DEVELOPMENT OF AN INJECTABLE AND ALIGNED GELLAN GUM-BASED HYDROGEL FOR REGENERATIVE MEDICINE.

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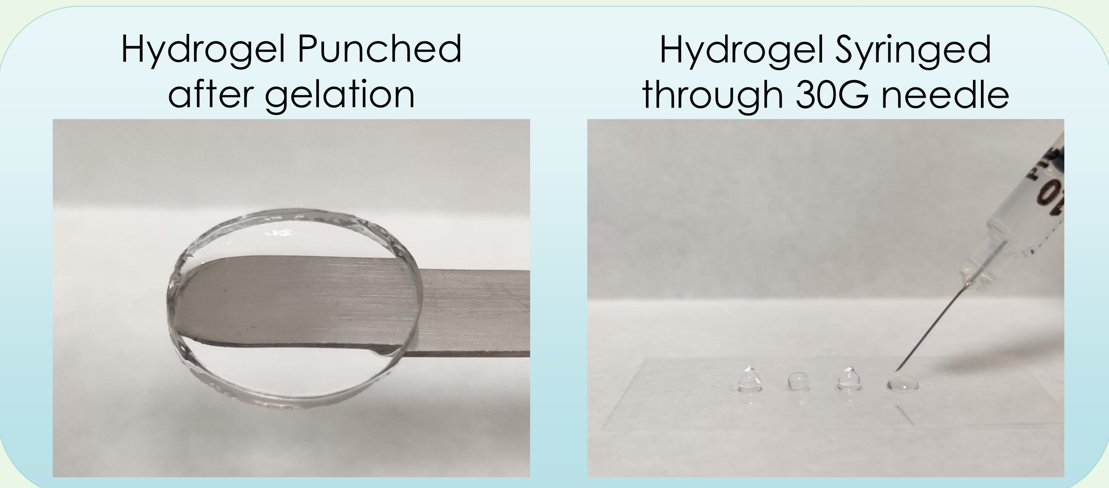
INTRODUCTION: Controlled cellular alignment plays a crucial role in the microarchitecture of many human tissues dictating their biological and mechanical function[1]. Nowadays a multitude of techniques have been described to control cellular alignment, but the resulting 3D aligned scaffolds usually need invasive surgery to be implanted. Among the biomaterials, injectable hydrogels could be transplanted via minimally invasive injection, but without displaying any aligned structure. In the present work, we develop a novel tunable and injectable system starting from gellan gum (GG), a biocompatible bacteria-derived polysaccharide[2], that can achieve an oriented architecture upon a magnetic field application thanks to the presence of magnetized collagen fibers embedded into the GG matrix.

