Supplementary materials

Sponge-like scaffolds for colorectal cancer 3D models: substrate driven difference in micro tumors morphology

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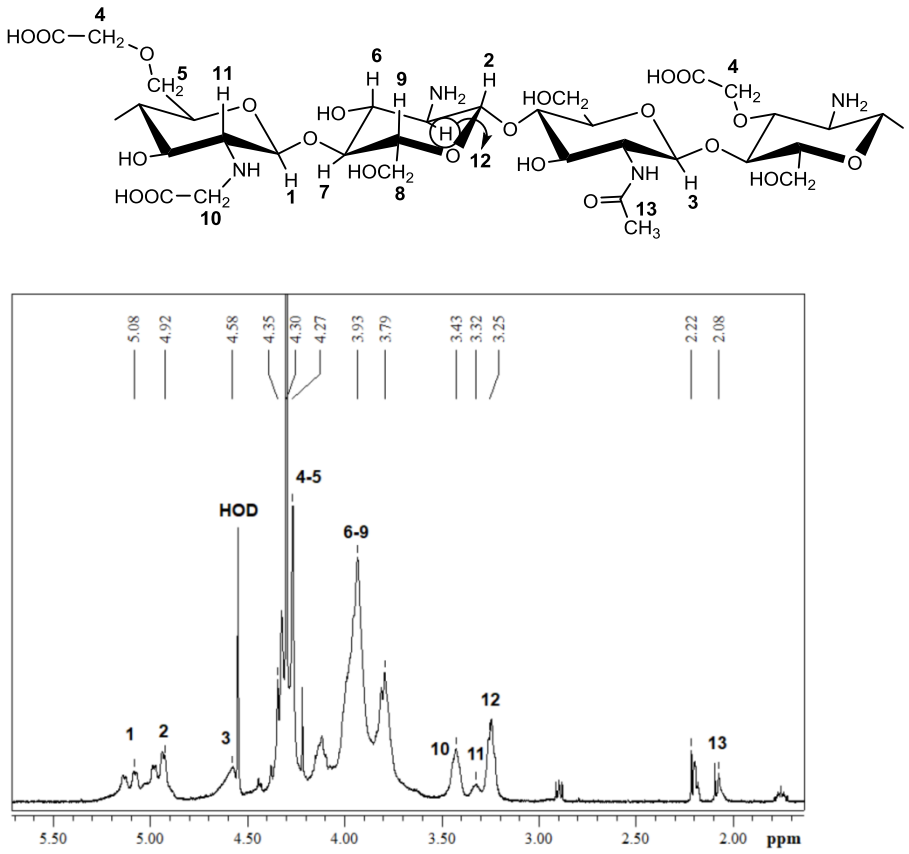


Figure S1. 400 MHz ^{1}H NMR spectra of N,O-(carboxymethyl)chitosan

Table S1. Monomer composition of carboxymethyl chitosan (CMC)

DA[1]	DS_{tot}[2]	N-DS[3]	O-DS[4]	Monomer composition			
				NH_2	NHR	NR_2	$NHCOCH_3$
0.25	1.49	0.29	1.20	0.46	0.29	0	0.25

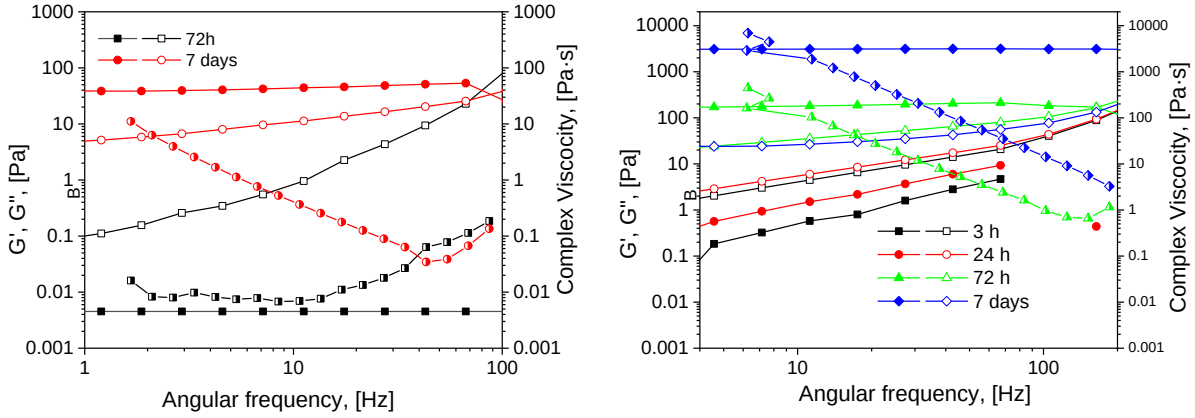
[1] - Degree of acetylation, [2] - Degree of carboxyalkyl substitution (total), [3] - Degree of N-carboxyalkyl substitution, [4] - Degree of O-carboxyalkyl substitution

