Electronic Supplementary Information for

Sustained drug release and cancer treatment by an injectable and biodegradable cyanoacrylate-based local drug delivery system

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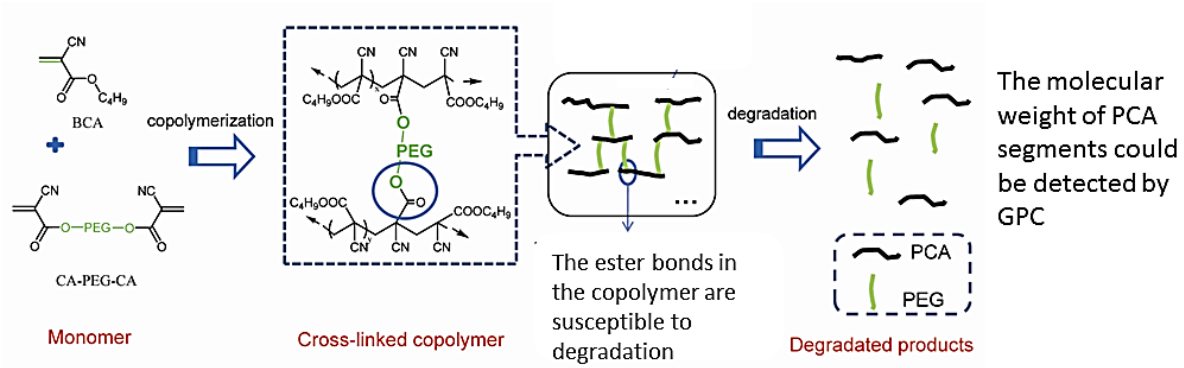


Fig. S1 Graphical scheme of the biodegradation mechanism of the cross-linked CA copolymer.

As had been proved and discussed in our former work, [29] poly(ethylene glycol)-di(cyanoacrylate) (CA-PEG-CA) was designed as the cross-linking agent to combine with BCA. The cross-linked copolymer would be degraded into linear or branched segments of PEG and poly-cyanoacrylate (PCA) chains at the ester sites in the main chains. Although the molecular weight of the copolymer could not be detected in GPC analysis as they were cross-linked copolymer (swelling rather than dissolution), the molecular weight of the degradation products could be detected. It was proved that the molecular weight of degraded PCA segments was related to the composition of BCA/CA-PEG-CA (A preferred formulation LKJ11 showed that all the degraded segments were less than 4 kDa). Their chemical structure had been proved by ^{1}H-NMR and MODI-TOF. Besides, it was also proved that the degraded PCA segments could be directly excreted via the renal filtration process once their molecular weights were small enough, which meant that this degradation and excretion mechanism had little concern with the breaking down of C-C bonds in the PCA chains under biological conditions.

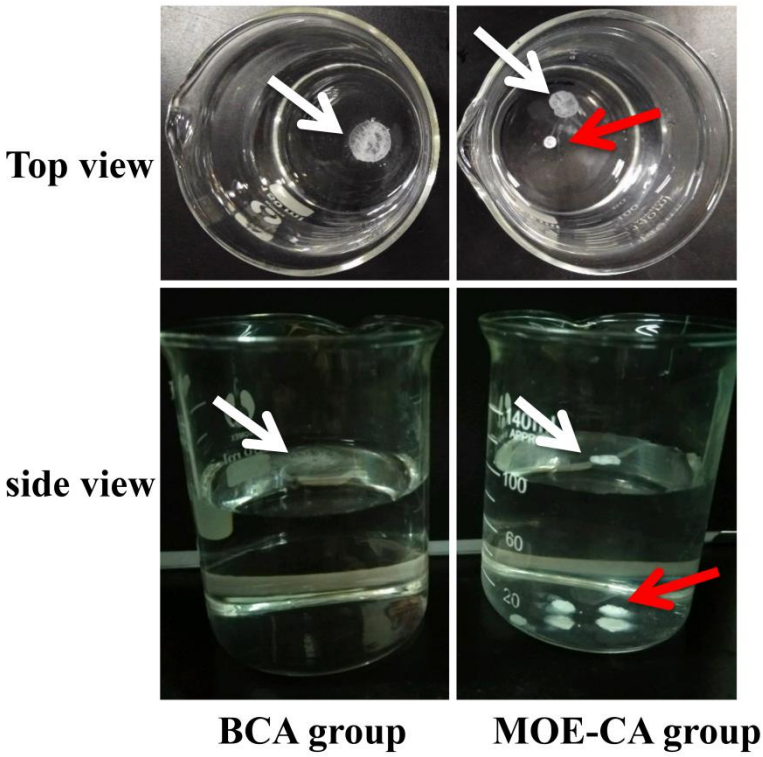


Fig. S2 Film formation test results for the BCA group (left) and the MOE-CA group (right).The white arrow indicates the hydrophobic film. The red arrow indicates the solid glue block sinking at the bottom.

These results indicated that the BCA monomers could polymerize into a polymer film that could float on the solution surface. Meanwhile, only a portion of the MOE-CA monomers formed a floating film, and the others sank to the bottom as a solid glue block. This finding implied the higher hydrophobicity of BCA compared to MOE-CA, as the BCA monomer could spread sufficiently on the water surface for polymerization into a film.

