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Original
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Abstracts
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whereas the subsequent resolution of inflammation and promotion of vascularization are mediated by M2 macrophages. However, this balance is dysregulated at a genetic level in chronic wounds, increasing the M1 macrophage presence, enhancing inflammation, and reducing vascularization. While collagen-based (CG) scaffolds show promise for wound repair, additional functionalization is required to overcome this dysregulation and elicit chronic wound healing.[1] microRNAs (miR) regulate gene expression in many biological processes, including inflammation and angiogenesis in wound healing.[2] miR-155 inhibition, in particular, has shown anti-inflammatory outcomes in vivo, while increasing pro-angiogenic marker expression.[3] Thus, we propose that targeted delivery of miR-155 inhibitor from CG scaffolds to macrophages will promote M2 polarization, effectively attenuating inflammation. Additionally, we assessed the interaction between miR-155-treated macrophages and endothelial cells, and their ability to enhance angiogenic processes necessary for chronic wound healing.

Methods
Nanoparticles were synthesized by complexation of miR-155 and non-viral GAG-binding enhanced transduction (GET) peptide.[4] miRNA-activated scaffolds were formed by soak-loading miR nanoparticles onto lyophilized CG scaffolds. The effect of miR delivery on macrophage polarization towards non-polarized (M0) and pro-inflammatory (M1) phenotypes was assessed using THP-1 monocytic cells. Cell phenotype and polarization was assessed by confocal microscopy through staining and quantification of pro-inflammatory (CD80) and anti-inflammatory (CD206) markers. The effect of macrophage polarization on angiogenesis was evaluated through macrophage-endothelial cell interactions via paracrine signaling in migration and tube formation assays. Analysis of the expression of key inflammatory and angiogenic markers, including TNF-α, IL-10 and VEGF, was examined by PCR and ELISA.

Results
miRNA-activated CG scaffolds increased the expression of anti-inflammatory and angiogenic markers, IL-10 and VEGF, from M0 and M1 macrophages over 7 days. Increased CD206+ and reduced CD80+ macrophage numbers were observed on M0 and M1-seeded scaffolds, respectively. Similarly, reduced circularity was quantified from M0 and M1 macrophages on miRNA-activated scaffolds with greater circularity indicating a pro-inflammatory phenotype. Endothelial cell migration and vascular-like structure organization was enhanced when exposed to the conditioned media from miRNA-activated scaffolds.

Conclusions
In this work, we show that miRNA-activated CG scaffolds enhance an anti-inflammatory M2 phenotype confirmed through gene expression and the quantification of CD206+ and CD80+ expression. We show that the secretome from these macrophage-seeded miRNA-activated scaffolds enhance pro-angiogenic processes in endothelial cells, essential for the vascularization of chronic wounds. Taken together, this data indicates that miRNA-activated scaffolds enable M2 macrophage polarization; key for inflammation reduction, promotion of angiogenesis, and the resolution of chronic wounds.

1 McGrath et al., ACS Appl. Mat. Inter., 2023
2 Banerjee et al., Adv. Exp. Med. Biol., 2015
3 Ye et al., IJLEW, 2017
4 Raftery et al., Biomaterials, 2019

Topic: Gene therapy
Subtopic: Biomaterial-guided gene therapy
Type: Oral presentation

TERMIS25_1217 - Immune cell-membrane derived vesicles as smart nanovectors for gene delivery

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Introduction/Objectives
Nowadays, the new frontier of cancer therapy is represented by immunotherapies which include several approaches exploiting the immune system's ability to target, and attack cancer cells. In particular, cancer immunotherapy based on engineered T cells has emerged as an effective tool in the treatment of cancer. In this frame, one of the main goals of nanomedicine for the advancements of immunotherapy is focused on the generation of smart nanovectors to achieve therapeutic gene delivery in patient's immune cells, thus conferring to the engineered cells the ability to fight against patient-specific cancer. However, the design of these smart nanovectors required to adopt innovative strategies able to confer enhanced targeting properties, thus considering the presence of biological barriers such as tumor heterogeneity, clearance by immune system, osmotic pressure, and stromal microenvironment. In this scenario, this work was focused on the development of cell-membrane derived nanovesicles by exploiting their ability to mimic both biological and functional features of the native cells, thus ensuring to nanoparticles self-recognition, immune system evasion and long-time circulation *in vivo*.

