Triggering the activation of Hippo ON-state pathway in the study of Tenosynovial Giant Cell Tumor cells

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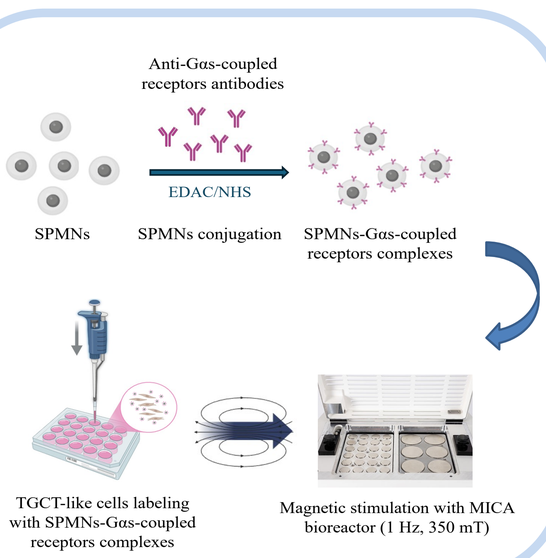
INTRODUCTION

Tenosynovial Giant Cell Tumor (TGCT) although benign, represents a locally aggressive neoplasm affecting synovial joints, characterized by abnormal synovial proliferation, leading to chronic pain, and high recurrence rates. This tumor is driven by overproduction of CSF1, primarily by neoplastic fibroblast-like synoviocytes (FLS) cells. A major obstacle in TGCT research is the lack of physiologically relevant preclinical 3D models that recapitulate tumor-stroma interactions and mechanotransduction cues, which drive disease progression [1]. The Hippo YAP/TAZ pathway plays a critical role in tumorigenesis and is frequently dysregulated in various tumors, making it a potential target for anticancer therapies [2].

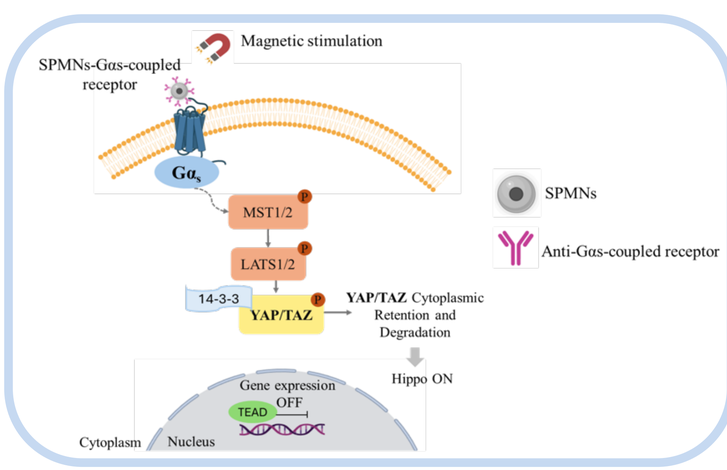
Herein we propose to use controlled mechano-magnetic forces on superparamagnetic nanoparticles (SPMNs) complexes, according to [3], for studying the Hippo pathway, by targeting $G\alpha_s$-coupled receptors, to inhibit YAP/TAZ nuclear translocation. Furthermore, the we developed a 3D microtissue composed of TGCT cells and FLS cells to mimic the TGCT microenvironment, thus allowing to recreate cells crosstalk and facilitating the evaluation of the proposed magnetically targeted therapy.

METHODOLOGY

1. SPMNs conjugation with antibodies

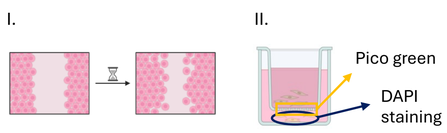


2. Remote activation of Hippo ON state signaling pathway

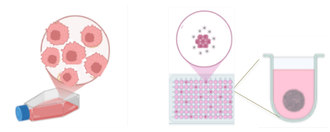


Magnetic stimulation with MICA bioreactor (1Hz, 350mT) vs non-stimulated conditions

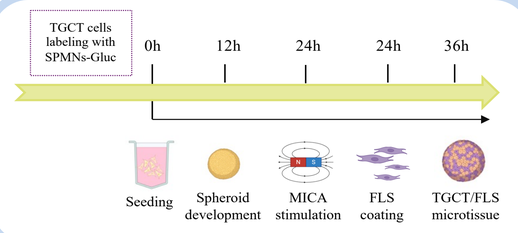
3. Assessment of the Tumoral migration/proliferation