Table S2. Calculations of reagents quantities for chitosan cryogels fabrication

Chitosan molecular weight - 163 g/mol

BDDGE molecular weight - 202 g/mol

BDDGE:chitosan molar ratio	3% chitosan solution, g	60% BDDGE solution, g
2:1	5	0.62
1:1	5	0.31
1:2	5	0.155
1:4	5	0.0775



(**a**) (**b**)

Figure S2. Evolution of mechanical spectra of 3% CMC (a) and chitosan (b) solutions after addition of cross-linker BDDGE: filled symbols – storage modulus (G'), open symbols – loss modulus (G''), half-filled – complex viscosity storage modulus (G'), open symbols – loss modulus (G''), half-filled – complex viscosity

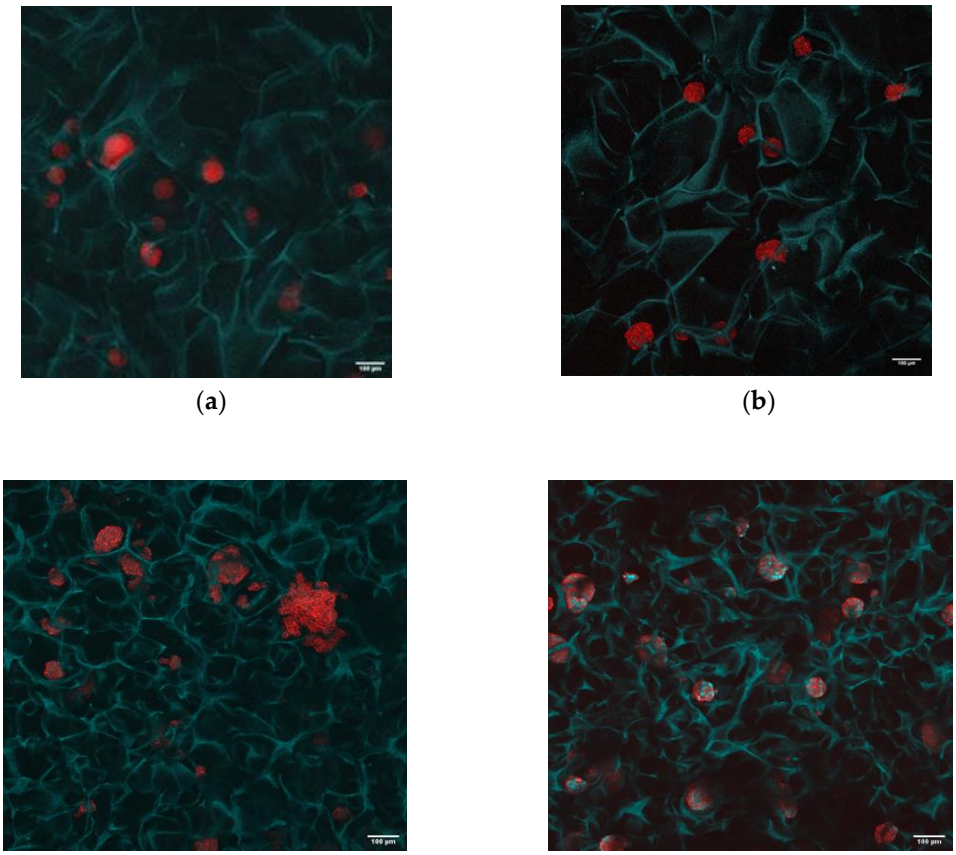


Figure S3. Confocal laser scanning microscopy (CLSM) images of CMC- cryogels with HCT 116 cells after 7 days (a), and 14 days (b) of cultivation (BDDGE:polymer ratio of 1:2) and after 3 days (c), and 7 days (d) of cultivation (BDDGE:polymer ratio of 2:1), scale bar – 100 μm.