DIALYSIS METHOD FOR β-1,3-GLUCAN GELATION PROVIDES HIGH BIOCOMPATIBILITY OF β-1,3-GLUCAN/HA BONE SCAFFOLD

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Introduction

Beta-1,3-glucan (curdlan) is a non-toxic, bacterial, and linear polymer which possesses ability to form firm and flexible gel [1]. It was shown that thermally obtained curdlan gel is a suitable component of bioactive bone substitute [2] as well as biocompatible bone scaffold [3]. The aim of this study was to evaluate whether dialysis method for curdlan gelation is suitable for fabrication of biocompatible and bioactive bone scaffold.

Materials and Methods

The β-1,3-glucan/HA scaffold (glu/HA D) composed of 8 wt.% curdlan and 80 wt.% HA granules was made via dialysis method against $CaCl_2$ as described in details in Patent No. P.415936 [4]. Microstructure of glu/HA D scaffold was visualized by SEM. The cell-biomaterial interactions were assessed by evaluation of osteoblast (hFOB 1.19 and MC3T3-E1 cells) viability, adhesion, and proliferation in direct contact with glu/HA D scaffold. In turn, bioactivity of glu/HA D scaffold was estimated by measurement of changes in ionic composition of culture medium as well as by evaluation of *in vitro* apatite-forming ability after soaking in SBF.

Results and Discussion

The cytotoxicity assay demonstrated that the viability of both hFOB 1.19 and MC3T3-E1 cells seeded on glu/HA D scaffold was high and exceeded 70% in comparison with control cells (TABLE 1). Moreover, it was proved that number of cells grown on the scaffold increased with time indicating that glu/HA D material promoted osteoblast survival and proliferation (FIG. 1). As revealed by ion reactivity test (FIG. 2), glu/HA D scaffold released huge amount of Ca^{2+} ions to the culture medium, what positively affected cell-scaffold interaction and also apatite-forming ability *in vitro*. SEM analysis (FIG. 3) demonstrated the occurrence of characteristic crystals on the glu/HA D scaffold already on the 14th day of experiment. EDS analysis (FIG. 3) confirmed results obtained with SEM and showed that observed layer was composed of calcium phosphate with Ca/P ratio ranging from 1.7-1.72, which is similar to Ca/P ratio in hydroxyapatite (1.67).

TABLE 1. Viability of osteoblast cells seeded on glu/HA D scaffold (assessed after 24-h culture).

	Viability [% of control ± SD]
hFOB 1.19 cells	71.30 ±1.82
MC3T3-E1 cells	87.72 ± 6.31

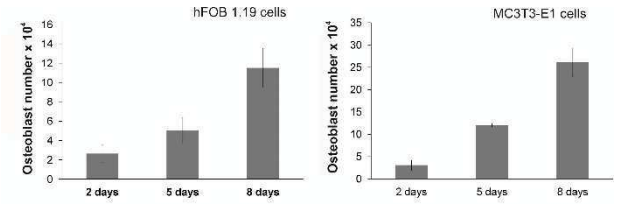

FIG. 1. Evaluation of osteoblast cells growth on glu/HA D scaffold.

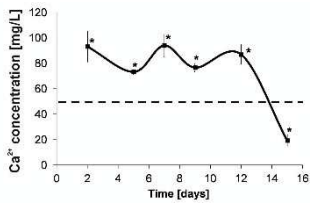

FIG. 2. Changes in Ca^{2+} concentration during 15-day of glu/HA D soaking in culture medium. Dotted line – ion concentration in fresh culture medium. *significantly different result compared to fresh medium (unpaired *t*-test, Graph Pad Prism 5).

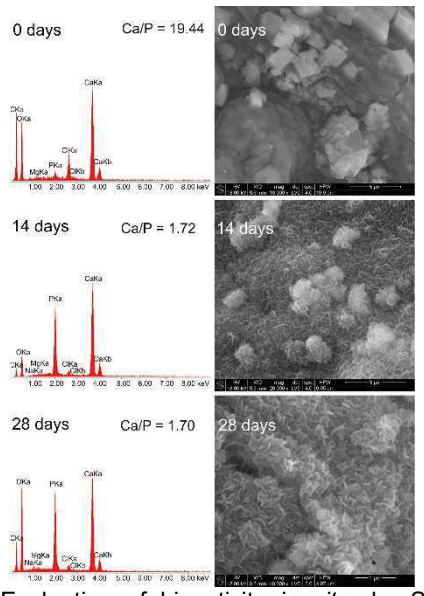

FIG. 3. Evaluation of bioactivity *in vitro* by SEM/EDS analysis during 28-day of glu/HA D soaking in SBF.

Conclusions

Within this study it was demonstrated that dialysis method for curdlan gelation may be successfully used for HA-based biomaterial fabrication. Produced glu/HA D scaffold releases huge amount of Ca^{2+} ions to the surrounding environment, what positively affects osteoblast viability, adhesion, and growth. Moreover, glu/HA D scaffold possesses ability to form apatite layer on its surface. Thus, considering biocompatible and bioactive properties of glu/HA D scaffold, it may be concluded that it is a promising biomaterial for bone tissue engineering.

Acknowledgments

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