4. Optimization of 3D model and characterization



5. Proof-of-concept of SPMNs-based therapy in microtissues



RESULTS

SPMNs Functionalization

Particles	Zeta Potential (mV)	Size (nm)
SPMNs	-12,45 ± 3,15	279,2
SPMNs-Glucagon	-11,02 ± 2,12	486,5
SPMNs-Adrenergic	-9,26 ± 3,01	297
SPMNs activated	-11,18 ± 2,89	279,7

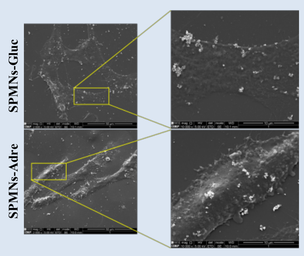
DLS assay

Particles charge and size increased upon carbodiimide conjugation with antibodies

Samples	Supernatant protein concentration (µg/mL)		Efficiency (% protein on MNPs)
	pre coating	post coating	
SPMNs-Glucagon	249,53	221,11	88,23
SPMNs-Adrenergic	97,57	50,05	85,24

BCA protein assay

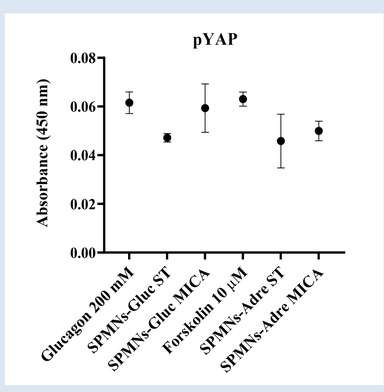
BCA technique revealed higher conjugation efficiency in the Gluc condition



