Electronic Supporting Information (ESI)

Evaluation of RGD functionalization in hybrid hydrogels as 3D neural stem cell culture systems

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FT-IR spectrum of PAA-RGD. Figure S1 shows FT-IR spectra of RGD tripeptide and PAA functionalized RGD. In polymer spectra, RGD amide I and II signals are recognizable at 1670 cm^{-1} and 1530 cm^{-1} and triazole bond presents peak at 1445 cm^{-1}: this confirms the correct PAA functionalization.

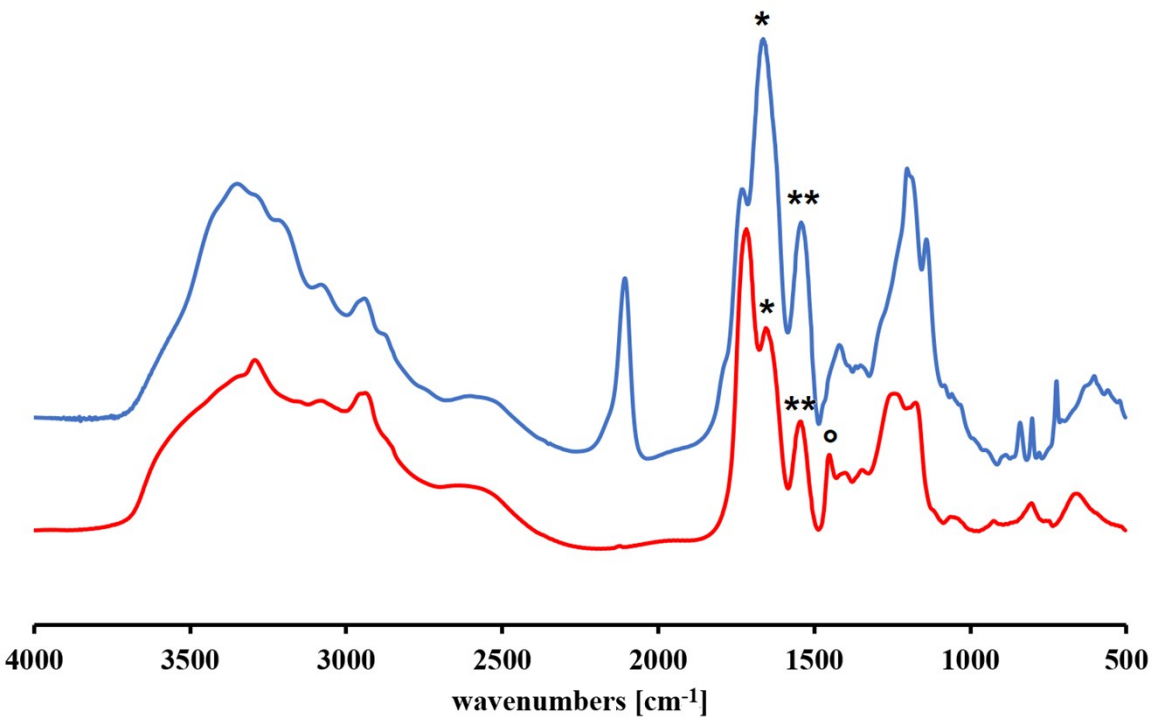

Figure S1. FT-IR spectra of RGD (blue) and PAA modified with tripeptide (red). Signals of RGD amide I (*) and amide II (**) are detectable in both spectra. In polymer IR spectrum, triazole signal (°) is highlighted.

Propidium iodide staining in *in vitro* NSC samples. Figure S2 shows the percentage trend of dead cells in laminin, HG and HG-RGD samples, over time. Percentage of PI labelled NSCs was increasing faster in laminin sample than in hydrogels systems.

