https://doi.org/10.17952/37EPS.2024.P2076

37th EPS European Peptide Symposium
14th IPS International Peptide Symposium
25 - 29 August 2024
Florence, Italy

A BMP-2-derived bioactive peptide conjugated to chitosan improves osteoblast proliferation and antibacterial properties of membranes for oral surgery

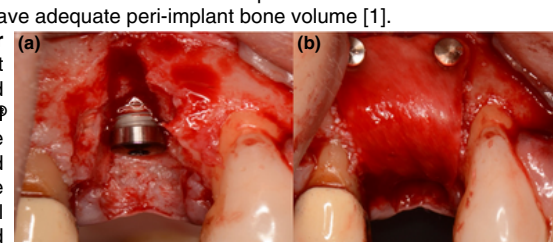
Cristian Balducci[1], Annj Zamuner[1,2], Martina Todesco[2], Andrea Bagno[2], Antonella Pasquato[1], Giovanna Iucci[3], Federica Bertelà[3], Chiara Battocchio[3], Luca Tortora[3,4], Luca Sacchetto[5], Paola Brun[6], Eriberto Bressan[5], Monica Dettin[1]

[1] Department of Industrial Engineering, University of Padova, [2] Department of Civil, Architectural and Environmental Engineering, University of Padova, [3] Department of Science, Roma Tre University, [4] National Institute for Nuclear Physics, INFN Roma Tre, [5] Department of Neurosciences, Section of Dentistry, University of Padova, [6] Department of Molecular Medicine, University of Padova

INTRODUCTION

About 3 billion people suffer from partial or total edentulism and alveolar bone resorption. To achieve a favourable prognosis in implant-supported rehabilitations, it is crucial to have adequate peri-implant bone volume [1].

Guided Bone Regeneration (GBR) uses **barrier membranes** in combination with bone substitutes to block out non-osteogenic cells, thereby improving bone recovery and growth at defect sites [2]. Commercial **OsseoGuard® membranes**, made of formaldehyde-cross-linked bovine type I collagen, are resorbable in 6-9 months and can be moulded and applied dry or wet. Despite their benefits, these membranes sometimes fail to adequately promote vertical alveolar bone growth, leading to suboptimal bone quality and density.



(a) implant insertion with persistence of bone defect and exposure of implant coils (b) defect correction with GBR technique: compensation of the defect with particulate heterologous bone and covering with collagen membrane fixed with 2 titanium pins.

BACKGROUND

Chitosan (CS) is known for its biocompatibility and antibacterial features; it supports coagulation, hemostasis, and osteoblast proliferation and differentiation. In previous studies, we demonstrated that **the fragment (48-69) of BMP-2 (GBMP1α)** enhances human osteoblast calcium deposition, adhesion, proliferation, and the expression of important osteoblast genes, and guides mesenchymal stem cells differentiation towards an osteoblastic phenotype [3].

AIM

This study aimed to engineer and evaluate the efficacy of modified barrier membranes by enhancing their bioactivity for alveolar bone tissue regeneration and their antibacterial properties. Specifically, membranes covalently functionalised with **chitosan (CS)** and **chitosan functionalized with the peptide GBMP1α (CS + GBMP1α)** were investigated to promote bone growth.

METHODS

1 Solid phase SxGBMP1α peptide synthesis

- Fmoc chemistry
- RP-HPLC for purification
- mass spectrometry to confirm peptide identity

S-x-GBMP1α H-S-x-P-F-P-L-A-D-H-L-N-S-T-N-H-A-I-V-Q-T-L-V-N-S-NH_2

x: spacer = α-7-aminoeptanoic acid
S: N-terminal Serine

N-terminal Serine oxidation

GBMP1α ald OHC-CO -x-P-F-P-L-A-D-H-L-N-S-T-N-H-A-I-V-Q-T-L-V-N-S-NH_2

aldehyde group

2 Chitosan functionalization with GBMP1α ald peptide

OHC-CO -x-P-F-P-L-A-D-H-L-N-S-T-N-H-A-I-V-Q-T-L-V-N-S-NH_2 + 70% deacetylated chitosan

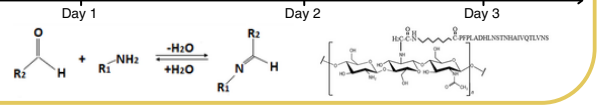
GBMP1α 1 mg/mL in H_2O
+
1:1 (w/w) vs GBMP1α of CS 15,59 mg/mL in 0,2M AcOH + 58% EtOH RT and pH 5,1
+
1:1 (w/w) vs GBMP1α of $NaBH_3CN$
24h magnetic stirring

Bring pH to 7
Double the volume with EtOH 4°C
10 min magnetic stirring
4°C overnight reaction

