

CONTROLLED RELEASE OF ACTIVE SUBSTANCE AFTER DOUBLE LOADING OF DEMINERALIZED CANCELLOUS BONE

Cobzac Vitalie¹, Jian Mariana¹, Maritoi Tatiana¹, Baranetchi Iana¹, Malcova Tatiana¹, Nacu Viorel¹

¹Laboratory of Tissue Engineering and Cell Cultures, *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova.

Introduction. Demineralized bone graft (DBG) preparation involves substantial growth factor loss due to prolonged liquid exposure during demineralization, marrow and fat removal, and pH restoration. Albumins bind various endogenous substances and drugs in blood, and their incorporation into demineralized bone enhances regeneration. This study investigates the controlled release of biologically active substances from primary DBM loading after secondary bovine serum albumin (BSA) impregnation.

Materials and methods: Demineralized cancellous bone was obtained from bovine iliac bone using 0.5 M HCl and processed per Human Tissue Bank protocols. To assess controlled release, primary coating was performed using Methylene Blue (MB) 10X solution (BioGnost, Croatia). Thirteen DBG samples were vacuumed at 400 mBar, then immersed in 150 ml of 325 µg/ml MB. Four groups were established based on secondary loading: (1) Positive control (MB+dH₂O), (2) MB+10%BSA, (3) MB+20%BSA, and (4) Negative control (MB only). DBG weights did not significantly differ across groups ($p > 0.2$). After secondary vacuum loading with BSA, samples were frozen at -80°C and freeze-dried. MB release was assessed during 33 days by spectrophotometry at 570 nm (BioTek Instruments, USA).

Results and Conclusions. The MB release differed significantly ($p < 0.05$) between control groups from 4 hours to day 8, with higher release in the negative control. After this period, no significant difference was observed ($p > 0.05$). Compared to the positive control, MB release in experimental groups did not differ within the first 6 hours (MB+10%BSA) or 21 hours (MB+20%BSA) ($p > 0.05$). However, from this point until day 6, MB release was significantly higher in the positive control ($p < 0.05$) before declining ($p > 0.05$). MB release in the negative control was significantly higher than in experimental groups for the first 11 days ($p < 0.05$), with no significant differences observed across groups from day 11 to day 33 ($p > 0.05$) (Fig. 1).

Keywords: controlled release, active substance, double loading, demineralized cancellous bone.

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