Sequentially loaded biofunctional demineralized bone matrix with therapeutic agents

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Introduction: Bone grafting remains a key therapeutic strategy for repairing skeletal defects, yet the demineralization of bone matrix results in the loss of native osteoinductive factors. This study evaluates a protocol for dual sequential vacuum-loading, for incorporating into bovine cancelous demineralized bone matrix (DBM) of vancomycin (VA) and human albumin (HA), to enhance bioactivity and modulate release behavior. DBM was sequentially loaded with VA (100 and 200 mg/ml) followed by HA (10% or 20%). Material properties were assessed through swelling ratio, elastic modulus, enzymatic degradation, HPLC-based VA release, MTT assay on rat osteoblasts and antibacterial activity.

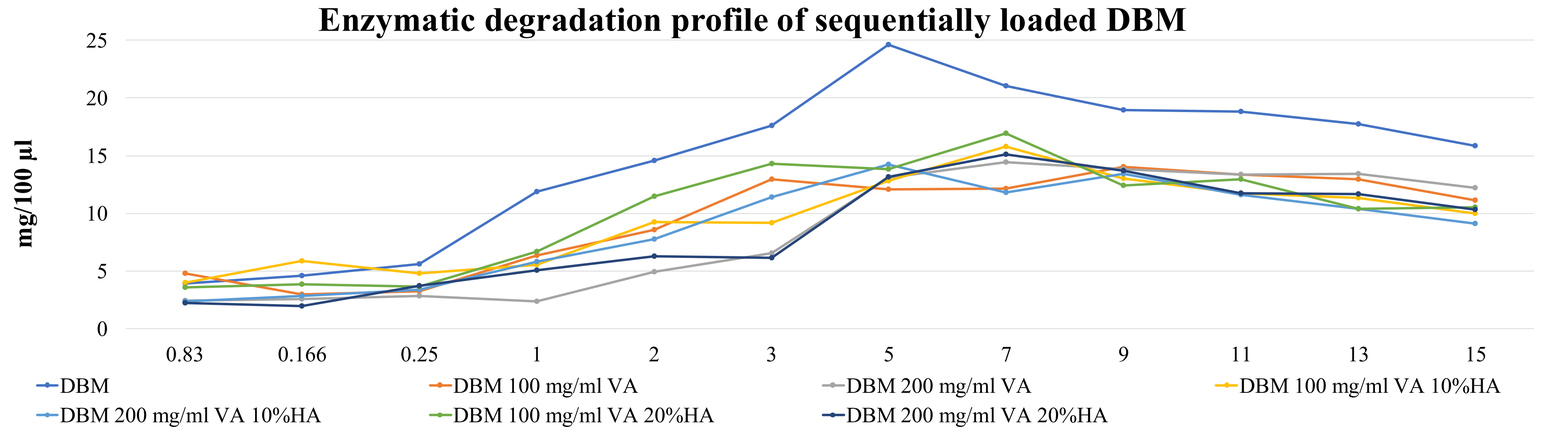
Results: Samples with high VA concentration had a reduced liquid absorption capacity ($p<0.05$), while 20% HA partially restored hydration properties, suggesting stabilizing protein–matrix interactions. VA incorporation significantly increased the elastic modulus ($p<0.05$), and all loaded samples exhibited reduced enzymatic degradation rates compared to native DBM ($p<0.05$). HA did not significantly modify VA release kinetics ($p>0.05$), while MTT assays demonstrated good cytocompatibility at 24 hours across all groups, with a moderate decrease at 48–72 hours due to high VA concentration. However, HA improved cell viability by 15–25%, particularly in high-VA samples, indicating a protective effect. Antibacterial testing confirmed strong VA mediated inhibition against *Staphylococcus aureus* and *Enterococcus faecalis* (30 mm), while *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* remained unaffected, consistent with intrinsic resistance profiles.