Methods
To this aim, immune cells were collected and subjected to first hypotonic cell lysis and differential centrifugation for purification of plasma membranes. Cell plasma membranes were subjected to the extrusion method by using nanoporous membrane filter for the nanovesicle's synthesis. Dot blot, dynamic light scattering analysis (DLS) and transmission electron microscopy (TEM) were useful techniques for evaluating the surface protein's retention, the particle's average size and the morphology structure. To examine the cellular uptake, cell membrane derived nanovesicles were co-incubated with pre-seeded immune cells for different time points and analysed by using flow cytometry and confocal fluorescence microscopy.

Results
Physical characterizations confirmed the successful production of monodispersed immune derived vesicles in the nanometric size range. Dot blot analysis confirmed the retention of protein exposed on nanovesicle's surface. TEM analysis revealed the core-shell structure as expected in a lipid bilayer-coated particle. Data obtained from flow cytometry showed a high internalization rate of synthetized nanovesicles withing immune source cells. The cellular uptake was further confirmed by confocal microscopy.

Conclusions
The well-shaped and nanometric immune derived nanovesicles retained lipid molecules and proteins on their surface which allowed them the homotypic targeting and the high cellular uptake within source cells. The further step is focused on the optimized production of nanovesicles encapsulating plasmid DNA with the aim to efficiently engineer T cells to be employed for targeted immune cancer therapy.

Topic: Gene therapy
Subtopic: Gene delivery
Type: Oral presentation

TERMIS25_1401 - Development of Functionalized Polymer-Lipid Hybrid Nanoparticles for Skeletal Muscle-Targeted RNA Delivery in Spinal and Bulbar Muscular Atrophy (SBMA) Therapy

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Introduction/Objectives
Spinal and bulbar muscular atrophy (SBMA) is a rare X-linked disorder caused by mutations in the androgen receptor gene, and characterized by progressive muscle weakness, wasting, and spasms, significantly reducing patient quality of life [1]. Current therapeutic strategies are limited, necessitating the development of targeted and effective treatments. Recently, artificial microRNAs (amiRs) were designed to downregulate overexpressed genes implicated in SBMA pathogenesis, such as lysine-specific demethylase 1 and protein arginine N-methyltransferase 6 [2]. Initial studies have demonstrated that amiRs improve motor function in SBMA murine models [2]. However, amiRs were delivered using viral vectors, which poses safety concerns and limits clinical translation. To address these challenges, we designed and patented polymer-lipid hybrid nanoparticles (NPs) as a promising miRNA delivery platform with high biocompatibility, high loading efficiency, and potential for surface functionalization. This study focuses on developing polymer-lipid hybrid NPs functionalized with a skeletal muscle-specific peptide (MSP) for targeted amiRs delivery to skeletal muscle cells.

Methods
Polymer-lipid hybrid NPs functionalized with MSP were prepared based on a patented method. Optimization of the functionalization involved conjugating MSP to polyethylene glycol spacer (MSP-PEG), followed by MSP-PEG grafting to NP surface via click chemistry (PEG-MSP NPs). The aim was to enhance peptide exposure and cellular uptake. The nanoparticles were characterized for hydrodynamic size, zeta potential, and functionalization efficiency. *In vitro* internalization studies were performed using C2C12 murine myoblasts and differentiated myotubes, comparing PEG-MSP NPs with non-functionalized NPs through fluorescence microscopy and flow cytometry. Dose-dependent cell viability assays were also performed.

Results
PEG-MSP NPs showed a hydrodynamic size of ~200 nm, a zeta potential of -18.7 mV, and 80% functionalization efficiency. In C2C12 myoblasts, PEG-MSP NPs exhibited high internalization (99.2% Cy5+ cells; 12,925 RFU fluorescence intensity). Higher internalization of PEG-MSP NPs versus non-functionalized NPs was confirmed in tests with differentiated myotubes, better reproducing physiologically relevant conditions. High cell viability (>90%) was found for all tested concentrations.