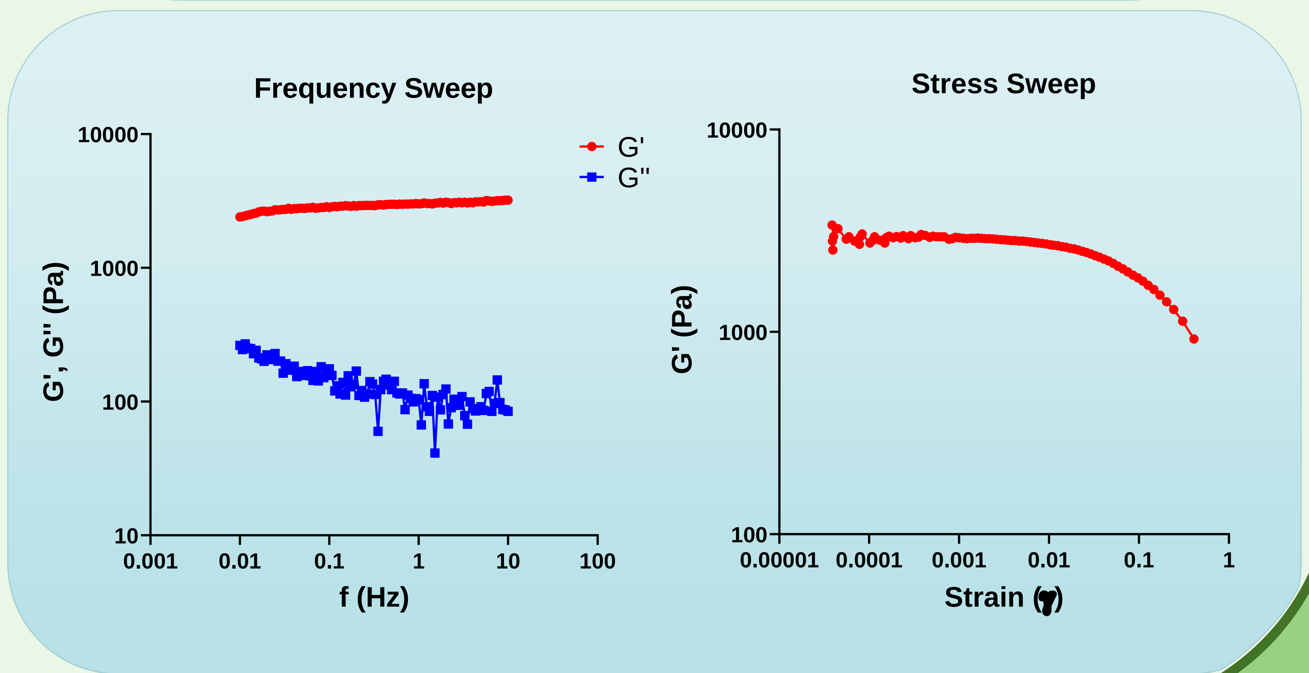
EXPERIMENTAL: GG-based hydrogels were fabricated using bivalent ions, normally present in Phosphate Buffered Saline and cell culture media by dropping the system through a 30G needle. The resulting system was implemented with hyaluronic acid (HA) and carboxymethyl-chitosan (CMC) (weight ratio between polymers and GG equal to 3:10 and 6:10). The resulting samples were characterized using rheology and the stability of hydrogels were investigated up to 21 days. Sodium citrate was added to super paramagnetic iron oxide nanoparticles (SPIONs) to improve their stability, then mixed with collagen type I (Coll) to obtain magnetic fibers (weight ratio between SPIONs and Coll equal to 1:1). This system was added to the hydrogel system to obtain an oriented architecture. The hydrogel biocompatibility was preliminarily screened on fibroblasts (BALB-3T3) by analyzing cell viability and cell morphology up to 7 days.

Hydrogel Characterization

GG-based hydrogel gelation occurs within minutes when dropped by 30G needle into cations containing solutions.

No changing in hydrogels shape and weight were detected up to 21 days. Rheological measurements confirmed that we obtained a gel endowed with a good resistance to deformation.