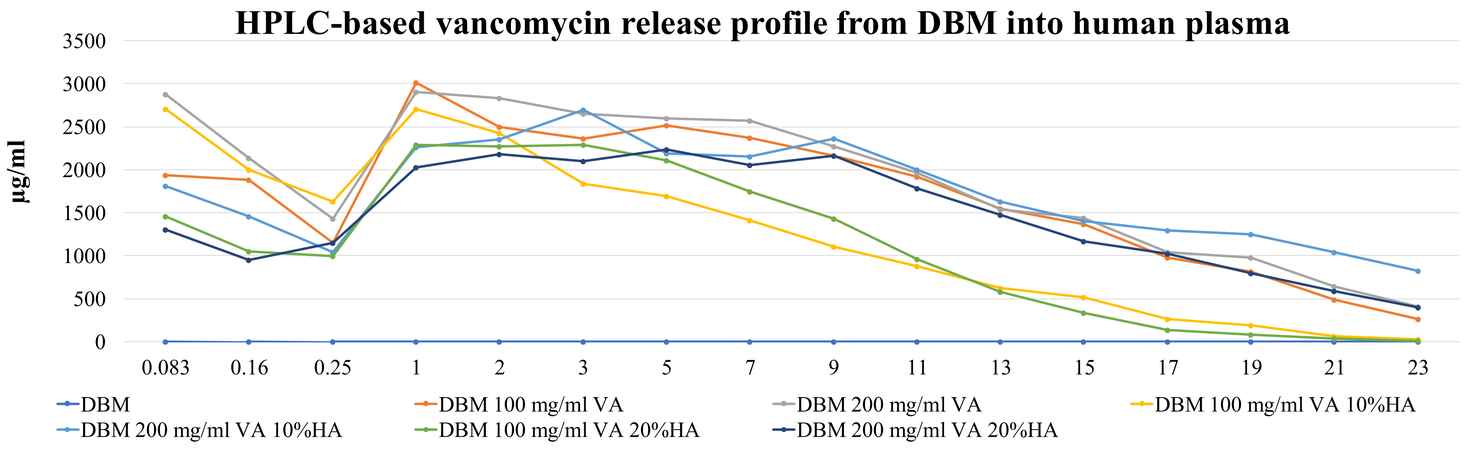


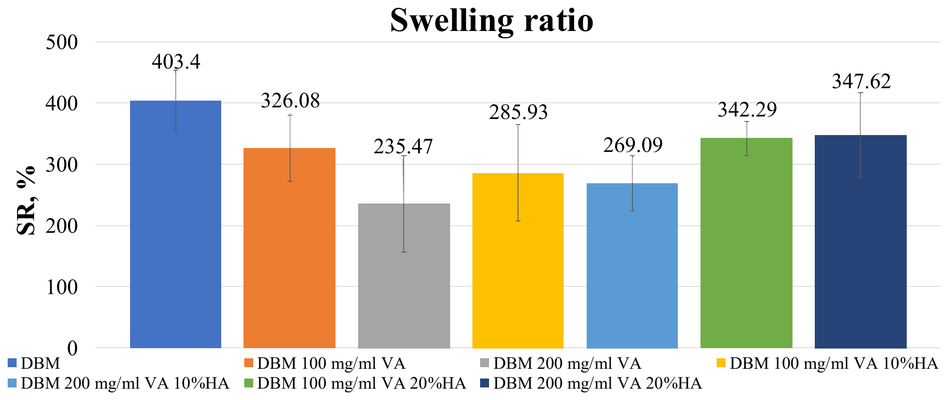
Figure 1. Preparing of bovine cancelous DBM