Filter and dry under vacuum

R1: chitosan
R2: peptide

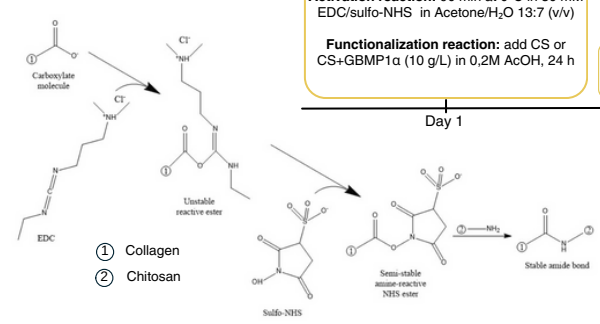
Day 1 — Day 2 — Day 3



3 Barrier membranes functionalization

COVALENT FUNCTIONALIZATION

Crosslinking agents
EDC/sulfo-NHS



Activation reaction: 90 min at 0°C in 30 mM EDC/sulfo-NHS in Acetone/H_2O 13:7 (v/v)

Functionalization reaction: add CS or CS+GBMP1α (10 g/L) in 0,2M AcOH, 24 h

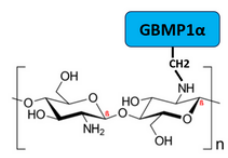
2 one-hour washes in H_2O
1 overnight wash

Remove water

Day 1 — Day 2 — Day 3

COVALENT FUNCTIONALIZATION

Epoxy linker
BDDGE



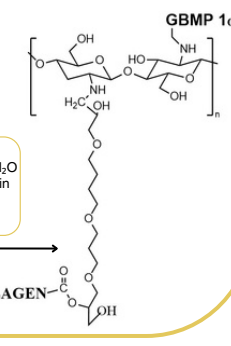
Membrane in chitosan or chitosan+GBMP1α 10 g/L in AcOH 0,2M. Freeze drying

5 ten-minutes washes in H_2O + 5 five-minutes washes in H_2O. Freeze drying

Add BDDGE in H_2O 1% w/w. 48h reaction

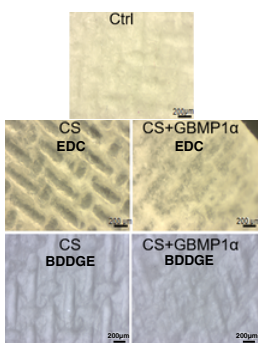
5 ten-minutes washes in H_2O + 5 five-minutes washes in H_2O. Freeze drying

