Foamed calcium phosphate bone cements with biosurfactants - cytotoxicity studies

Ewelina Cichoń[1]*, Małgorzata Krok-borkowicz, Aneta Zima[1], Joanna Czechowska[1], Szymon Skibiński[1], Piotr Pańtak[1], Elżbieta Pamuła[1], Anna Ślósarczyk[1]

[1] AGH University Of Science And Technology, Faculty Of Materials Science And Ceramics, Kraków, Poland
* Email: Ecichon@agh.edu.pl

Introduction

Foamed calcium phosphate bone cements (fCPCs) constitute a novel type of biomaterials. For their preparation as foaming agents usually surfactants are used [1]. In this study, we developed fCPCs prepared with the use of two biosurfactants – saponin from Quillaja bark and sucrose fatty acid ester S0112. These compounds are characterised by many interesting properties, such as antibacterial and anticancer activity. To our best knowledge, these biosurfactants have not been used as foaming agents for calcium phosphate bone cements yet. The purpose of this study was to determine the effect of biosurfactant additives in fCPCs on the cytotoxicity and proliferation of MG-63 cells.

Methods

fCPCs containing the biosurfactants: saponin (material fSAP) and sucrose ester (material fSuE) were developed and investigated. Cement without added biosurfactant (fCTRL) served as a control material. In the case of fSAP, the liquid phase consisted of a 2% aqueous solution of Na_2HPO_4 with the addition of saponin (SAP) in the amount of $10g \cdot L^{-1}$. After mechanical foaming of the liquid phase, it was mixed with the solid phase, which consisted of highly reactive alpha tricalcium phosphate (α-TCP). The solid phase of fSuE consisted of α-TCP mixed with sucrose ester at 7 mg per gram of α-TCP powder, whereas liquid phase was 2% aqueous solution of Na_2HPO_4. For all cements, the ratio of liquid to solid phase was $0.7\ g \cdot g^{-1}$. Materials were sterilized with ethylene oxide and subjected to indirect MG-63 cell studies. Cell viability and proliferation were studied with Alamar Blue assay, live/dead staining with calcein and propidium iodide and hematoxylin/eosin staining. Extracts from the cements were prepared according to ISO 10993 with 1:10 sample to medium ratio. The series of extract dilutions were prepared: 1 (undiluted), 2, 4, 8, 16, 32 times. Cells were seeded in 48-well plates at a concentration of 1x104 cells/well and after 24 hours prepared extracts dilutions were added. Live/dead staining and Alamar Blue assays were done on days 1, 3 and 7.

Results

On day 1 (Fig. 1A) for undiluted extracts, cell viability was low - less than 50% of control medium (MEM) for extracts from fCTRL and fSuE and for fSAP was close to zero. Cell viability for fCTRL and fSuE samples at 1:2 dilution was at similar level if compared to the control (cells cultured in MEM medium). The reduction of Alamar Blue in the case of fSAP twice diluted extract was lower than for MEM. This suggests that cells died after contact with saponin. On day 3 (Fig. 1B), reagent reduction increased for MEM, suggesting that the cells were proliferating. Undiluted extracts from all samples were lethal to MG-63 cells. Lack of cytotoxicity (>70% MEM) was observed for twice diluted extracts of fCTRL and fSuE materials, while for fSAP when the extract was diluted eight times. On day 7 (Fig. 1C), the lack of cytotoxicity similarly affected cells cultured in extracts from fCTRL and fSuE materials diluted twice but in the case of fSAP, four times diluted extract revealed no cytotoxicity. This suggests that the saponin is less toxic to cells over time. Cells incubated in twice diluted extracts proliferated and their number increased as compared to days 3 and 1. The results were confirmed also by live/dead and hematoxylin/eosin studies (Fig. 2-5).