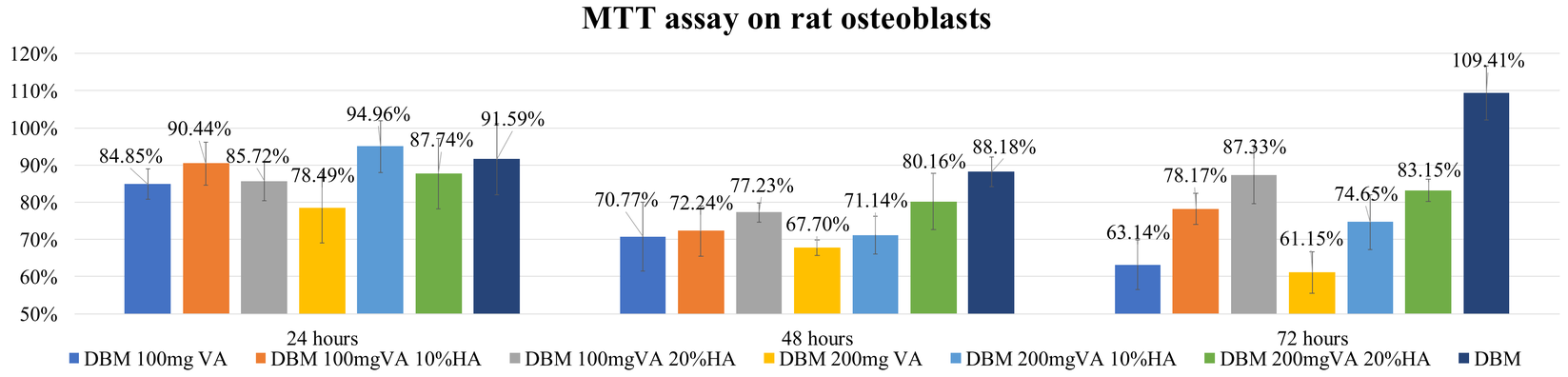


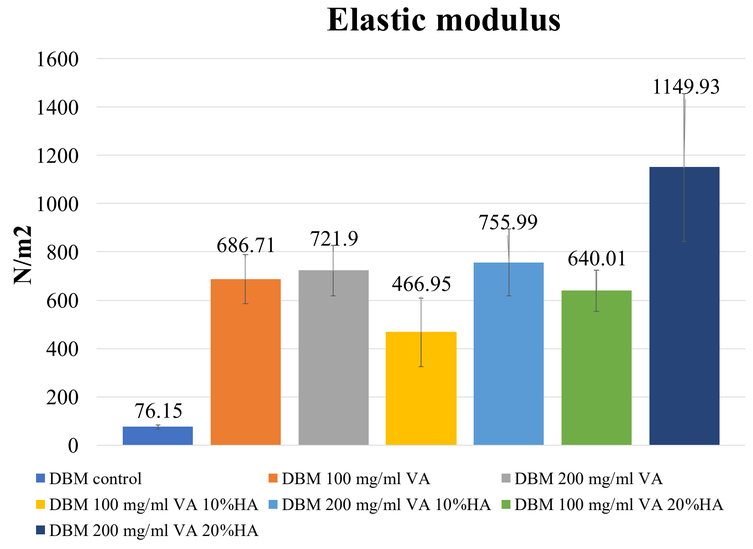
Figure 2. Sequentially loaded VA-HA DBM











Bacterial culture assay for vancomycin activity

SAMPLES	S. aureus (t. 209)		Kl. pneumoniae (t. 3534/51)		E. faecalis (t. ATCC 19433)		E. coli (t. ATCC 25922)		P. aeruginosa (t. ATCC 27853)	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Control– DBM	+	+	+	+	+	+	+	+	+	+
DBM 100mg VA	29 mm	29 mm	3 mm	3 mm	28 mm	28 mm	+	+	+	+
DBM 100mg VA 10%HA	28 mm	28 mm	2 mm	3 mm	28 mm	28 mm	+	+	+	+
DBM 100mg VA 20%HA	30 mm	30 mm	3 mm	3 mm	30 mm	30 mm	+	+	+	+
DBM 200mg VA	30 mm	30 mm	2 mm	2 mm	30 mm	30 mm	+	+	+	+
DBM 200mg VA 10%HA	29 mm	29 mm	2 mm	2 mm	29 mm	29 mm	+	+	+	+
DBM 200mg VA 20%HA	30 mm	30 mm	2 mm	2 mm	30 mm	30 mm	+	+	+	+

+ bacterial culture growth
– absence of bacterial culture growth

Conclusion: The sequential loading with VA and HA preserves cytocompatibility, maintains mechanical stability, ensures sustained antibiotic functionality, and does not impair VA release, making this strategy a promising approach for enhancing DBM-based grafts with combined structural and antibacterial benefits.

Acknowledgement: The authors are acknowledging the support of the project 29ROMD/20.05.2024: "Nanostructured bone grafts with predetermined properties" and to the Romanian Government for providing access to the research infrastructure of the National Centre for Micro and Nanomaterials through the National Program titled "Installations and Strategic Objectives of National Interest"