Day 1 — Day 2 — Day 3 — Day 5

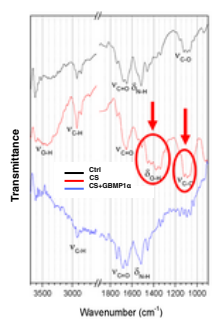


RESULTS

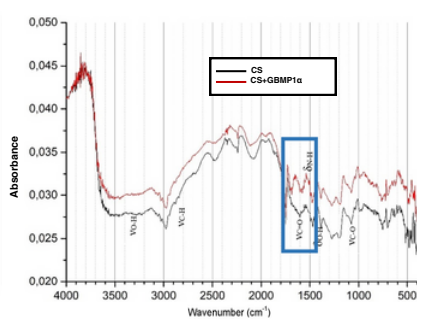
Optic microscopy



FT-IR EDC



FT-IR BDDGE



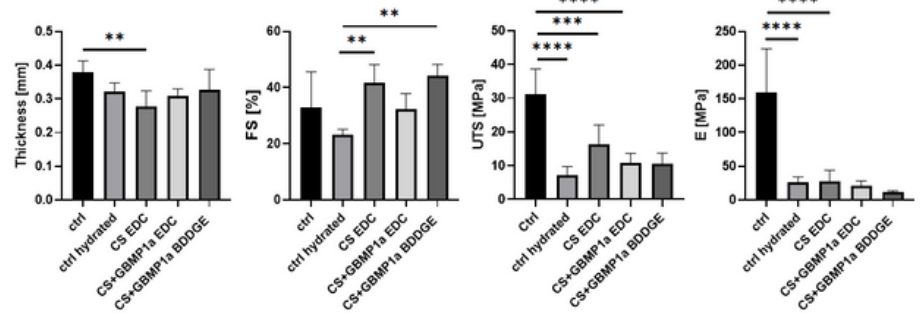
XPS EDC

	N/C
Ctrl	0,134
CS	0,109
CS+GBMP1α	0,029

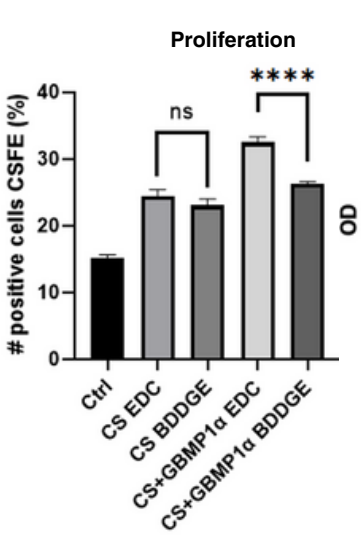
	N/O
Ctrl	0,54
CS	0,39
CS+GBMP1α	0,12

	O/C
Ctrl	0,246
CS	0,279
CS+GBMP1α	0.227

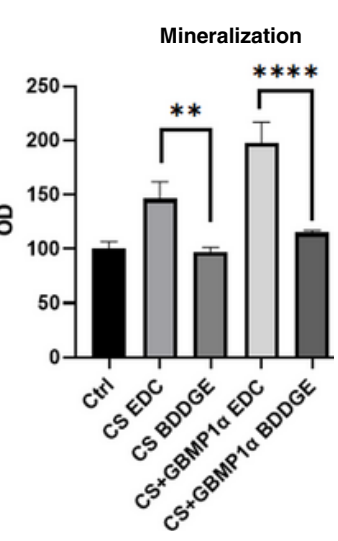
Uniaxial traction mechanical test



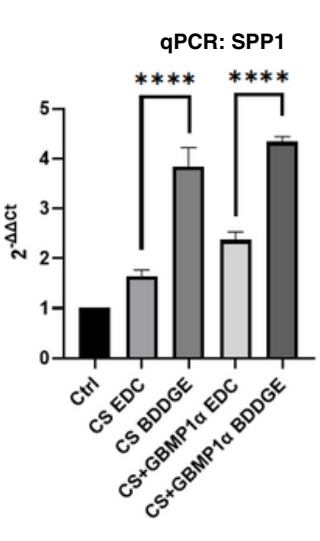
Proliferation



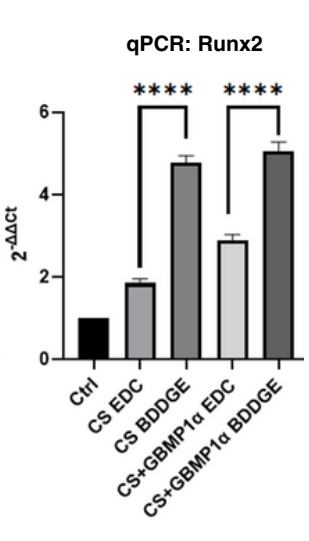
Mineralization



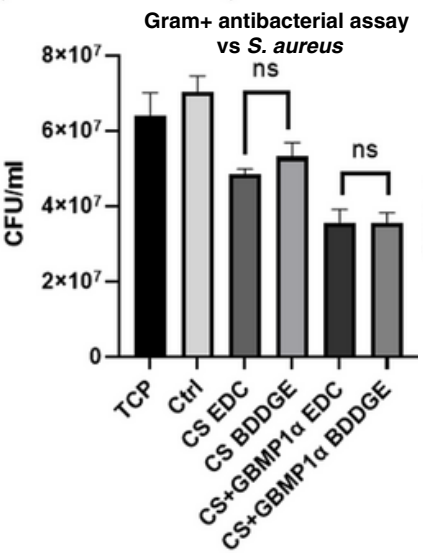
qPCR: SPP1



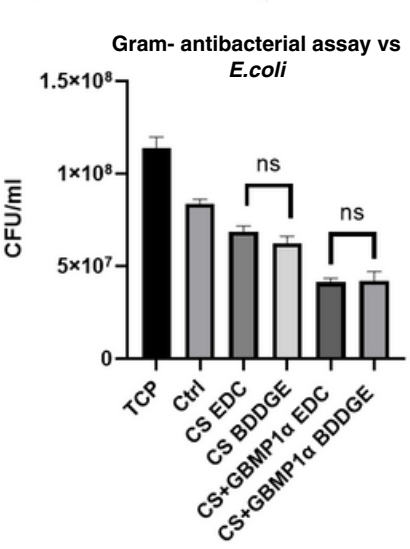
qPCR: Runx2



Gram+ antibacterial assay vs *S. aureus*



Gram- antibacterial assay vs *E.coli*



CONCLUSIONS

the modification of membrane surfaces was confirmed through optical microscopy, FT-IR, and XPS analyses. Biological *in vitro* tests (proliferation, mineralization, gene expression assays) with human osteoblasts showed notable enhancements in functionalized membranes, especially those with GBMP1α that in addition showed antibacterial activity against S. aureus and *E. coli*. EDC/sulfo-NHS method gave better results in proliferation and mineralization, while BDDGE method outperformed in SPP1 and Runx2 genes expression. Mechanical tests indicated functionalizations did not vary the mechanical properties of the membranes.

REFERENCES

[1] R. Gupta, et al, *Dental Implants*, StatPearls Publishing, Aug 2023
[2] I. A. Urban, et al, *Guided Bone Regeneration in Alveolar Bone Reconstruction*, Oral and Maxillofacial Surgery Clinics of North America, May 2019, pp. 331-338
[3] P. Brun, et al, *Chitosan Covalently Functionalized with Peptides Mapped on Vitronectin and BMP-2 for Bone Tissue Engineering*, Nanomaterials (Basel), Nov 2021; pp.2784.

CONTACTS

Cristian Balducci, PhD student - cristian.balducci@phd.unipd.it
Prof. Monica Dettin - monica.dettin@unipd.it