0.889 S = 0.017).

After 24 h of incubation, no statistically significant differences between the types of concentration were found.

After 48 h of incubation, there were statistically significant differences between the concentrations: control (M = 1.098; SD = 0.091)—25% (M = 1.014; SD = 0.027); control—50% (M = 0.917; SD = 0.030); control—100% (M = 0.835; SD = 0.035); 12.5% (M = 1.066; SD = 0.062)—50% (M = 0.917; SD = 0.030); 12.5% (M = 1.066; SD = 0.062)—100% (M = 0.835; SD = 0.035); 25% (M = 1.014; SD = 0.027)—50% (M = 0.917; SD = 0.030); 25% (M = 1.014; SD = 0.027)—100% (M = 0.835; SD = 0.035); 50% (M = 0.917; SD = 0.030)—100% (M = 0.835; SD = 0.035).

After 72 h of incubation, there were statistically significant differences between the concentrations: control (M = 1.217 SD = 0.081)—25% (M = 1.076; SD = 0.058); control—50% (M = 1.031; SD = 0.031); control—100% (M = 0.889; SD = 0.058); 12.5% (M = 1.16; SD = 0.048)—25% (M = 1.076; SD = 0.058); 12.5% (M = 1.16; SD = 0.048)—50% (M = 1.031; SD = 0.031); 12.5% (M = 1.16; SD = 0.048)—100% (M = 0.889; SD = 0.058); 25% (M = 1.076; SD = 0.058)—100% (M = 0.889; SD = 0.058); 50% (M = 1.031; SD = 0.031)—100% (M = 0.889; SD = 0.058).

Figure S1

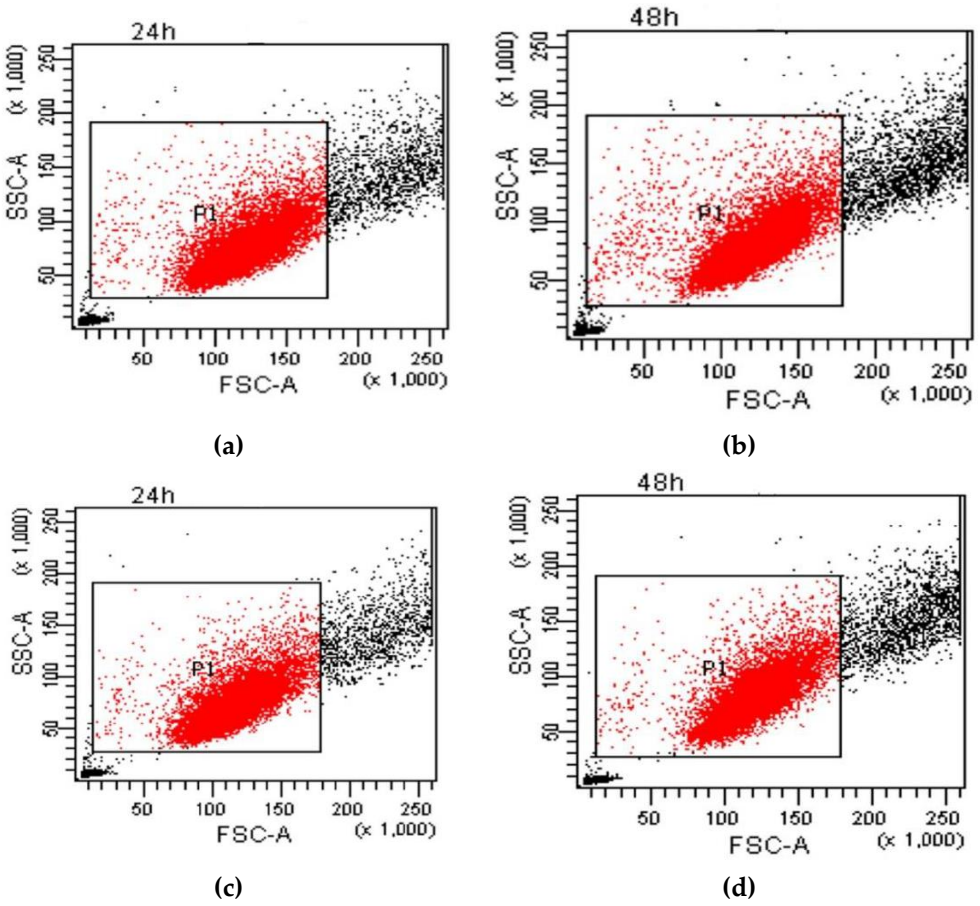

Figure S1. Morphological evaluation of L929 murine fibroblasts after exposure to extracts from the $Cu_{25}Ti_{75}$ thin film (**a** and **b**), and control (**c** and **d**); the test was performed after 24 and 48 h of incubation, respectively.