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BOOK OF ABSTRACTS

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Elucidating mechanism behind the impact of regulatory T cell-derived extracellular vesicles on osteogenesis

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Introduction: Regulatory T cells (Treg) play a crucial role in immune regulation and bone regeneration. Emerging evidence highlights the immunomodulatory potential of extracellular vesicles derived from Treg (Treg-EVs); however, their impact on osteogenesis remains understudied. This study aimed to assess how Treg-EVs modulate the osteogenic potential of bone marrow-derived mesenchymal stromal cells (BMSC), potentially through the PI3K/AKT pathway, a key regulator of osteogenesis.

Methods: Treg were isolated from the peripheral blood of healthy donors (n=6) following informed consent. Cells were expanded for 13 days, followed by 24 h of serum starvation. Conditioned media were then collected, and concentrated. Treg-EVs were isolated using size-exclusion chromatography and characterized by TEM, NTA, flow cytometry, western blot, and multiplex immunoassay. BMSC were treated with Treg-EVs (80 µg/mL), with or without a PI3K/AKT inhibitor. Metabolic activity, migration, and osteogenic differentiation were assessed.

Results: Uptake of Treg-EVs by BMSC was confirmed after 24 h. Treg-EVs significantly enhanced BMSC metabolic activity after 4 and 7 days, albeit insignificant when PI3K/AKT pathway was inhibited. Migration decreased at 48 and 72 hours, even when the pathway was inhibited. During osteogenic differentiation, Treg-EVs activated PI3K/AKT pathway from day 4. Furthermore, alkaline phosphatase activity, osteogenic gene expression, and mineralization (day 10) were enhanced in BMSC with Treg-EVs treatment, regardless of pathway inhibition.

Conclusions: Treg-EVs promote BMSC osteogenic differentiation, even in the presence of PI3K/AKT inhibition, suggesting a potential PI3K/AKT independent pathway. These findings provide insights into Treg-EVs mechanisms for enhancing osteogenesis and highlight their therapeutic potential in bone regeneration.

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Promoting Tissue Regeneration in Severe Burns with Spray-Delivered Adipose Stem Cells: An In Vitro Study

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Aim
This study investigates the regenerative potential of adipose-derived mesenchymal stem cells (ASC) delivered via a fibrin glue spray system, focusing on cell viability, proliferation, and immunophenotype.

Background
Severe burn wounds pose a significant challenge in regenerative medicine due to extensive tissue loss and the need for rapid, effective healing. ASC are known for their regenerative capabilities, including promoting angiogenesis, modulating inflammation, and enhancing tissue repair. Spray application offers a promising approach for uniform ASC delivery over large wound surfaces, potentially accelerating regeneration and improving clinical outcomes.

Methods
Human ASC were encapsulated in fibrinogen and sprayed using a fibrin glue spray system, with 1 or 1.8 bar pressure, at 10 or 20 cm distance, before adding thrombin. Casted ASC without spraying were used as control. During culture for 7 days, viability, morphology and proliferation of ASC were assessed using Live/Dead staining, metabolic activity assay, actincytoskeleton staining, and DNA quantification. Cellular immunophenotype was examined using flow cytometry.

Results
Sprayed ASC retained regenerative potential, with high viability, proliferation, and preserved morphology similar to the casted controls. The regenerative capacity was not diminished by spray delivery, as evidenced by increasing metabolic activity and stable immunophenotype across all conditions.

Conclusion
Spray delivery of ASC using fibrin glue is a viable method for regenerative therapy in burn wounds, preserving key regenerative properties crucial for effective tissue repair

Effects of functionalized nanodiamond particles on macrophages in 2D and 3D

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Introduction

Bone regeneration of large defects requires treatment ensuring interactions of immune and progenitor cells. Nanodiamond particles (nDP) have great functionalization potential to exert immune-driven pro-regenerative processes. While polycaprolactone (PCL) provides attractive biocompatibility, it lacks bioactivity. Therefore, we aimed at rendering PCL scaffolds immunomodulatory via coating with functionalized nDP. We compared effects of functionalized and pristine nDP on macrophages in dispersion and on nDP-coated PCL scaffolds.