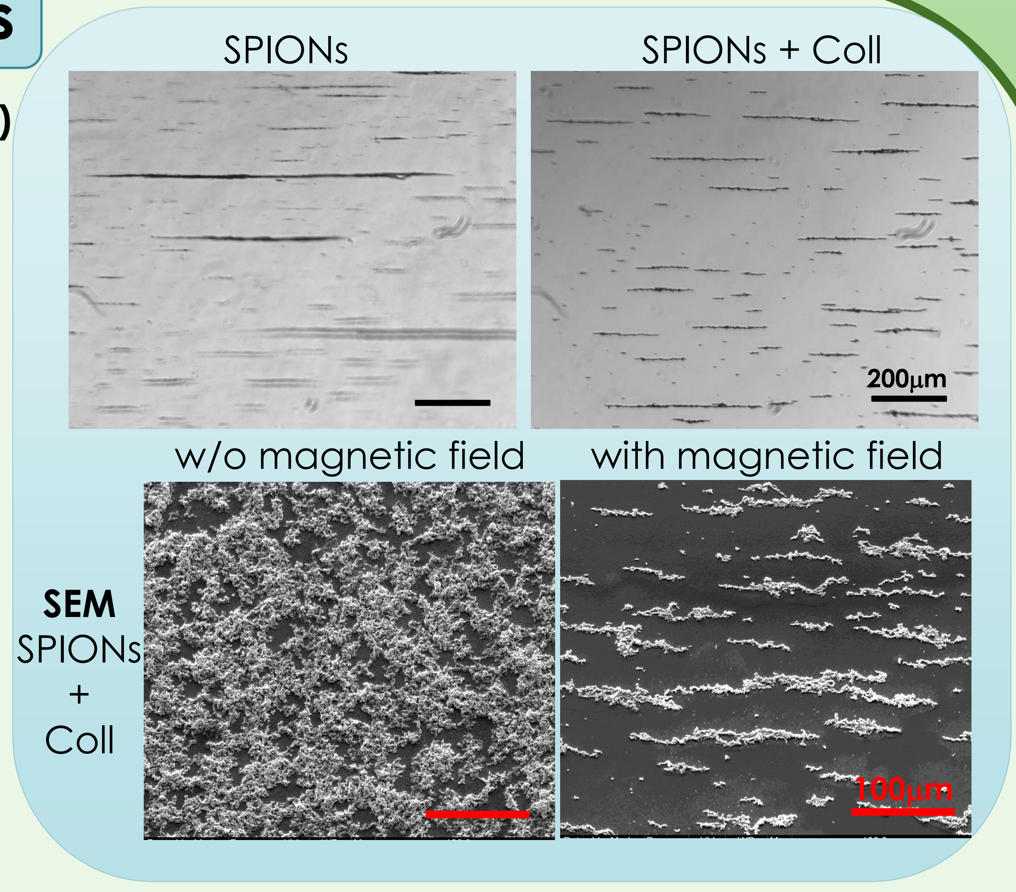




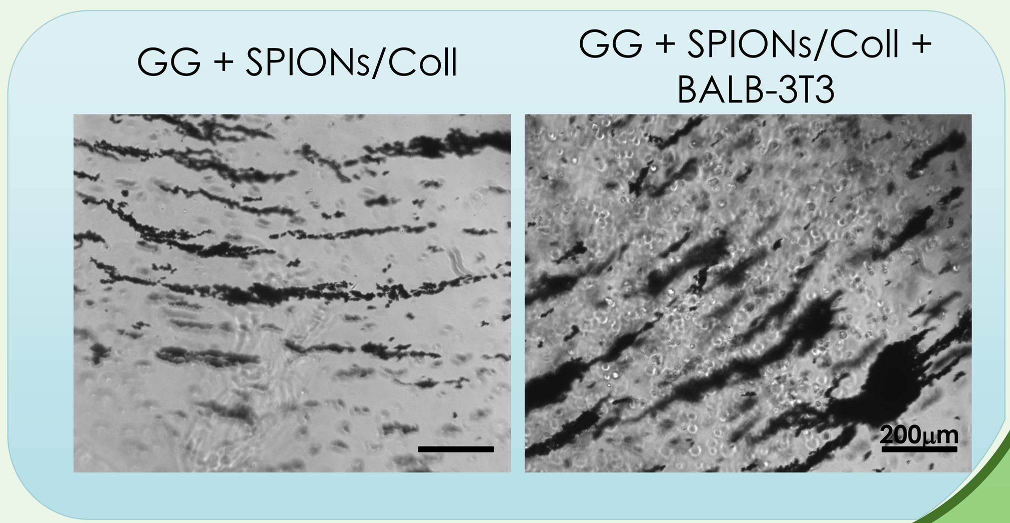
Magnetic Collagen Fibers

SPIONs coupled to collagen type I were able to form magnetic fibers easily oriented by applying a magnetic field (400mT) at a distance of ≈ 2 cm both in water solution (Fig. A) and when encapsulated into the GG-based gel (Fig. B) where fibers showed good stability after magnetic field interruption.