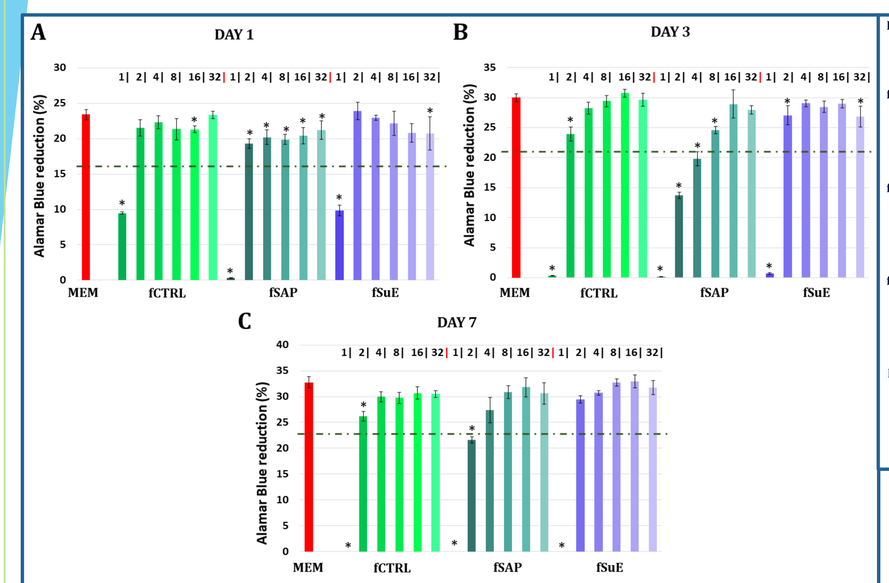

Figure 1. Viability (based on Alamar Blue assay) of MG-63 cells after contact with extracts from fCPCs on day 1(A), 3(B) and 7(C) after extract addition. Numbers (1, 2, 4, 8, 16, 32) stand for the extract dilution level.

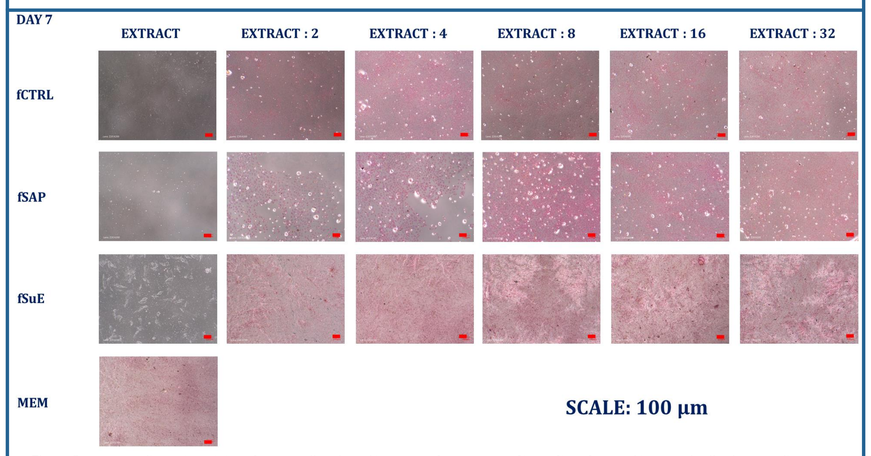

Figure 2. Hematoxylin-eosin staining of MG-63 cells cultured in extracts from cements fCTRL, fSAP, fSuE and in control cell culture medium (MEM) on day 7.

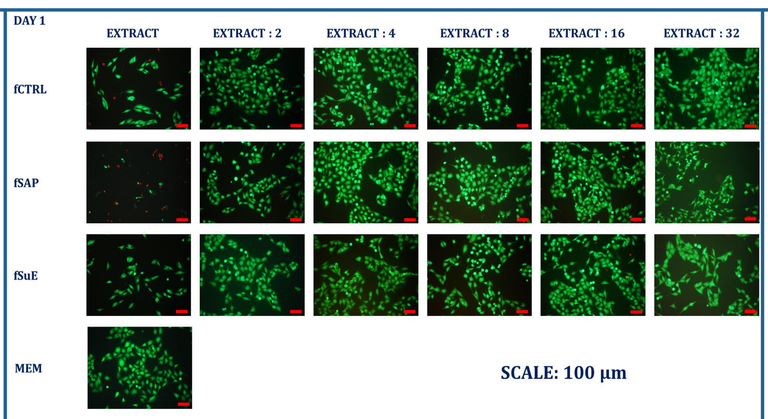

Figure 3. Live/dead staining of MG-63 cells cultured in extracts from cements fCTRL, fSAP, fSuE and in control cell culture medium (MEM) on day 1.