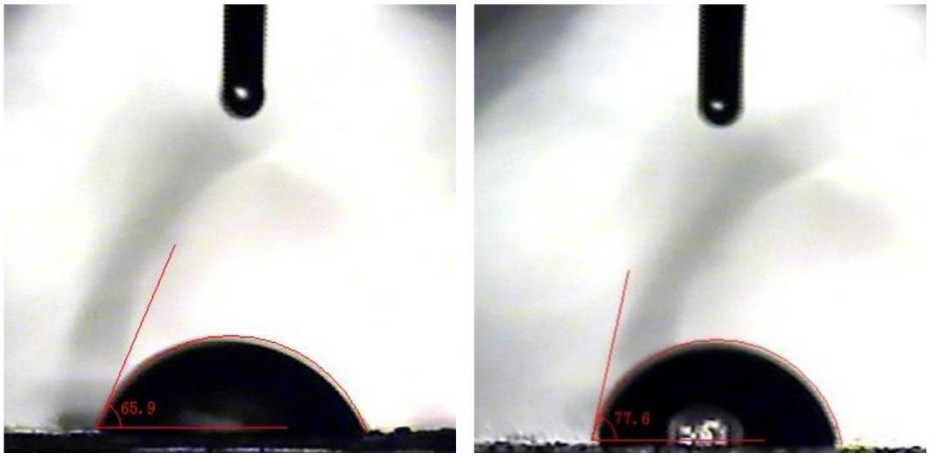


Fig. S3 Contact angle test of poly-MOE-CA (left) and poly-BCA (right).

The membrane of poly-MOE-CA had a lower contact angle (65.2 ± 2.60°) than that of poly-BCA (79.1 ± 4.50°), indicating that MOE-CA is more hydrophilic than BCA.

Table. S1 Shear tensile strength of the CA formulations

Group	Chemical composition	Weight ratio (wt%)	Molar ratio	Shear tensile strength (kPa) [a)]
A	MOE-CA/CA-TEG-CA	85.9/14.1	13.9/1	390 ± 45.8
B	MOE-CA/CA-TEG-CA	70/30	5.3/1	342 ± 37.3
C	MOE-CA/CA-TEG-CA	50/50	2.3/1	300 ± 50.5
D	BCA/CA-TEG-CA	85.9/14.1	13.9/1	466 ± 78.1
E	MOE-CA/CA-PEG600-CA	90/10	44/1	533 ± 117
F	MOE-CA/CA-PEG600-CA	73.9/26.1	13.9/1	508 ± 137
G	MOE-CA/CA-PEG600-CA	50/50	4.9/1	420 ± 114
H	BCA/CA-PEG600-CA	73.9/26.1	13.9/1	504 ± 66.5
I	MOE-CA/CA-PEG2000-CA	90/10	125.3/1	319 ± 46.9
J	MOE-CA/CA-PEG2000-CA	70/30	32.5/1	364 ± 76.0
K	MOE-CA/CA-PEG2000-CA	50/50	13.9/1	118 ± 32.1
L	BCA/CA-PEG2000-CA	50/50	13.9/1	186 ± 78.6
M	MOE-CA			331 ± 36.1
N	BCA			352 ± 31.1
O	CA-TEG-CA			145 ± 43.3
P	CA-PEG600-CA			129 ± 39.3
Q	CA-PEG2000-CA			22.4 ± 6.00

a) Six parallel experiments were performed to derive the means ± SD.

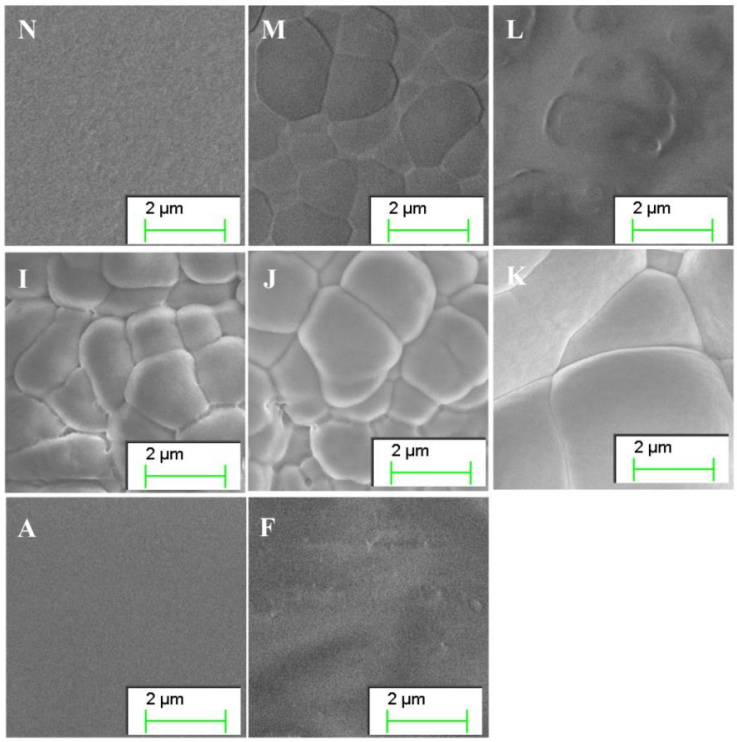


Fig. S4 The SEM test results. N, poly-BCA; M, poly-MOE-CA; L, poly-(BCA/CA-PEG2000-CA) molar ratio=13.9/1; I, poly-(MOE/CA-PEG2000-CA) molar ratio=125.3/1; J, poly-(MOE/CA-PEG2000-CA) molar ratio=32.5/1; K, poly-(MOE/CA-PEG2000-CA) molar ratio=13.9/1; A, poly-(MOE/CA-TEG2000-CA) molar ratio=13.9/1; F, poly-(MOE/CA-PEG600-CA) molar ratio=13.9/1.