(A)



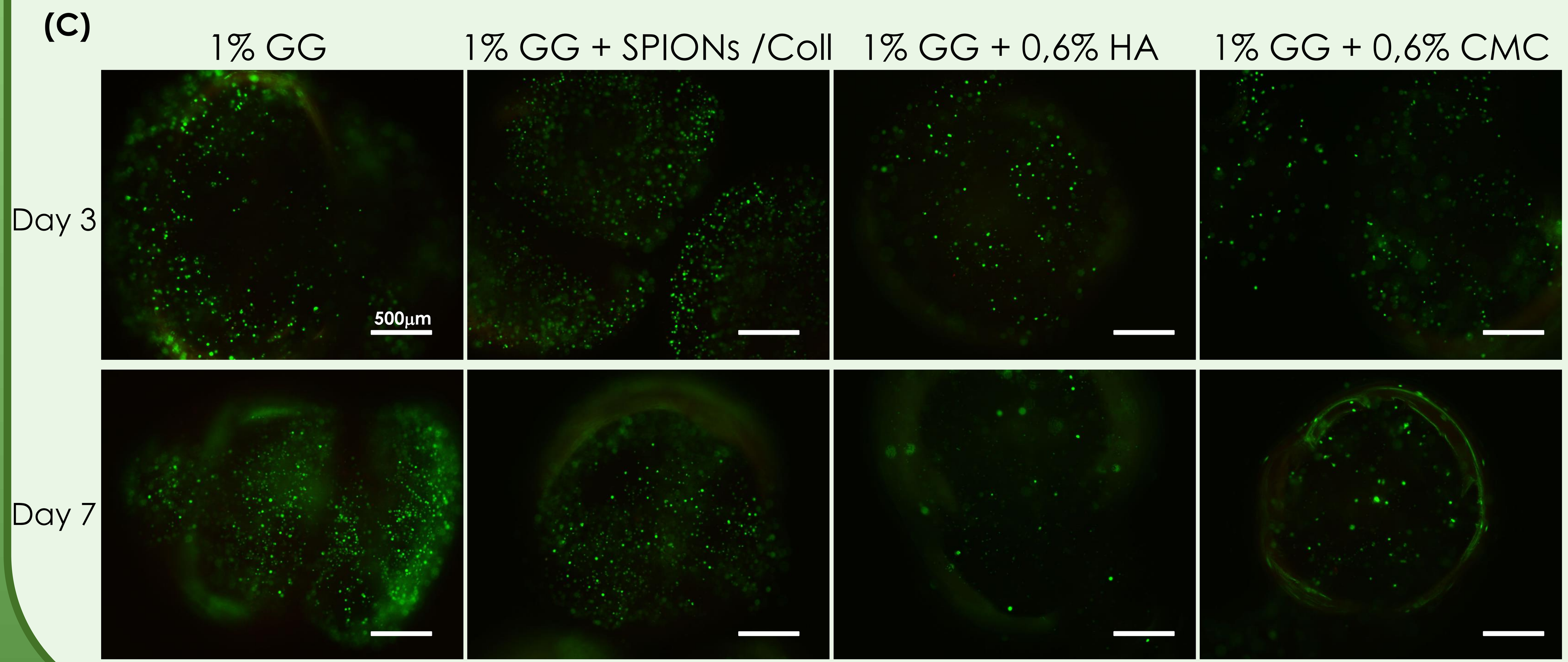
(B)