Conclusions
Results showed that PEG-MSP NPs represent a promising platform for RNA-based therapies, demonstrating superior delivery efficiency, high biocompatibility, and potential for targeted delivery to skeletal muscle cells. Future studies will focus on evaluating therapeutic efficacy of PEG-MSP NPs/amiRs *in vitro* using human myoblasts and *in vivo* in SBMA murine models, alongside their tissue biodistribution. In conclusion PEG-MSP NPs represent a safe and effective non-viral miRNA delivery system with the potentiality of transforming the treatment of SBMA and other skeletal muscle disorders.
Acknowledgments: This project is supported by ERC BIORECAR-EU H2020 GA 772168, ERC-2022-PoC2 POLIRNA GA 101113522 and AFM TELETHON SaveAR.

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[1] Chahin et al., 'Natural history of spinal-bulbar muscular atrophy', *Neurology*, 2008.
[2] Prakasam *et al.,* 'LSD1/PRMT6-targeting gene therapy to attenuate androgen receptor toxic gain-of-function ameliorates spinobulbar muscular atrophy phenotypes in flies and mice', *Nat Commun*, 2023.

Topic: Cells
Subtopic: Cell signaling
Type: Oral presentation

TERMIS25_1481 - Red Light Activated TGFB Signalling to Drive hPSC Chondrogenesis

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Introduction/Objectives
Osteoarthritis affects 7.6% of the global population and leads to articular cartilage defects.[1] To generate cartilage tissue analogues for defect site regeneration, chondrogenesis protocols direct human pluripotent stem cells (hPSCs) towards permanent articular chondrocytes *in vitro*. Activation of transforming growth factor beta (TGFB) family signalling at precise timepoints during differentiation is imperative to prevent deviation of cells to off-target lineages. Expensive and batch-variable growth factors provide limited control over cell signalling, particularly within 3D biomaterial scaffolds, and so achieving a stable articular chondrocyte phenotype remains a challenge. Optogenetic systems harness light to dynamically activate signalling pathways with spatial and temporal precision. Already, blue light activation of TGFB family signalling has been used to drive hPSC chondrogenesis, and shown potential to create 3D signalling gradients.[2-5] However, all systems are blue light activated. This limits clinical applicability due to photoxicity concerns, may interfere with photocrosslinking of biomaterial scaffolds, and means only one signalling pathway can be controlled at a time. To exploit the potential of optogenetics to fine-tune chondrogenic phenotype, this work aims to add an additional activation wavelength to the optogenetic toolkit.

Methods
We designed a red light activatable TGFB signalling system using PhyB and Pif photosensitive proteins coupled to the cytoplasmic domains of native TGFB receptors. Lentiviral vectors were constructed to engineer the system into a human chondrogenic cell line (TC28a2) , which contained a bioluminescent reporter for TGFB pathway activation.[5] To test activation, engineered cells were incubated in serum free medium, containing 1μM phycocyanobilin (PCB) for PhyB activation, and stimulated for 3 hours with 10ng/ml TGFB3 or 630nm light (3.4mW/cm^2). Cells were then imaged in an Alligator Luminescence System and expression of selected TGFB response genes was measured using RT-qPCR.

Results
RT-qPCR detection of engineered transgenes and confocal imaging of fluorophore tags confirmed expression of optogenetic TGFB receptors in TC28a2 cells. Compared to non-stimulated cells, TGFB and red light stimulated cells had strong bioluminescent signals, corresponding to TGFB activity, and upregulated TGFB response gene expression.

Conclusions
The results indicate that optogenetic TGFB receptors were successfully expressed in TC28a2 cells. Detection of TGFB signalling activity following red light stimulation suggests that it can effectively induce dimerization of optogenetic receptors to switch on TGFB signalling. This is evidence that we have, for the first time, engineered red light control of the TGFB signalling pathway in a human cell line, which may be more suitable for cartilage tissue engineering than existing blue light optogenetic systems. Future application in hPSC chondrogenesis could open opportunities for controlling multiple signalling pathways with different light wavelengths to better guide formation of a stable chondrogenic phenotype within cartilage tissue analogues.
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2. Humphreys *(et al.)*, ACS Synth. Biol. 9:3067–3078, 2020
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6. Woods *(et al.)*, Cells 10: 726, 2021

Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_917 - Electroactive polysaccharide-based click-hydrogels: targeting stimuli responsive biomaterials for skin wound healing

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