The SEM test was carried out to characterize the surface performance of groups A, F, I, J, K, M, and N in **Table 1.** The pure poly-MOE-CA membrane (group M) presented a less smooth and less compact morphology than that of poly-BCA (group N). Increasing the molar ratio of CA-PEG2000-CA showed an increasing uneven morphology with the characteristic of a deep gully in the shell (from groups I to J to K). The same tendency was also observed with increasing the PEG length (from groups A to F to K). These varied results might mainly be attributed to the different polymeric densities and microstructures. The nonhomogenized surface might increase the surface area and provide better accessibility for water diffusion to the ester bonds in the polymer main chains, leading to faster chain scission, which could further explain the better degradation rate of group M than N, group K than J and I, group K than F and A, and also group K than L.

Table. S2 Drug-release percentages of groups M, A, F, and K.

Time (h)	Drug-release percentage (%)			
	group M	group A	group F	group K
	MOE-CA	MOE-CA/CA-TEG-CA	MOE-CA/CA-PEG600-CA	MOE-CA/CA-PEG2000-CA
		13.9/1	13.9/1	13.9/1
0	0	0	0	0
2	1.64 ± 0.24	0.48 ± 0.20	1.38 ± 0.12	86.9 ± 11.1
4	1.96 ± 0.16	0.91 ± 0.20	2.33 ± 0.32	92.6 ± 8.32
6	2.56 ± 0.44	1.12 ± 0.28	2.67 ± 0.28	98.7 ± 4.56
12	4.40 ± 0.20	2.06 ± 0.68	3.65 ± 0.76	99.0 ± 4.67
24	5.52 ± 2.84	3.64 ± 0.48	9.08 ± 0.96	99.9 ±5.43
48	7.68 ± 1.52	5.08 ± 1.04	21.0 ± 8.72	100 ± 3.45
72	10.2 ± 1.20	7.56 ± 0.88	28.4 ± 12.5	
96	11.1 ± 0.36	9.84 ± 1.40	39.9 ± 11.3	
120	12.2 ± 0.40	11.9 ± 1.28	47.7 ± 8.28	
168	13.5 ± 0.16	14.8 ± 2.20	55.2 ± 6.60	
Apparent degradation rate (%)[a)]	26.7 ± 6.00	25.7 ± 3.80	79.5 ± 14.8	90.5 ± 3.54

a) Apparent degradation rate (100%) = $(W_0 – W_1) / W_0$. W_0 is the weight of the polymerized CA formulation before degradation, and W_1 is the weight of the residual glue block after degradation. Data were collected after degradation in PBS buffer (pH 7.4) for 14 days. Six parallel experiments were performed to derive the means ± SD.

Table. S3 Drug-release percentages of groups I, J, and K.

Time (h)	Drug-release percentage (%)		
	group I MOE-CA/CA-PEG2000-CA 125.3/1	group J MOE-CA/CA-PEG2000-CA 32.5/1	group K MOE-CA/CA-PEG2000-CA 13.9/1
0	0	0	0
2		1.75 ± 0.58	86.9 ± 11.1
4		8.57 ± 1.85	92.6 ± 8.32
6		14.4 ± 2.81	98.7 ± 4.56
12	1.43 ± 0.11	33.7 ± 8.99	99.0 ± 4.67
24	3.70 ± 0.67	58.9 ± 9.89	99.9 ±5.43
48	8.11 ± 1.09	89.7 ± 2.36	100 ± 3.45
72	14.2 ± 2.84	94.6 ± 3.14	
96	16.9 ± 0.86	98.6 ± 5.23	
120	19.3 ± 2.34	100 ± 4.83	
168	20.5 ± 4.53		
Apparent degradation rate (%)	17.0 ± 8.48	71.5 ± 4.95	90.5 ± 3.54

Table. S4 Drug-release percentages of groups G and F.

Time (h)	Drug-release percentage (%)	
	group G MOE-CA/CA-PEG600-CA 4.9/1	group F MOE-CA/CA-PEG600-CA 13.9/1
0	0	0
6	17.8 ± 5.96	2.67 ± 0.28
24	33.0 ± 0.68	9.08 ± 0.96
48	59.9 ± 3.90	21.0 ± 8.72
72	72.7 ± 6.79	28.4 ± 12.5
96	81.8 ± 2.62	35.3 ± 4.59
120	86.5 ± 4.38	42.8 ± 2.82
168	90.5 ± 1.06	49.7 ± 3.79
Apparent degradation rate (%)	98.0 ± 3.80	79.5 ± 14.8

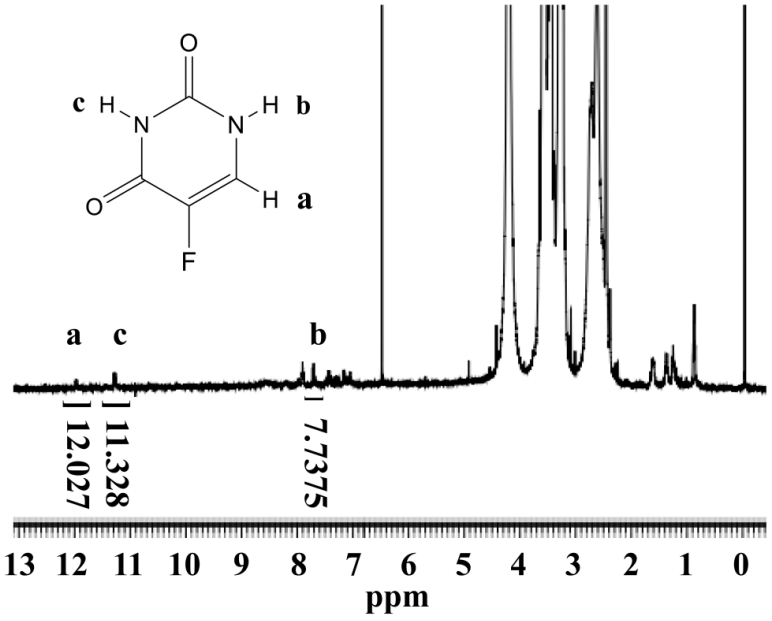


Fig. S5 ^{1}H-NMR of ***J-Fu-1.25*** after polymerization.