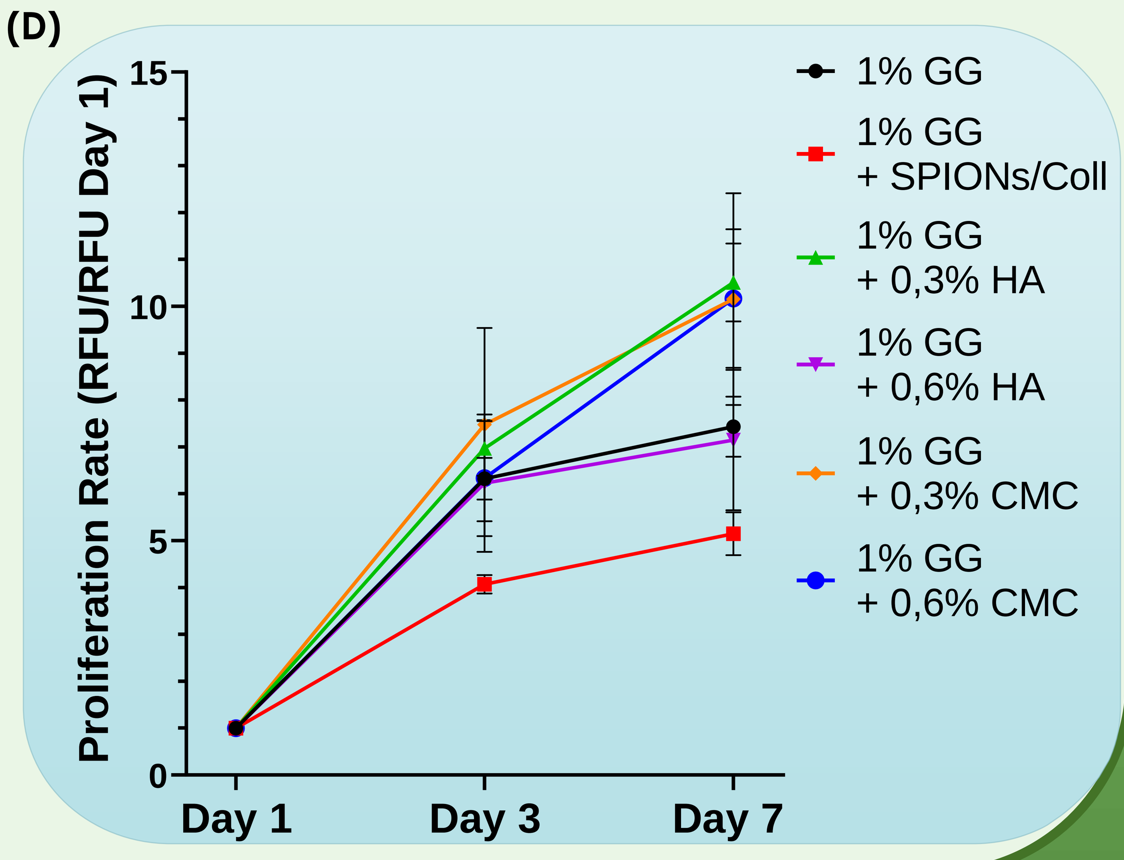
Cell Viability and Proliferation Evaluation

Hydrogels exhibited good biocompatibility without affecting cell viability, morphology (live/dead assay, Fig. C) and cell proliferation (presto blue assay, FIG. D) of fibroblasts.

(C)



(D)



MINISTERO DELLA DIFESA

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PON RICERCA E INNOVAZIONE

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CONCLUSIONS: In the current work, we developed a fully bioresorbable and biocompatible tunable system for regenerative medicine. This system can be injected in situ and fine-tuned remotely to achieve an aligned architecture by a simple external magnetic field application. It displays good biocompatibility with fibroblasts and is a promising system that could be implemented by adding bioactive compounds.

REFERENCES:
1. Rose J.C., et al., Hierarchical Design of Tissue Regenerative Constructs; Adv Healthc Mater, 2018; 7(6):e1701067.
2. Das M. et al., Hydrogels based on gellan gum in cell delivery and drug delivery; J. of Drug Deliv. Sci. and Tech, 2020; 56,A101586.