Osteoblast Response to Bioactive Borate Glass Surfaces for Titanium

Roger F. Brown, Trini King, Laxmikanth Peddi, and Richard K. Brow
University of Missouri-Rolla, Rolla, MO 65401

Introduction

Prompted by an earlier finding that some borate glasses adhere well to titanium,[1] we recently developed and tested a family of borate glasses for possible use as bioactive surface coatings on Ti6Al4V. Two of the new borate glasses produced, compositions designated B18 and H12, have a close thermal expansion match with titanium, form well-adhered bonds to titanium by enameling techniques, and develop a bioactive hydroxyapatite surface layer when soaked in simulated body fluid.[2] Here we report results of a companion *in vitro* study of the effects of these glasses on the growth, morphology, and function of two lines of osteoblastic cells with silicate 45S5 bioglass included as control samples.

Table 1. Composition (mole %) of glasses tested

Glass	B_2O_3	SiO_2	CaO	Na_2O	Al_2O_3	P_2O_5
B18	41.5	6.5	35.0	12.5	3.5	1.0
H12	40.0	7.5	40.0	8.0	2.0	2.5
45S5	-	46.3	26.9	24.3	-	2.5

Materials and Methods

Glass rods were prepared from reagent grade components mixed in the proportions listed in Table 1 using conventional glass processing techniques. The rods were sectioned to 1 mm discs, sonically cleaned in ethanol, and dry heat sterilized. Cell lines used included MC3T3-E1 murine pre-osteoblastic cells and UMR-106 rat osteosarcoma cells. Culturing was in alpha MEM medium supplemented with 10% fetal calf serum. A cell contact assay with MC3T3-E1 cells was included as an initial test of glass biocompatibility. Additional glass samples seeded with MC3T3-E1 cells were incubated for intervals of 2 to 6 days and then stained with Hoechst 33258 (1µg/ml) for fluorescent visualization of cell proliferation. The amount of borate released from the glasses was measured by ICP analysis. Alexa 488-phalloidin was used for fluorescent viewing of actin filaments in cells cultured on the glasses. Alizarin red staining was used to detect formation of mineralized nodules by UMR-106 cells cultured on the test glasses.

Results and Discussion

Cells were seen growing uniformly along the interface of the borate glasses after incubations of 1 and 3 days with morphology similar to cells at the interface of 45S5 glass. Related measurements of the growth of MC3T3-E1 cells seeded directly onto borate and 45S5 glass discs indicate the cells proliferate on both glass types although their density on the borate glasses was approximately half that of cells on control 45S5 glass (Table 2). The lower proliferation on the B18 and H12 glass appears attributable to the release of borate ions. The ICP analyses reveal borate levels of 2.5-3 mM in the medium. Separate tests show addition of this amount of borate causes about a 40% reduction in cell density during a 4-day culture.

Table 2. MC3T3E-1 cell growth on test glasses. Values are cells/mm^2 ± sd (n=5); inoculum was 300 cells/mm^2.

Glass	Day 2	Day 4	Day 6
B18	157 ± 10	846 ± 167	1135 ± 204
H12	180 ± 96	698 ± 92	1128 ± 169
45S5	484 ± 104	1413 ± 156	2259 ± 106

Other analyses indicate normal cell morphology and behavior on the borate glass surface despite lower cell density. Alexa 488-phalloidin staining reveals similar orientation of actin filaments in MC3T3-E1 cells grown on the borate and 45S5 glasses (Fig. 1) suggesting no cytoskeletal change by exposure to the borate glass.

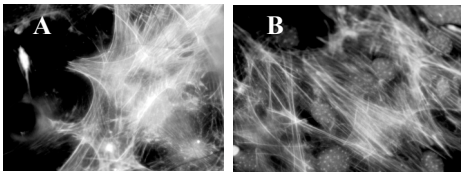

Fig.1. Phalloidin visualization of actin cytoskeletal filaments in MC3T3-E1 cells cultured for 6 days on (A) 45S5 glass disc and (B) H12 borate glass disc.

Additional evidence of normal osteoblast behavior on the borate glass emerged from the tests for mineralization. UMR-106 cells formed Alizarin red-positive mineralized nodules on both borate and 45S5 glasses in response to addition of inorganic phosphate to induce mineralization.[3] These findings are indicative of osteoblast function.

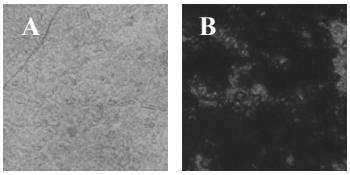

Fig 2. Alizarin Red staining of UMR-106 cultures grown on H12 glass for six days (A) without and (B) with added 5 mM phosphate.

Conclusions

Results of this study indicate that the two borate glasses are biocompatible and permit normal bone cell function. These findings together with previous evidence that B18 and H12 glasses adhere to Ti and exhibit bioactivity suggest these borate glasses may be useful as coatings to enhance osseointegration of titanium alloy implants.

References

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Acknowledgements

Project supported by NIH Grant 1 R15 AR050018-01.