Conclusions
In conclusion, this study successfully developed an innovative chitosan-based, 3D-printed buccal delivery system capable of achieving unidirectional peptide release. The patch demonstrated promising outcomes regarding its release profile, mechanical integrity, and mucoadhesive performance. The strategic integration of a permeation enhancer within the mucoadhesive layer further amplified peptide absorption, highlighting the system's potential to effectively address and overcome the limitations associated with traditional peptide delivery methods.

Topic: Tissue-Specific Focus
Subtopic: Bone
Type: Oral presentation

TERMIS25_589 - 3D melt electro-written polycaprolactone membranes covalently functionalized with PLGA microparticles as a tunable drug release mimicking the physiological function of native periosteum

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Introduction/Objectives
Around 2.2 million bone grafting procedures after fracture are performed every year, with 5–10% of them undergoing some form of healing disturbance, including nonunion. This leads to a substantial economic burden on healthcare systems and major clinical consequences for patients. Despite the intensive research on biomaterials, no satisfactory regenerative outcome has been found combining the osteoinductive and osteogenic capabilities, mimicking physiological processes orchestrated by the periosteum[3] in bone fractures. The incorporation of bioactive molecules such as bone morphogenic protein rhBMP2 alone or in combination with other molecules, has improved the healing process but developed adverse side effects. In this study, we examined the regenerative potential of combining loaded poly (lactic-co-glycolic acid) microparticles, PLGA-MPs, with rhBMP-2 and/or vancomycin, in a previously designed melt electro-written poly ε-caprolactone (PCL) scaffold, via covalent binding. The regenerative potential was evaluated *in vitro* and *in vivo* in a non-union model developed in Sprague-Dawley rats.

Methods
Multiple emulsion solvent evaporation method using Total Recirculating One-Machine System (TROMS) was used to produce PEGylated and carboxylated MPs (PLGA-PEG-COOH MPs). A range of concentrations between 200 to 1500 μg/ml rhBMP-2 (InductOs 12 mg) was tested. MPs size characterization was performed by electron microscopy and laser diffractometry (Mastersizer). Medical graded poly ε-caprolactone (PCL), (PURABSORB 12) was used for the synthesis of the two parts of the implant, the inner rigid core scaffold, generated by fused deposition modeling (FDM), and the surrounded mesh, produced by 3D Melt Electro Writing printing technology (MEW), resembling the natural position of bone and periosteum. Surface chemical modification of MEW PCL membranes was performed via aminolysis for MPs immobilization, verifying the presence of primary amine groups by a colorimetric assay. Aminated MEW PCL membranes were subjected to covalent binding with PLGA-PEG-COOH MPs and the specific covalent union was confirmed by Fourier-transform infrared spectroscopy (FTIR). The bioactivity of PCL membrane with MPs loaded with rhBMP-2 was assessed for osteogenic induction of human periosteum stem cells (hPMSCs) and in the non-union *in vivo* model analysed by histology and μCT. The same analytic procedure was followed for the vancomycin.

Results
TROMS formulation rendered homogeneous particles of 10 microns. rhBMP2 was efficiently encapsulated in PLGA-PEG-COOH MPs reaching maximum values of 64.07± 5.20%. PLGA-PEG-COOH MPs showed a percentual release of up to 73.25± 4.34 of the total content, with 50% of the content released within the first 24 hours. MPs were well distributed along the PCL fibeŕs surface showed by electron microscopy. Osteogenic potential was corroborated by Alizarin Red staining and molecular analysis for BGLAP and SPP1 compared to untreated PCL membranes. The μCT performed at 10 weeks of the in vivo model showed that increased doses of rhBMP-2 (0.6 and 1.2 μg) resulted in greater tissue regeneration. The vancomycin encapsulation efficacy (up to 63.43%) and release (68.62± 1.25%) were settled but the in vivo model is still pending.

Conclusions
We have developed a system that can act as a native periosteum by delivering the critical elements into critical-size bone defects with significant therapeutic and translational potential.

Topic: Tissue-Specific Focus
Subtopic: Womens health
Type: Oral presentation

TERMIS25_742 - Natural polymer-based antibacterial nanocoating for silicone breast implants

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Introduction/Objectives
The demand for new tailored materials to address specific needs and limitations of several biomedical applications is continuously growing. Layer-by-layer self-assembly (LbL) allows to develop nanostructured and tunable surface coatings for biomaterials[1,2], improving biological behavior while preserving their native bulk properties. This research aims to develop a natural-based antimicrobial and biocompatible coating for silicone breast implants to reduce the risk of capsular contraction, which can foster the onset of Breast-Implant Associated Anaplastic Large Cell Lymphoma in severe cases[3,4].

Methods
Natural polymers were selected as polyelectrolytes. Antioxidant-enriched pectin (PEC) paired with methylglyoxal (MGO) compound (the antibacterial component of Manuka Honey) was used as polyanion, while the polycation was the antibacterial chitosan (CHI). A 16-layer LbL coating (PEC/CHI) was manufactured in mildly acidic (pH 5.5) aqueous environment on Sylgard184 silicone samples. A genipin-based crosslinking strategy within chitosan layers to improve the stability of the coating was also tested. Two MGO-containing coatings were investigated: one with MGO included in all PEC layers (PEC8MGO) and the other with MGO only in the last five layers (PEC5MGO). XPS, optical profilometer and QCM analyses were performed to monitor the coating deposition and growth, while antibacterial properties were assessed against gram-positive *Staphylococcus epidermidis* (NCTC 11047), one of the most abundant microorganisms found in infected silicone breast implants.