Methods

Pristine milled nanodiamond particles (nDP^{m}) were functionalized with Biotin (nDP^{Bio}), positively- or negatively-charged dipeptide (nDP^{+} or nDP^{-}, respectively), and used for coating PCL scaffolds. Monocytes differentiated into macrophages for 6 days were exposed to nDP or seeded on nDP-coated PCL scaffolds. After 24h, cytokine release, gene and surface marker expression were analyzed with Bioplex, qPCR and flow cytometry.

Results

mRNA expression of IL1B, IL10 and CCR7 was upregulated in nDP^{+} and nDP^{-} ($p<0.01$) compared to nDP^{m} and nDP^{Bio}. IL10 and CCR7 were downregulated in nDP^{+}_PCL but upregulated in nDP^{-}_PCL compared to nDP^{m}_PCL. IL10 was upregulated in nDP^{-}_PCL compared to nDP^{+}_PCL ($p<0.05$).
CD80 surface expression was highest in nDP^{+} and nDP^{m}_PCL. CD206 was downregulated ($p<0.05$) in nDP^{Bio} compared to nDP^{m}; this trend was also seen on PCL. Cytokines IL-1b, TNF-α, IL-6 and IL-10 were increased in nDP^{+} and nDP^{-}. Cytokine release in nDP^{Bio}_PCL was reduced compared to nDP^{m}_PCL, TNF-α was also reduced in nDP^{+}_PCL and nDP^{-}_PCL.

Conclusion

nDP^{+} and nDP^{-} induced strongest immune reactions. Coating with nDP^{m} augmented their immunomodulatory potential, while functionalized nDP retained immunomodulatory effects on PCL. nDP^{Bio}_PCL mitigated pro-inflammatory responses.

Rethinking limbal stem cell deficiency

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Introduction
Limbal stem cell deficiency (LSCD) is characterized by the dysfunction or loss of limbal epithelial stem cells, leading to impaired wound healing, corneal neovascularization, opacification, and vision impairment. Current treatments are insufficient, underscoring the need to better understand disease mechanisms across LCSD etiologies.

Purpose
To compare and characterize models of 3 different LSCD etiologies to identify shared and distinct pathogenic features. The goal is to improve understanding of disease mechanisms using this to improve therapeutic approaches.

Method
LSCD will be induced in the animal models using three approaches: mechanical corneal epithelial removal, alkali burn, and suture placement. Corneal wound healing, vascularization, and corneal opacity will be assessed clinically prior to tissue harvesting. Immunofluorescence will be used on tissue sections using antibodies against keratin 12 (K12, corneal epithelium marker) and keratin 13 (K13, conjunctival epithelium marker), and ΔNp63 (limbal stem cell marker) to assess epithelial phenotype and limbal stem cell presence.

Results
In the alkali burn model, K13 expression was detected on the central cornea, indicating conjunctivalization due to limbal barrier failure. ΔNp63 staining confirmed the presence of limbal stem cells in the basal epithelium of healthy eyes. Marker expression patterns support the reliability of selected antibodies in detecting conjunctivalization and limbal stem cell presence.

Conclusion
These applied immunohistochemical markers are effective in characterizing epithelial changes associated with LSCD. These approaches will be used to further define disease mechanisms, and response to treatment, across different experimental models.

Marine Biological Coproduct as a Source of Hydroxyapatite (HA) for Dental Pulp Regeneration

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Background
The dental pulp supplies nutrients and detects potential pathogens, so its loss will make the tooth fragile. Traditionally, infected dental pulp is removed and replaced with inorganic materials. Using regenerative endodontics, inflamed/necrotic pulp tissues are replaced with regenerated pulp-like tissues to revitalize teeth. The multi-doped ion composition and micro- and nano-scale architecture of marine-derived hydroxyapatite (HA) make it particularly promising for dental pulp regeneration. Hence, the study investigates the odontogenic potential of dental pulp stem cells (DPSC) when exposed to HA doped with manganese (Mn), strontium (Sr), and zinc (Zn).