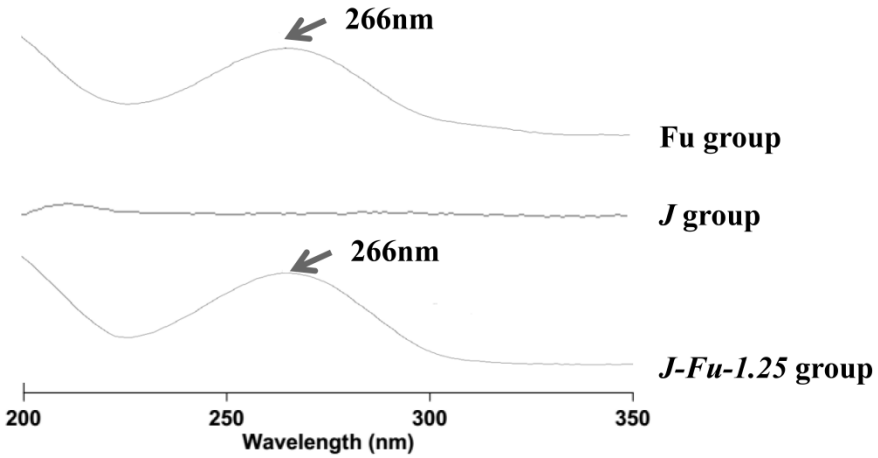


Fig. S6 The UV results of 5-Fu alone and the saline extracts of polymerized ***J*** and ***J-Fu-1.25***.

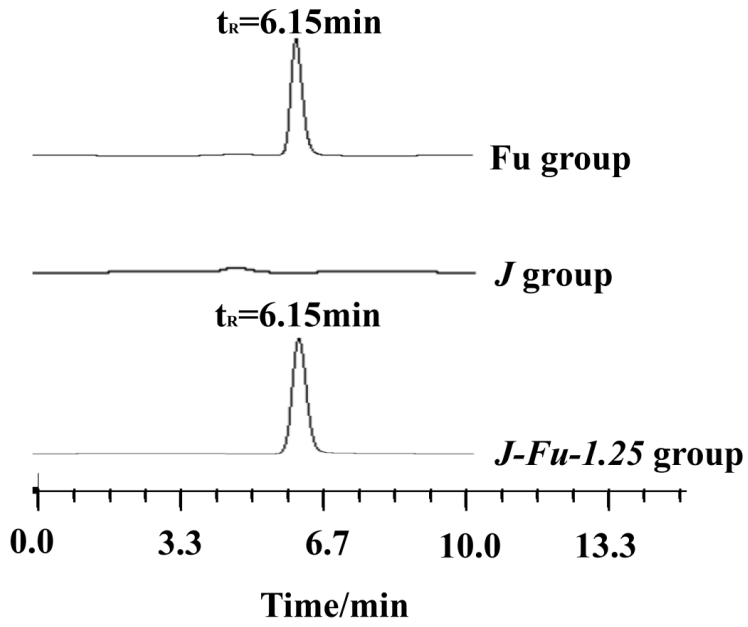


Fig. S7 The HPLC results of 5-Fu alone and the saline extracts of polymerized ***J*** and ***J-Fu-1.25***.

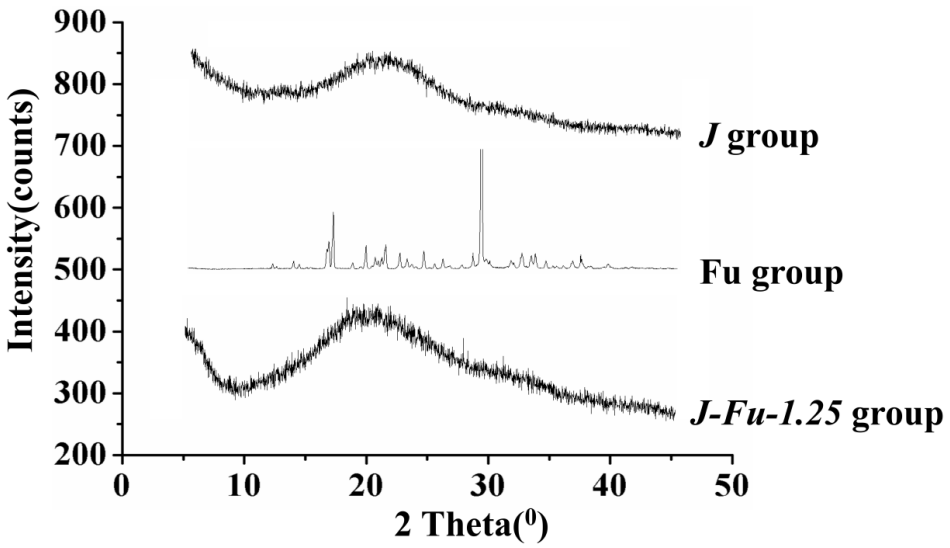


Fig. S8 The XRPD analysis results of 5-Fu alone and polymerized ***J*** and ***J-Fu-1.25***.