SEM analysis

Successful cell membrane target of SPMNs

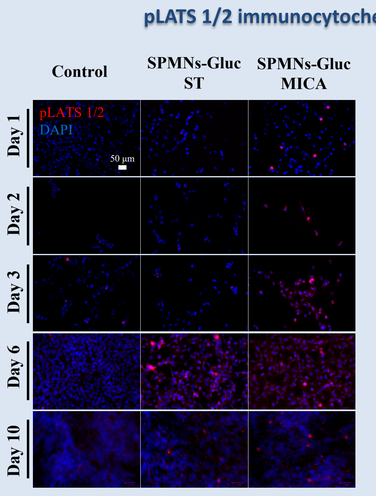
ELISA assay



SPMNs-Gluc induces YAP phosphorylation

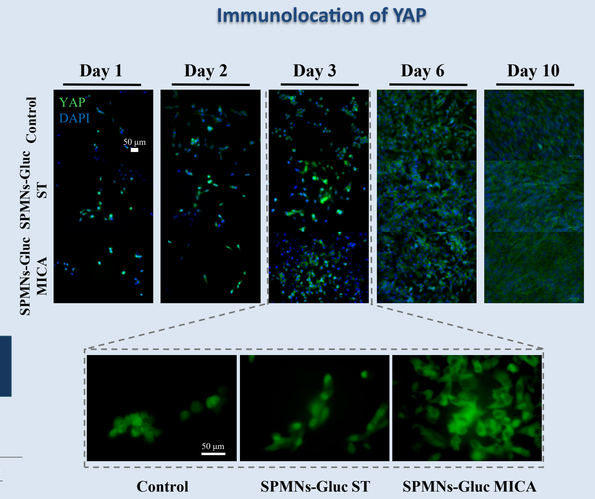
Assessment of Hippo core kinases

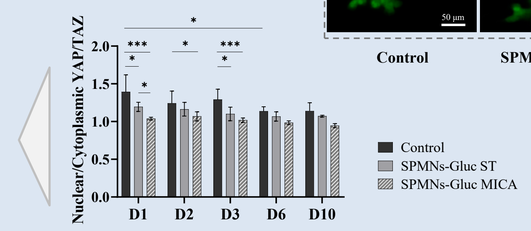
pLATS 1/2 immunocytochemistry



Increase on pLATS within the Gluc-MICA condition

Immunolocation of YAP

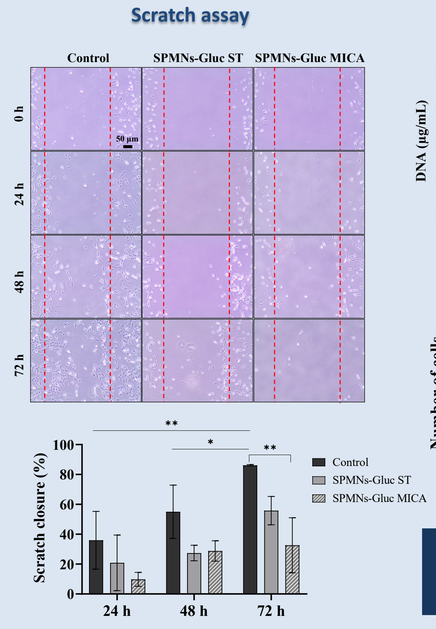




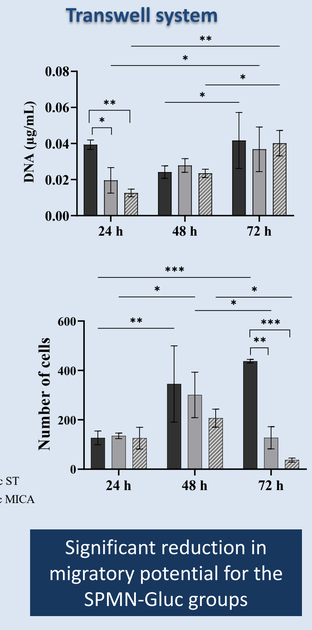
Nuclear-cytoplasmic shuttling of YAP in the SPMNs-Gluc MICA condition

Tumoral migration upon Hippo ON state remote activation

Scratch assay

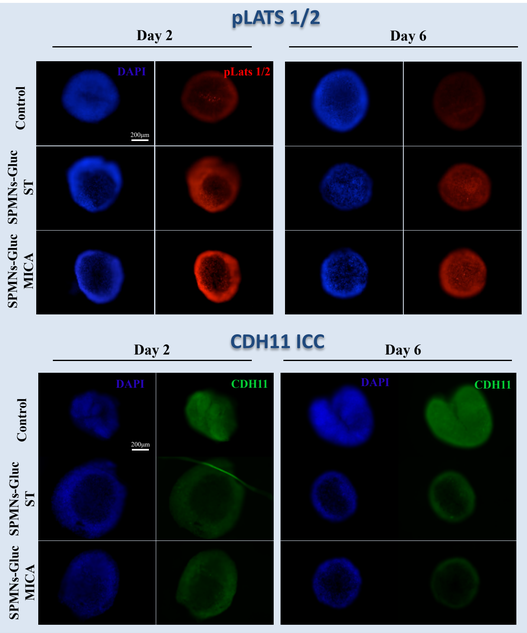


Transwell system



Significant reduction in migratory potential for the SPMN-Gluc groups

Proof-of-concept of the proposed therapy in TGCT engineered microtissues



Retention of YAP in the cytoplasm seems to be stimulated by phosphorylation of pLATS1/2

Activation of Hippo pathway can exert an inhibitory effect in TGCT proliferative behavior

CONCLUSION

Targeting Gαs-coupled receptors signaling in TGCT-like cells labeled with SPMNs-Glucagon and exposed to a controlled magnetic field, activates the glucagon receptor, suggesting restoration of the Hippo ON state, confirmed by the majority of cells showing cytoplasmatic staining of YAP/TAZ and LATS. The developed 3D microtissues developed to better mimic the TME, supported the conclusion obtained from 2D cultures, namely that stimulation of SPMNs-Glucagon activates a LATS kinase-dependent Hippo signaling cascade, and thereby LATS entails modulation of nuclear-cytoplasmic shuttling of YAP. This approach restrains the proliferative tumorigenic cell phenotype, offering a promising remote-controlled therapeutic avenue for TGCT.

References: [1] A. C. Chandler, et al., *Journal of Orthopaedic Research®* 2022, *40* (8), 1918-1925;
[2] M. Santucci, et al., *J Med Chem* 2015, *58* (12), 4857-73;
[3] A. I. Gonçalves, et al., *Nanomedicine: NBM* 2018, *14* (4), 1149-1159.

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