Methods
In this study, HA was doped with different concentrations of Mn, Sr, and Zn. Dental Pulp Stem Cells (DPSC) were cultured up to 21 days. Cell viability, proliferation and morphology were assessed *in vitro* using live/dead stain, PicoGreen quantification assay and PrestoBlue. Additionally, cell morphology was examined using a confocal microscope. Cell differentiation was measured by quantitative real-time reverse transcriptase-polymerized chain reaction analysis (qRT-PCR), alkaline phosphatase test and Alizarin red staining.

Results
The findings demonstrated the effect of marine HA doped with varying concentrations of strontium (Sr), zinc (Zn), and manganese (Mn) and their influence on cellular proliferation, differentiation, and calcium deposition. Specifically, Sr 3% and Zn 1.5% showed promising outcomes on proliferation and differentiation respectively using multiple assays.

Conclusions
Marine HA promoted DPSC proliferation and differentiation in a concentration-dependent manner. Sr and Zn doping, at specific levels, shows strong potential for dental pulp regeneration.

An *in vitro* study of mesenchymal stem cell characteristics and paracrine activity after spray administration in fibrin sealant

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Introduction
Severe burn wounds (>20% total body surface area) are debilitating injuries requiring novel therapeutic approaches. Preclinical studies and clinical reports on mesenchymal stem cell (MSC) show promising results, but their limited availability and the need to cover large areas highlight the importance of an efficient delivery method. This study evaluates MSC viability and function after spray application in fibrin sealant for burn wound treatment compared to cast cell application.

Methods
Human bone marrow-derived MSCs (hBMSC) were sprayed in a two-component fibrin sealant at varying pressures and distances and compared to cells that were cast. Viability was assessed with trypan blue exclusion test and propidium iodide (PI) staining. Stem cell surface markers were analyzed by flow-cytometry post-spraying. A scratch wound assay with conditioned medium from sprayed vs. cast hBMSCs was conducted to evaluate wound paracrine-mediated wound effects.

Results
Trypan blue staining showed good viability in all groups directly after spraying, though PI staining indicated reduced viability with higher spray pressures. Flow cytometry analysis showed that hBMSC surface marker expression remained preserved post-spraying. Conditioned medium from sprayed hBMSCs improved wound closure in the scratch assay, suggesting retained paracrine activity.

Conclusion
Spraying hBMSCs in fibrin sealant maintains their viability and function, offering a promising administration method for burn wound therapy. Further studies are needed to optimize spraying parameters for maximum therapeutic efficacy.

Evaluation of a Novel Injectable Dendritic Hydrogel for Bone Tissue Engineering Applications

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Background
Regenerating critical-sized bone defects remains a significant clinical challenge. Polymeric biomaterials, particularly hydrogels, are increasingly used to support bone regeneration. This study aims to evaluate the biocompatibility and osteoconductive properties of a novel hydrogel based on dendritic-linear-dendritic (DLD) polymers functionalized with micro-hydroxyapatite (HA) for bone tissue engineering, using both *in vitro* and *in vivo* approaches.

Methods
The DLD hydrogel was evaluated for cytocompatibility and its ability to support osteogenic differentiation of bone marrow mesenchymal stem cells (BMSC) *in vitro*. Its *in vivo* bone regenerative potential was assessed using a rat critical-sized calvarial defect model. The HA-functionalized hydrogel was injected into the defect site alone or combined with BMSC and/or bone morphogenetic protein 2 (BMP-2). Bone regeneration was monitored over 8 weeks using *in vivo* CT and micro-CT imaging, followed by quantitative morphometric analysis.

Results
In vitro, the DLD hydrogel maintained high BMSC viability and promoted osteogenic differentiation, as evidenced by elevated expression of osteogenic markers and mineralized matrix formation after 28 days. *In vivo*, all hydrogel-treated groups demonstrated enhanced bone formation compared to untreated control. The combination of hydrogel with BMP-2 achieved the highest bone volume/total volume (BV/TV) and bone mineral density (BMD). Notably, the HA-functionalized hydrogel alone or combined with BMSC also promoted bone regeneration.

Conclusions
The novel DLD hydrogel exhibits excellent biocompatibility and supports osteogenesis both *in vitro* and *in vivo*. Its injectable nature and compatibility with biological additives make it a promising platform for bone tissue engineering applications.

References

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