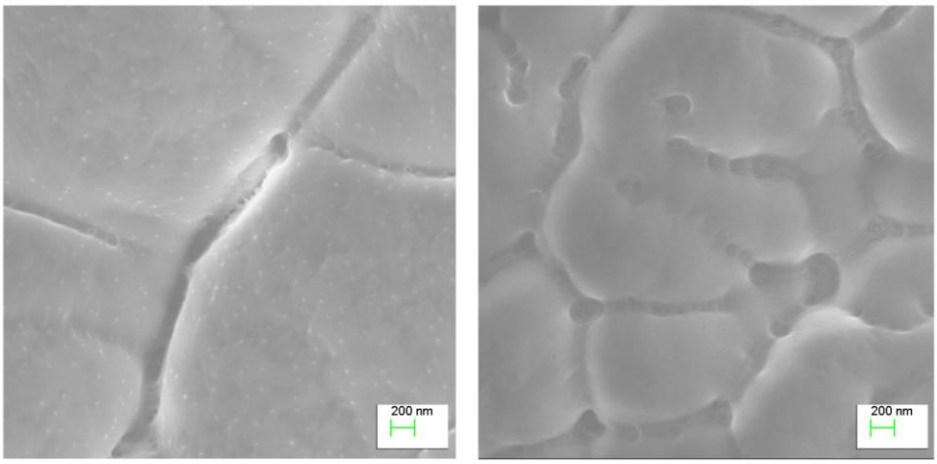


Fig. S9 The SEM test results of polymerized ***J-Fu-1.25*** (left) and ***J*** (right) (5.00KV, 20.00KX).

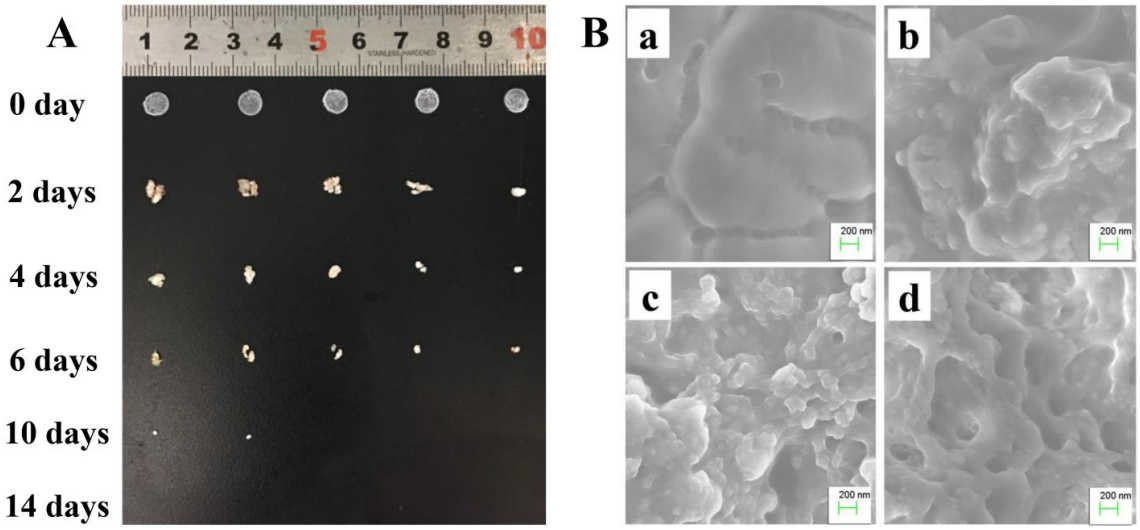


Fig. S10 The *in vivo* biodegradability test results. A) Residue glue. B) SEM test results of polymerized *J-Fu-1.25* during the *in vivo* degradation test. a, before degradation; b to d 2 days, 4 days, and 6 days after degradation, separately (5.00KV, 20.00KX).

In total, 36 female SD mice with an average weight of 220 g were used. A 20-mg sample of the J-Fu-1.25 was polymerized into a solid glue block with a round shape (with a diameter of 60 mm) and was subcutaneously implanted into the backs of the mice. The mice were sampled to observe the degradability of glue residues at 2 days, 4 days, 6 days, 10 days, 14 days, and 30 days post-injection; six mice were sacrificed at each time point. The degradation result of the polymers showed that as time passed, the solid glue block gradually degraded; and after about 10 days, the glue almost disappeared. The SEM results gave further evidence for the degradation and bioerosion course of the copolymer, as time passed, more holes and deeper gullies appeared in the copolymer.

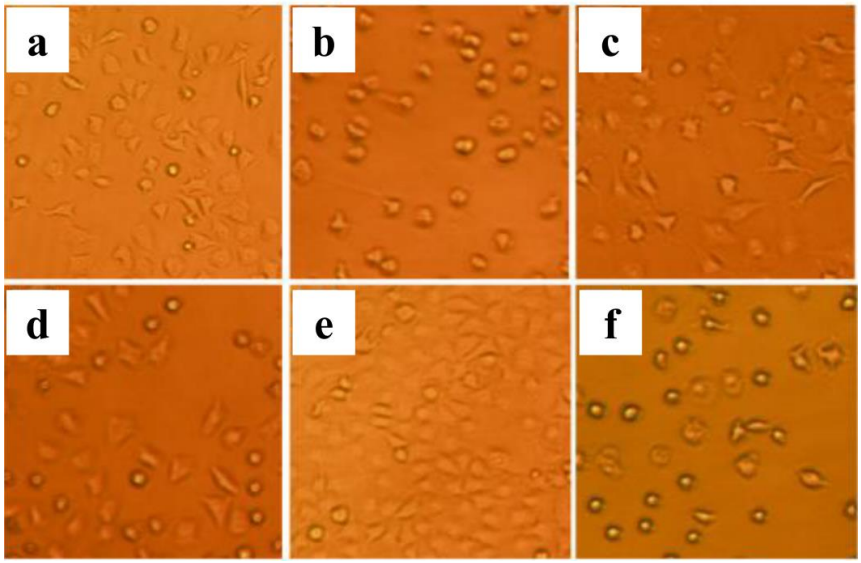


Fig. S11 Cell observation of the *in vitro* anticancer test results under a microscope. A, ***J*** group; B, ***J-Fu-1.25*** group; C, ***J-Fu-0.625*** group; D, ***J-Fu-0.3125*** group; E, Normal group; F, 5-Fu group.