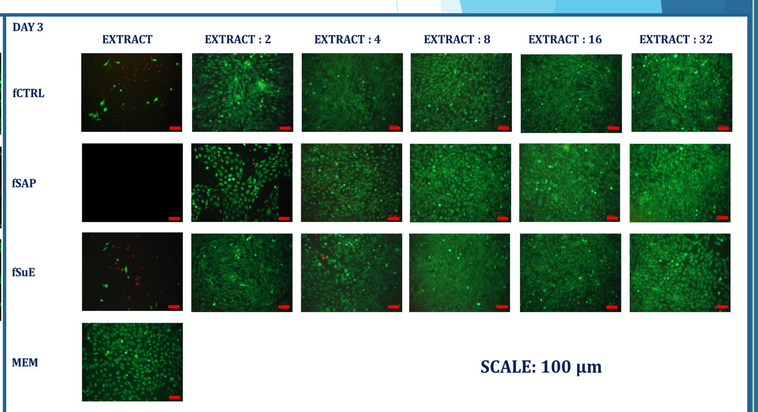

Figure 4. Live/dead staining of MG-63 cells cultured in extracts from cements fCTRL, fSAP, fSuE and in control cell culture medium (MEM) on day 3.

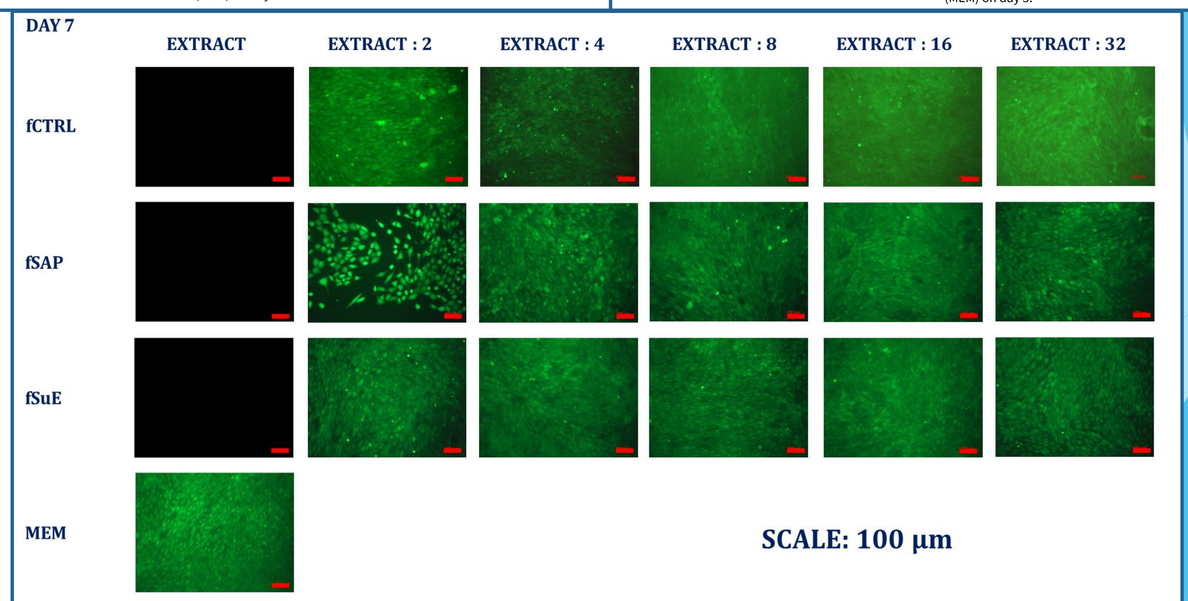

Figure 5. Live/dead staining of MG-63 cells cultured in extracts from cements fCTRL, fSAP, fSuE and in control cell culture medium (MEM) on day 7.

Conclusions

Cytotoxicity studies showed that among two used biosurfactants, saponin was more cytotoxic. Although cell viability was significantly lower for this surfactant as compared to control, one should keep in mind that in tissue environment there is a constant exchange of fluids, so even harmful leached molecules can be neutralized by the cells and the concentration of the toxic substance locally decreases over time. Saponins in several studies have shown promising cytotoxicity profiles suggesting potential use in cancer treatment. The MG-63 cell line is derived from osteosarcoma, a representative of malignant tumours. Its cytotoxicity towards MG-63 may be due to anticancer activity of saponin. Four times diluted extracts from all obtained cements with the surfactant addition resulted in the same cell viability as compared to MEM. Cells incubated in twice diluted extracts proliferated and their number increased over time. Interestingly, the material with sucrose ester addition (fSuE) increased the cell viability. This fact can be taken into consideration when developing novel foamed cements.

References

[1] Cichoń, E., Mielan, B., Pamuła, E., Ślósarczyk, A., & Zima, A. (2021). Development of highly porous calcium phosphate bone cements applying nonionic surface active agents. *RSC Advances*, *11*(39), 23908-23921.

Acknowledgements

This study was supported by the National Science Centre, Poland (2017/27/N/ST8/00913) EC acknowledges financial support from the National Science Centre, Poland under doctoral scholarship no. 2019/32/T/ST5/00207.