Gellan gum-based Spongy-like Hydrogels depict improved Cellular Performance

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Statement of Purpose: Gellan gum (GG) hydrogels can be formed by the addition of ions or by temperature decrease with a sol-gel transition. These mild processing conditions are particularly attractive to encapsulate cells that, in addition to the hydrogels particular properties shared by GG hydrogels, support their exploitation for tissue engineering applications [1]. Despite their attractiveness in physically resembling cells original microenvironments, hydrogels are highly hydrophilic matrices. Bounding of water molecules to the polymer backbone along with their anionic character contribute to repulse the adhesion of negatively charged cells [2] and to the lack of cell adhesion sites in hydrogels, unless cell adhesive features are inserted within the polymer backbone [3]. The complexity and the costs of processing hydrogels with biofunctionalities maintain the trail for innovative and straightforward methodologies to attain hydrogels with cell adhesive features. Thus, this work proposes a methodology to obtain GG-based structures that, upon hydration and in comparison to original hydrogels, depict physical features of both sponges and hydrogels, with improved physical stability and mechanical performance, and more importantly, cell adhesive character.

Methods: Different GG-based hydrogels formulations (Table 1) were prepared as previously described [1], with some modifications. Hydrogels were frozen to form salt and water crystals, and freeze-dried to obtain the dried polymeric networks. Spongy-like hydrogels were formed by hydration of the dried polymeric networks by dropwise addition of PBS or a cellular suspension prepared in adequate culture medium. The morphology of the precursor hydrogels and spongy-like hydrogels was analyzed by cryo-scanning electron microscopy (cryo-SEM) and the microstructure of the dried polymeric networks was characterized by micro-computed tomography (μ-CT) and SEM. Hydrogels and spongy-like hydrogels were characterized regarding their water content, mechanical strength, as well as, degradation rate in PBS, water and in the presence of hyaluronidase. Adipose stem cells (hASCs), keratinocytes (hKCs) and umbilical cord vein endothelial cells (HUVECS) were respectively isolated from lipoaspirates, skin samples and umbilical cord, all from human origin, following standard procedures. SaoS-2 osteoblastic cell line was purchased from ECCC. After 3, 7 and 14 days, cell adhesion was evaluated after phalloidin/DAPI staining and cell viability by calcein/PI. Cell seeding efficiency and cell proliferation was also determined by DNA quantification.

Results: The pore diameter was higher for the spongy-like hydrogels relatively to the hydrogels, 200-400 μm in contrast to 20 μm, as observed by cryo-SEM. Likewise, the mechanical strength was higher for the spongy-like hydrogels. These parameters were tuned not only by varying the processing conditions but also by changing polymer(s) concentration (Table I).

Table I – Microstructure and mechanical strength analysis by μ-CT and compressive stress, respectively.

	Porosity (%)	Pore size (μm)	Compressive modulus (KPa)		
	DPN	DPN	Hyd	DPN	SlH
GG 0.75 %	85 ± 6	325 ± 145	9 ± 2	210 ± 31	11. ± 5
GG 1.25 %	83 ± 6	268 ± 112	7 ± 4	1278 ± 439	20 ± 11
GG-HA 1 %	82 ± 9	252 ± 146	11 ± 3	494 ± 294	77 ± 38
GG-HA 2 %	80 ± 6	185 ± 90	23 ± 10	225 ± 52	34 ± 21

Dried polymeric networks (DPN), Hydrogel (Hyd), Spongy-like hydrogel (SlH), Hyaluronate (HA). Results are presented as mean ± S.D. (n>7).

After deformation, spongy-like hydrogels were able to completely recover their shape within 15 min, in contrast to hydrogels which break upon small deformations. GG-based dried polymeric networks were able to uptake from 1700 to 3100% of water within 15 minutes, forming the spongy-like hydrogels, reaching the maximum and a plateau after 3 days. Nevertheless, these values were lower than the ones observed for hydrogels. The degradation rate of the spongy-like hydrogels were also tuned; the incorporation of HA lead to a 40% increase of mass loss within 28 days, *in vitro*, especially in the presence of hyaluronidase. When incorporating cells, both hydrogels and spongy-like hydrogels were able to sustain cells viability up to 14 days of culture. However, independently of the phenotype, cells only adhered and exhibited their typical morphology in the spongy-like hydrogels. Moreover, cells were also able to proliferate within the 3D spongy-like hydrogels matrix (Fig.1).

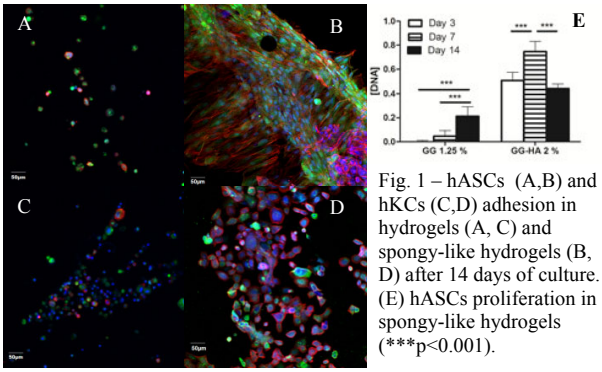

Fig. 1 – hASCs (A,B) and hKCs (C,D) adhesion in hydrogels (A, C) and spongy-like hydrogels (B, D) after 14 days of culture. (E) hASCs proliferation in spongy-like hydrogels (***p<0.001).

Conclusions: An innovative, low cost and simple processing methodology to obtain GG-based spongy-like hydrogels that retain hydrogels significant water content but overcome their limited mechanical performance and their incapacity to sustain cell adhesion is herein presented. Most interestingly, the off-the-shelf availability and stability of dried polymeric networks, precursors of the spongy-like hydrogels, constitute an advantage for its clinical application simply after hydration.

References: 1.Oliveira, JT. et al. (2010). J Biomed Mater Res A.; 2. **93**(3): p. 852-63. 2.Chang, HI. et al. (2011) Regenerative Medicine and Tissue Engineering - Cells and Biomaterials; 3.Silva, NA. et al. (2012) Biomaterials. **33**(27): p. 6345-54.