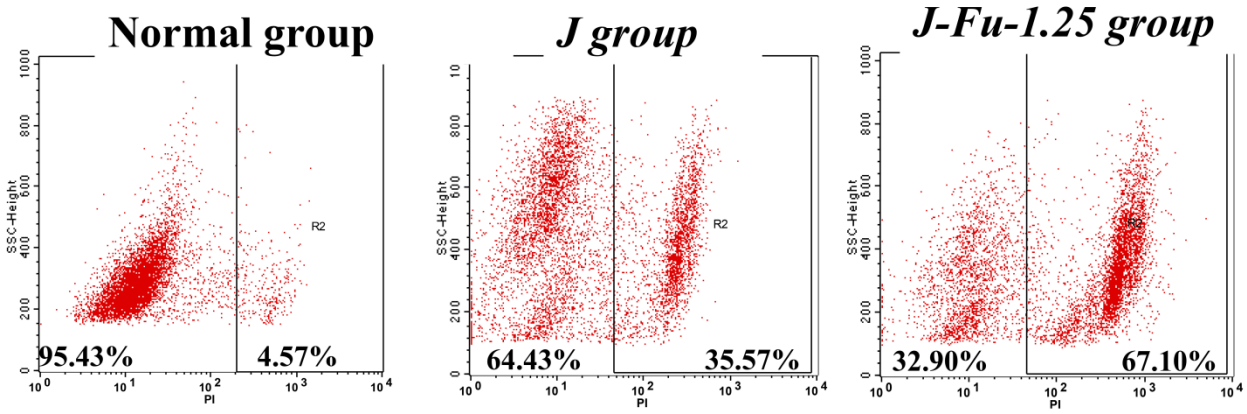


Fig. S12 Flow cytometry assays results.

Fig. S13 Solid glue collected from tumors.

Three sampled tumor were cut open to collect the solid glues 1 minute after injection, and the solid glues were photographed as an evidence of the polymerization.

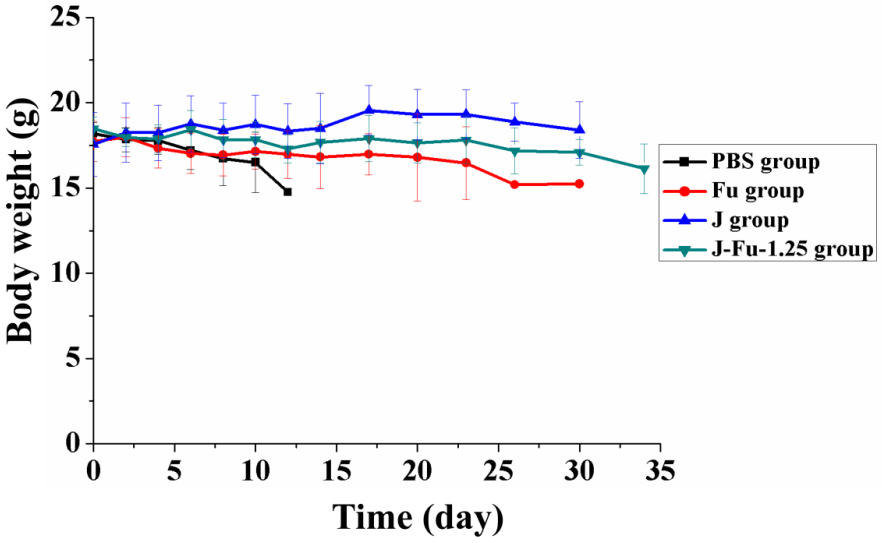


Fig. S14 Mouse weights of the different groups at certain time points; each point is the mean value.

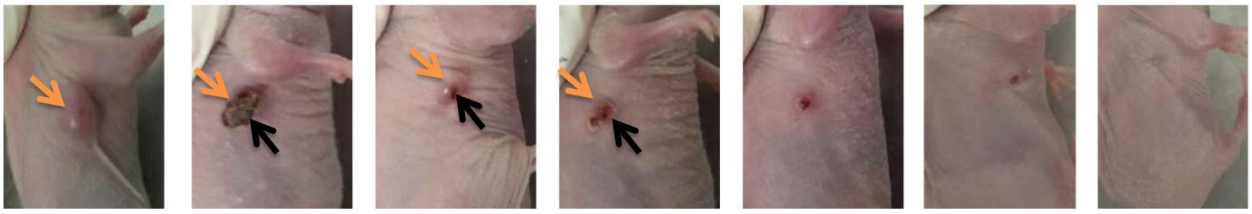


Fig. S15 Photographs of a mouse injected with ***J-Fu-1.25***. The photographs were taken at 0 days, 6 days, 10 days, 14 days, 17 days, 21 days, and 28 days post operation (from left to right). The orange arrow points to the tumor site, and the black arrow indicates necrosis.