Results
QCM estimated the coating final thickness at ∼100 nm. The deconvolution of the C1s peaks in the XPS spectra confirmed the presence of chitosan and the successful crosslinking reaction. The topographical reconstructions showed a 4-fold increase in surface roughness for nanocoated silicone. The coating stability (crosslinked and non-

crosslinked) was tested both in physiological (PBS) and acidic conditions (pH 5.5) up to 48h, wettability and FTIR analyses demonstrated that polyelectrolytes were still present on the surface. The coating with the highest amount of MGO (PEC8MGO) led to a 1-log *S. epidermidis* CFU reduction in 24h. Bacteria metabolic activity was tested via XTT assay, suggesting that MGO-coated samples mildly reduce cell viability compared to plain silicone. Observing LIVE/DEAD bacteria staining after 24h of culture, the number of live cells decreased on all nanocoated samples compared with the bare silicone.

Conclusions
A nanoscale coating for silicone-based biomaterials was successfully developed. Microbiological results suggest that the coating exerts a bacteriostatic action against *S. epidermidis* colonies, resulting in a reduction of bacteria proliferation and adhesion. There is currently an ongoing 2D *in-vitro* study on normal human dermal fibroblasts (NDHF), stimulated via a cocktail of cytokines to simulate an inflamed state, with the aim of establishing the efficacy and dose-dependent action of three natural antioxidants (polyphenols quercetin and epicatechin, and trolox as vitamin E-analogue) for possible LbL integration within the coating.

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Topic: Biomaterials
Subtopic: Functionalized, stimuli responsive biomaterials
Type: Oral presentation

TERMIS25_899 - De novo design of highly specific integrin α5β1 agonists for tissue engineering applications

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Introduction/Objectives
Cell adhesion to biomaterials is a critical step for successful implant integration into the body. However, most current biomaterials do not inherently promote cell adhesion, necessitating their functionalization with adhesion motifs such as extracellular matrix proteins. The clinical use of full-length proteins such as fibronectin (FN) is challenging due to their difficulties in large-scale production. Conversely, widely used RGD (Arg-Gly-Asp) peptides and their derivatives often lack specificity, resulting in limited desired cellular responses. Here, we aimed to computationally design a novel protein based on the interaction of FN and its primary target, integrin α5β1. Our goal was to create a stable, easily producible protein capable of effectively promoting cell growth, offering a potential alternative to current cell-adhesive molecules used in tissue engineering.

Methods
Based on the interaction between FN and α5β1, we computationally designed novel proteins from the interacting RGD peptide. To this end, ferredoxin topologies were chosen as protein scaffold templates due to their ability to accommodate multiple loops for α5β1 interaction while providing highly stable structures. Two approaches were employed: (1) designing ferredoxins around the RGD loop of FN while creating new interactions with α5β1, and (2) grafting the RGD loop into pre-built computational protein scaffold libraries that were further optimized for α5β1 interactions. Following screening and optimization, the most promising designs were expressed in bacteria and successfully produced in high yields. Affinities and specificities were assessed by biolayer interferometry. The leading candidate was evaluated for its ability to promote cell attachment, spreading and proliferation when covalently immobilized on titanium discs (as 2D model) and polyethylene glycol (PEG) hydrogels (as 3D model). Finally, NN-grafted titanium implants were tested in vivo for osteointegration in a rabbit model.

Results
The best candidate, named NeoNectin (NN), demonstrated high specificity for integrin α5β1 without detectable binding to other relevant integrins. Notably, NN induced the active conformation of integrin α5β1 resembling the interaction of FN, a result not previously achieved with RGD variants. The novel NN supported robust attachment, spreading and proliferation of different bone cell types on titanium discs, comparable to FN. In contrast, cells on RGD-coated titanium discs displayed a more rounded morphology. We also observed complete cell spreading on PEG hydrogels functionalized with our novel NN. In vivo, NN remained active upon implantation, outperforming FN- and RGD-grafted titanium implants. NN-grafted titanium implants exhibited the highest bone volume-to-total volume ratio, bone-implant contact and new bone formation.

Conclusions
We successfully designed and produced a de novo protein by computational methods able to mediate the attachment and spreading of cells to a level comparable to FN, but easier to produce and much more stable. We anticipate that NN shows a great potential as a substitute for FN and RGD in different biomaterials, with applications in tissue engineering and biomedicine.

Topic: Biomaterials
Subtopic: Functionalized, stimuli responsive biomaterials
Type: Oral presentation

TERMIS25_1186 - Innovative cryo-patches with engineered spider silk proteins for enhanced bone defect repair

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Introduction/Objectives
Bone defects resulting from trauma, tumor resection, or congenital abnormalities, pose significant challenges in clinical practice. Current treatments have several limitations, including limited availability of autografts and donor site morbidity, failing to restore bone tissue and leading to further complications that need to be tackled. Bioengineered spider silk has an enormous potential to develop innovative materials with added biological function. The fusion with Bone morphogenetic proteins (BMPs) like BMP7 is an innovative method that promotes osteogenesis and bone turnover. In this study, we investigate the potential of bioengineered spider silk proteins (6mer) fused with an osteogenic protein (BMP7) aiming to simultaneously induce bone tissue regeneration as bone substitutes in segmental bone defects.

Methods
Herein, we developed new cryo-patches by combining bioengineered spider silk protein with BMP7 (2%, 6mer-BMP7), as well as