Influence of zinc oxide enriched bioglass on proregenerative properties of chitosan based biocomposites for bone tissue regeneration

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Introduction

The rapid development in the field of biocompatible materials forces the introduction of new solutions for the regeneration process of bone tissue. Recent studies show the great importance of bioglass which make improve the structure of bone regenerative biocomposites. Additionally, zinc ions can have beneficial effect on cell condition. In this study different kinds of zinc oxide enriched bioglasses were combined with chitosan to prepare solid biocomposites..

Aim

The aim of the study was to determine the cytotoxic, pro-regenerative properties of bioglasses and bioglass-chitosan solid composites.

Materials and methods

Cytotoxic and pro-regenrative effects of bioglass: 12 – well plates with 1.5 ml of medium per well and inserts with or without bioglass and 0.5 ml were preincubated in 34°C, 90% of humidity and 5% CO_2 to the time of cells' seeding. Next 2 x 105 hFOB 1.19 cells were seeded into each well. Plates were incubated for 48 h in previous descripted conditions then proliferation and cytotoxicity test were conducted.

Cytotoxic and proregenrative effects of composites

Direct method: Prior the experiment the composites were inserted into 24 well plates and immersed in 1 ml of culture medium for 1 hour. Sterilized Raschig rings were placed on the top of the composites followed by hFOB 1.19 cell seeding. For each well $5x10^4/cm^2$ hFOB were seeded inside the Raschig ring.

Plates were incubated for 1 hour in 34°C and 5% CO_2 to allow cells attachment to the composites. After 1 hour rings were removed, and cells were incubated for 48 h. After that time the cell viability was estimated by **mitochondrial activity measurement** (WST-1 test) and cytotoxicity was measured by the **leakage of lactate dehydrogenase** (LDH cytotoxicity assay).

Indirect method: Different composites were inserted into 24 – well plates and immersed in 1,1 ml culture medium for 24 hours in 34°C and 5% CO_2. After that time the extract was collected. Day prior the experiment hFOB 1.19 cell were seeded at density $5x10^4/cm^2$ on 24 – well plates. On the day of experiment medium from wells were removed and collected extracts were added on the cells and then incubated for 48 h 34°C and 5% CO_2. After incubation time the cell viability was estimated by **mitochondrial activity measurement** (WST-1 test) and cytotoxicity was measured by the **leakage of lactate dehydrogenase** (LDH cytotoxicity assay).

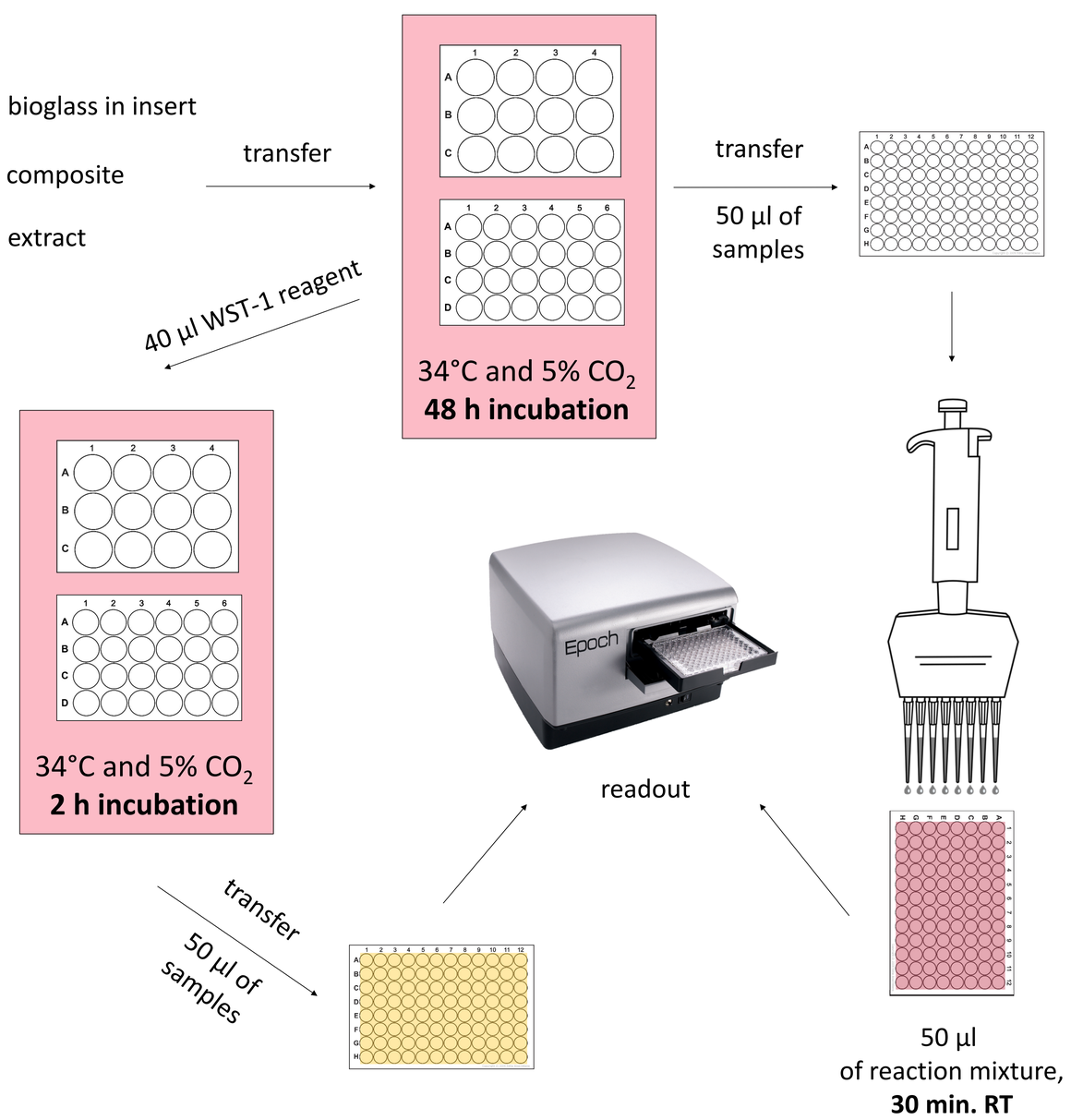


Fig. 1. Short experiment protocol.