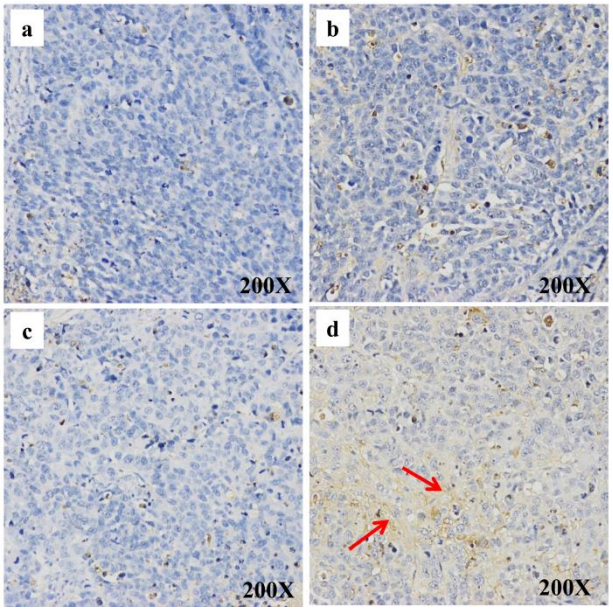


Fig. S16 TUNEL staining of immunohistochemical sections of tumors at 14 days post operation. a, PBS group; b, 1.25% 5-Fu in PBS group; c, J group; d, J-Fu-1.25 group. The images were obtained by a Leica microscope at 400× magnification. The red arrows represent positive expression stained by 3, 3′-diaminobenzidine.

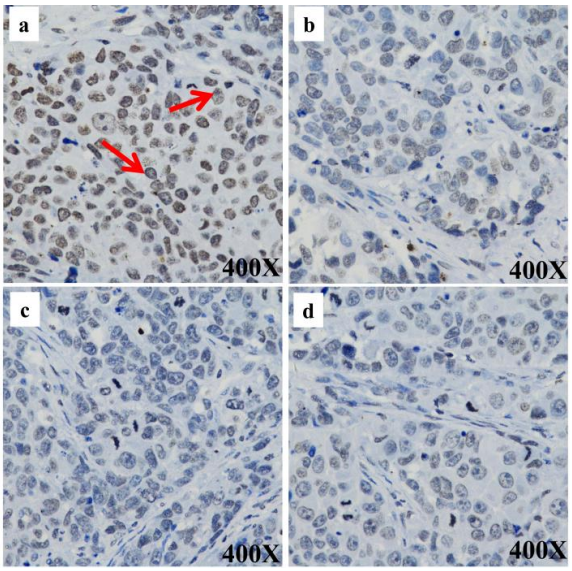


Fig. S17 p53-stained immunohistochemical sections of tumors at 14 days post operation. a, PBS group; b, 1.25% 5-Fu in PBS group; c, J group; d, J-Fu-1.25 group. The images were obtained by a Leica microscope at 400× magnification. The red arrows represent positive expression stained by 3, 3′-diaminobenzidine.

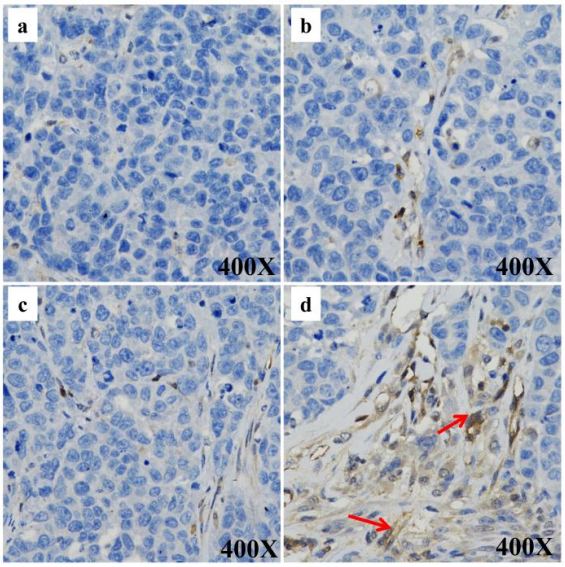


Fig. S18 Caspase-3-stained immunohistochemical sections of tumors at 14 days post operation. a, PBS group; b, 1.25% 5-Fu in PBS group; c, J group; d, J-Fu-1.25 group. The images were obtained by a Leica microscope at 400× magnification. The red arrows represent positive expression stained by 3, 3′-diaminobenzidine.

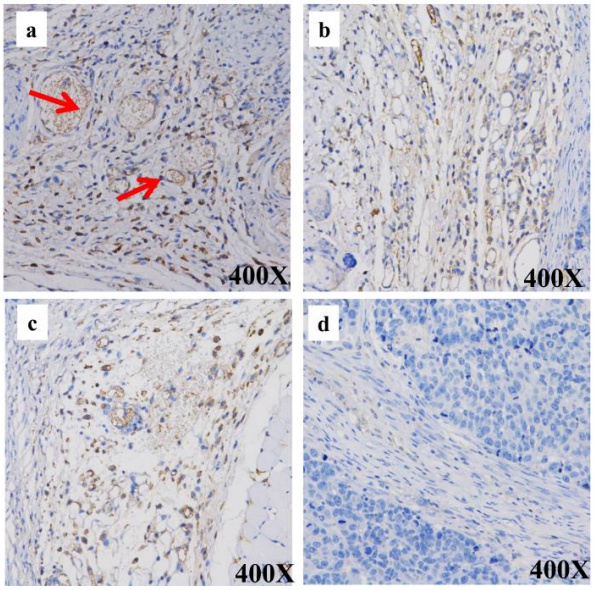


Fig. S19 CD31-stained immunohistochemical sections of tumors at 14 days post operation. a, PBS group; b, 1.25% 5-Fu in PBS group; c, J group; d, J-Fu-1.25 group. The images were obtained by a Leica microscope at 400× magnification. The red arrows represent positive expression stained by 3, 3′-diaminobenzidine.