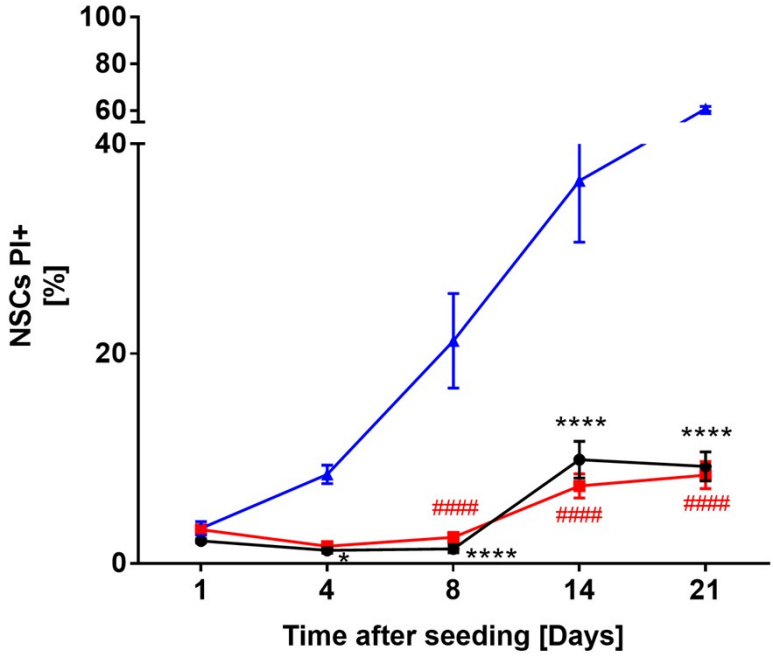

Figure S2. Graph representing the PI stained NSCs in laminin (Δ, blue), HG (⭘, red) and HG-RGD (ᘓ, black) samples, over time. Statistical analysis: two-way ANOVA followed by Tukey's post hoc test. Mean ± SEM are reported; n = 6 per group. (*) $p < 0.05$ *vs* laminin, (****) $p < 0.0001$ *vs* laminin; (####) $p < 0.0001$ *vs* laminin.

Hydrogel biocompatibility. No-toxic effects inherent in the produced scaffolds HG and HG-RGD were tested using LDH assay. HG and HG-RGD samples, whitout cell seeding, were submerged in NPCs growth medium and incubated at 37°C, until 1, 4, 8, 14 and 21 days. Then, the obtained conditioned media were used as growth medium for NSC cultures. LDH release (Figure S3) showed that synthetized biomaterials did not influence cell behavior and the proliferating phase: reference sample (CTRL), constituted by the standard growth NSCs medium, presented the same percentage of LDH release of the samples treated with the HG or HG-RGD conditioned medium.

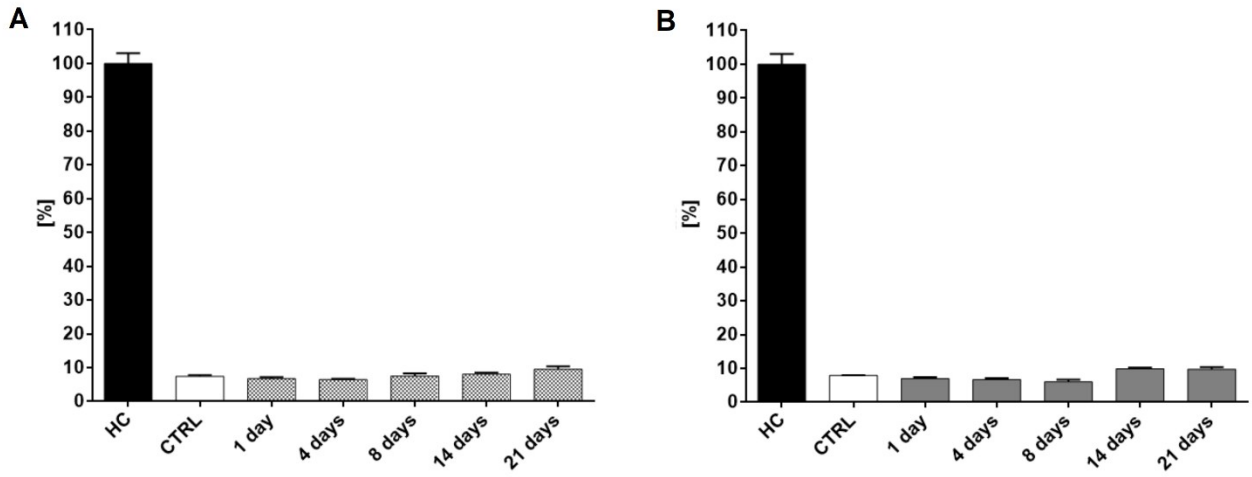

Figure S3. LDH assay for NSCs treated with hydrogel HG (A) and HG-RGD (B) conditioned medium, at different time points. HC represents high LDH release control performed with NSCs treated with 10% Triton X-100; CTRL is LDH release of NSCs in standard growth medium.