

OSTEOINDUCTIVE PROPERTIES OF GEL-DERIVED BINARY CaO-SiO₂ AND TERNARY CaO-P₂O₅-SiO₂ BIOACTIVE GLASSES

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Introduction

Bioactive glasses have been long recognized for their ability to integrate well with bone tissue and support bone tissue formation and remodelling. However, the emerging new feature of this biomaterial group is the ability of some bioactive glasses to induce osteogenic differentiation of mesenchymal precursor cells *in vitro* and/or *in vivo* without the necessity of additional cell treatment with osteogenic growth factors. Such osteoinductive material properties are promising for the clinics as their implantation would not require supplemental treatment with osteogenic compounds. In this work we have examined osteoinductive properties of PLGA-based composites containing gel-derived bioactive glasses differing in their CaO:SiO₂ ratio. They were used as growth surfaces for human BMSC cultures that were not treated by any other, but materials, osteogenic compounds. Cells were examined at different culture times for the expression of osteogenic growth factors (BMP-2), early osteogenic transcription factors (Runx-2 and Osterix), alkaline phosphatase (ALP) activity and extracellular matrix mineralization. The direct effects of surface chemistry or the ions released to the culture medium due to material surface activity were evaluated for their osteoinductive properties. Also, the materials were pre-incubated in simulated body fluid (SBF) before cell culture to compare cell responses with those cultured on "as-prepared" materials.

Materials and Methods

Bioactive glasses from the binary CaO-SiO₂ and ternary CaO-P₂O₅-SiO₂ systems were obtained by the sol-gel process (SBG, gel-derived bioactive glasses) and incorporated into PLGA basically as described by Zagajczuk et al. [1]. TABLE 1 lists all compositions of SBGs examined in this study. The thin SBG-PLGA films were then sterilized by 70% ethanol and UV, and fitted into 24-well culture plates. Human BMSC were obtained from iliac crest of adult patients and, after expansion, cells were seeded directly on the studied material surfaces or they were cultured on standard tissue culture plastic and exposed to the culture medium previously used for the materials incubation. Some materials were pre-incubated in SBF for three days before cell culture. Human BMSC were examined for mRNA expression (real-time PCR) of BMP-2, Runx-2 and Osterix after 2 culture days, cell number (MTS assay) and ALP activity (biochemical kinetic assay) after 7 culture days and extracellular matrix mineralization (Alizarin Red S staining) after 14 culture days.

TABLE 1. PLGA-based composites containing SBGs of listed chemical composition [mole%].

Symbol of material	SiO ₂	CaO	P ₂ O ₅
A1	40	60	-
D1	60	40	-
T1	50	50	-
S1	80	20	-
A2	40	54	6
D2	60	36	4
T2	47	47	6
S2	80	16	4
PLGA-control	-	-	-
SiO ₂ -control	100	-	-

Results and Discussion

Culture analyses showed that practically each studied material induced osteogenic response of cells despite the cells were not treated with any osteogenic supplements. Increased calcium content in SBGs corresponded with increased BMP-2 mRNA levels and decreased Runx-2 levels, especially for binary SBGs. Notably, BMP-2 and Osterix mRNA levels were significantly higher in cells grown on substrates containing SBGs derived from ternary, P₂O₅ containing system, but ALP levels were comparable in cell cultured on substrates containing either binary or ternary SBGs. Preincubation of materials in SBF eliminated most of the observed differences in osteogenic response of cells, suggesting that the changes in the chemistry of scaffolds can significantly affect the response of cells, particularly if the materials are used "as prepared" without pre-incubation in SBF. We also found that the "ions extracts" collected from tested materials were capable to stimulate extracellular matrix mineralization in human BMSC cultures. This implies that the ions released from these materials on contact with cells and physiological fluids may play the key role in osteogenic cell responses.

Conclusions

Our studies suggest osteoinductive properties of gel-derived bioactive glasses derived from the binary CaO-SiO₂ and ternary CaO-P₂O₅-SiO₂ systems. The CaO:SiO₂ ratio in SBGs may influence the overall response of cells to SBG-containing materials and this is plausibly related to the ions released from these materials. It thus becomes possible to modulate cell responses depending on the SBG chemistry. On the other hand, incubation of materials in SBF before their exposure to the cells and tissues may change the biological outcome as SBF incubation moderates ions release and cell response.

Acknowledgments

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References

- [1] Zagajczuk B, Dziadek M, Cholewa-Kowalska K and Łączka M „Effects of chemical compositions on the structure and the bioactive properties of SiO₂-CaO and SiO₂-CaO-P₂O₅ bioactive glasses” (25th Conference on Biomaterials in Medicine and Veterinary Medicine – Abstract and Poster).