Results

- Non-toxic effects in LDH test were detected for all tested bioglasses and most of tested biocomposites,
- Biocomposites tested by indirect method were non-toxic and most of them showed beneficial effect on cell proliferation which exceeded 130% of control cells measured in WST-1 test,
- In direct method, that takes into consideration direct contact of cells with porous material, showed decreased proliferation and only one composite caused cytotoxic effect greater than 30%,
- Increased cytotxicity for composite 2/952000 P5Zn2II reached value 48,4 ± 1,5% and it is corelated with decreased proliferation (56,9 ± 0,5% of non treated control cells),
- Low cytotoxicity and decreased proliferation in mitochondrial activity test present in experiments conducted using direct method suggest occurance of additional mechanisms responsible for reduced proliferation.

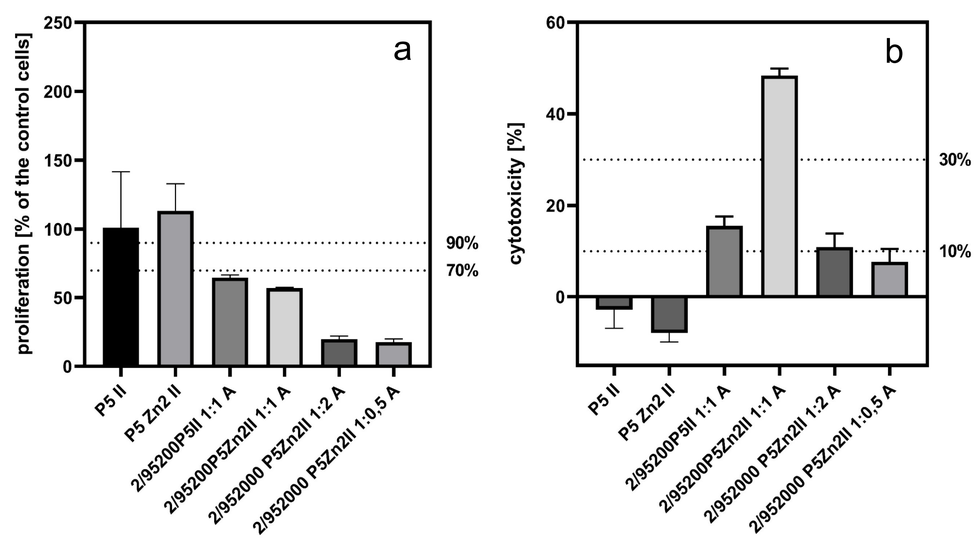


Fig. 2. Comparison of results received for bioglass and composites tested in direct method with use of a) WST-1 test, b) LDH test.

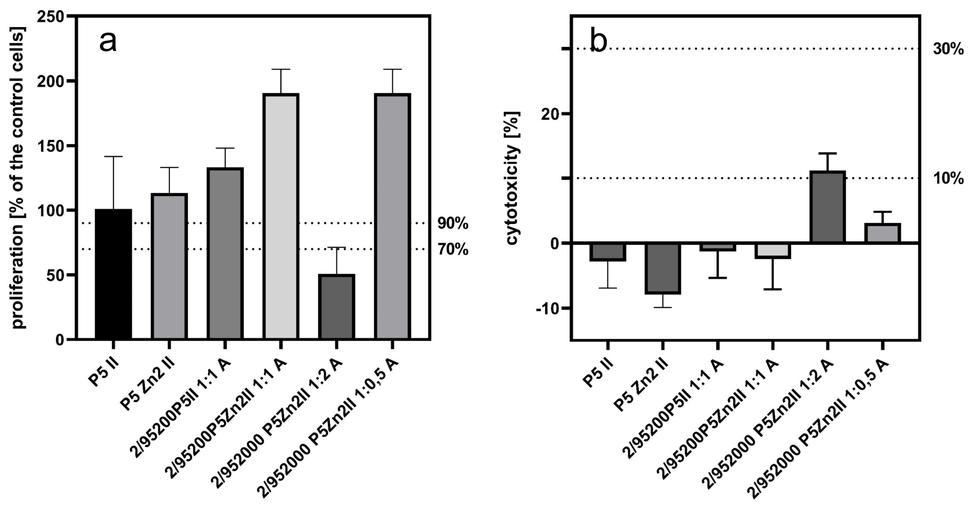


Fig. 3. Comparison of results received for bioglass and composites tested in indirect method with use of a) WST-1 test, b) LDH test.

Conclusions

Bioglasses and chitosan-bioglass biomaterials are intensively tested for use in regenerative medicine. Addition of zinc oxides to bioglass improve properties of biomaterials and support tissue regeneration. This effect depends on concentration of ions released from the material and the molecular structure of the biocomposite. Therefore, further research should be carried out to evaluate most beneficial concentration of zinc oxide for bone regeneration and the most optimal spatial scaffold to support cells growth.

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Literature

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