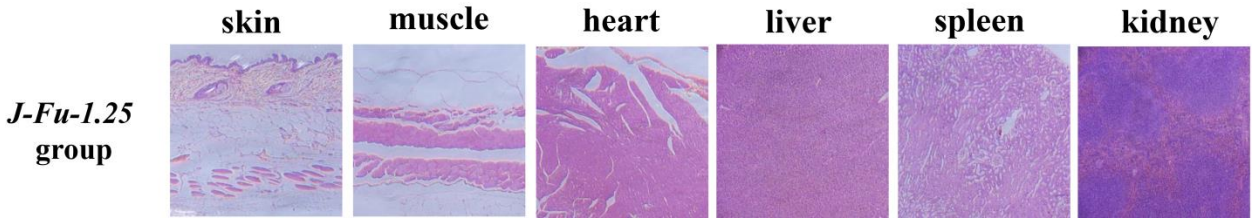


Fig. S20 H&E-stained histological sections of organs in the subcutaneously implanted ***J-Fu-1.25*** group at 30 days post operation. The images were obtained by a Leica microscope at 200× magnification.

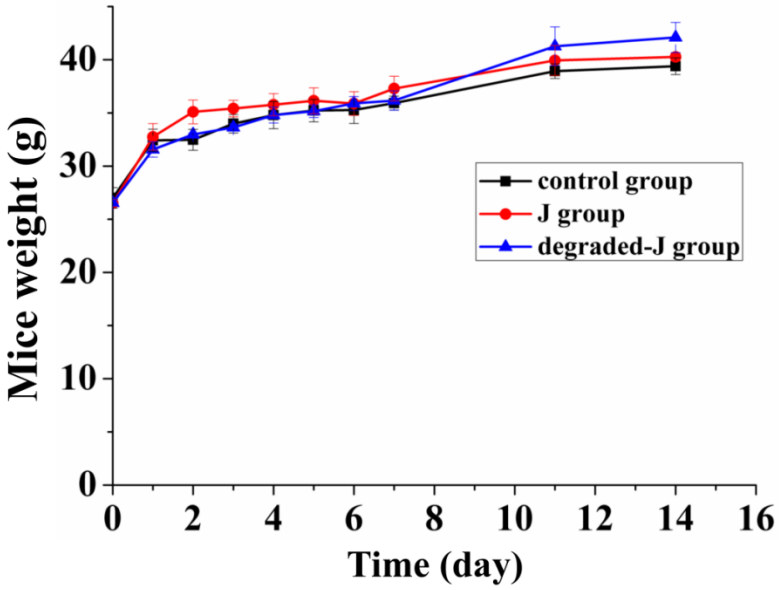
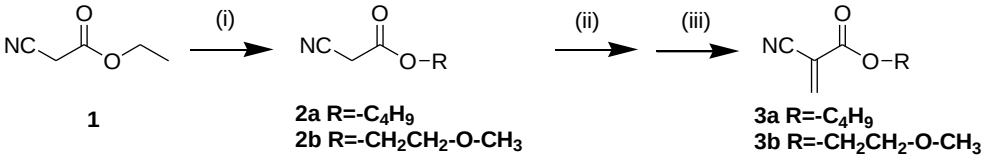
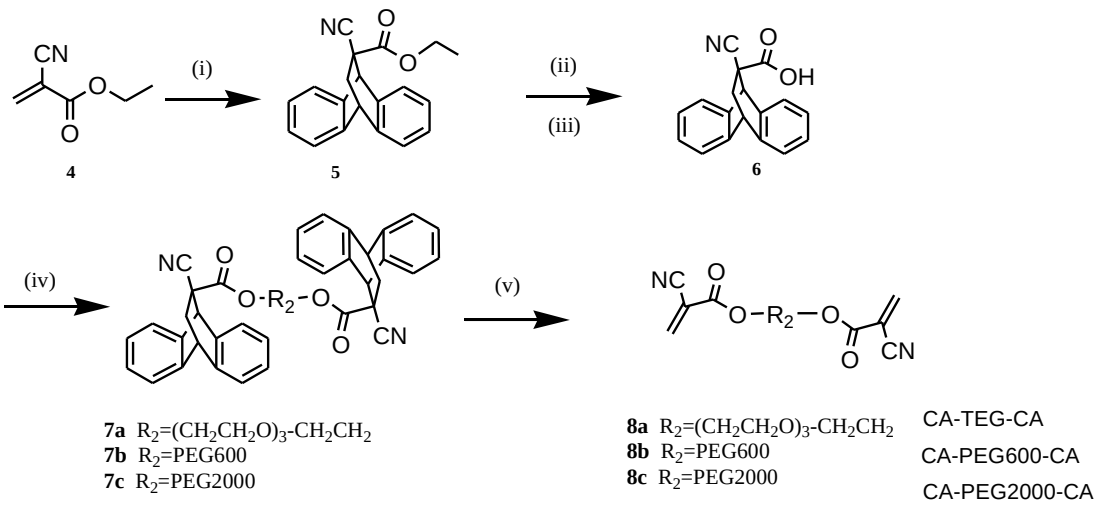


Fig. S21 Mice weight in acute toxicity test.



Scheme S1. The synthesis of MOE-CA (**3b**) and BCA (**3a**). Conditions: (i) 2-Methoxyethanol, tetrabutyl titanate, reflux; vacuum distillation. (ii) Paraformaldehyde, hexahydropyridine in 1,2-dichloroethane, azeotropic dehydration; 3 h. (iii) Pintsch process; rectification.



Scheme S2. The synthesis of CA-PEG-CA (**8a–8c**). Conditions: (i) Anthracene, toluene, reflux, 48 h; (ii) KOH; (iii) HCl; (iv) EDCI, DMAP; (v) Maleic anhydride, xylene, reflux, 6 h.