Supplementary Information

Exploring the match between the degradation of the ECM-based composites and tissue remodeling in full-thickness abdominal wall defect model

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Methods and Results:

Fig. S1 showed the schematic drawing of the Cy5.5@ECMB composites preparation. The NIR fluorescent Cy5.5@ECMB composites were prepared by conjugating Cy5.5 NHS ester to amino groups of the ECM-based composites.

To engineer a highly bright scaffold, various labeling ratios were analyzed by measuring fluorescence signals. As shown in Fig. S2, the highest fluorescence signal was observed when the mole ratio of fluorescent dye against amino groups was around 1 (labeling ratio ≈ 0.581 , as presented in Table S1), and we used this labeling ratio to prepare the Cy5.5@ECMB composites for further experiments. As shown in Fig. S3, the NIR fluorophore Cy5.5 NHS ester presented stable physicochemical and optical properties in collagenase buffers.

The modulus of samples was measured with a speed of 1 mm/min at room temperature using a Shimadzu EZ-LX Universal Testing Machine with a 50 N maximum load cell. The samples were cut into 1×4 cm rectangular size. The load and displacement were recorded and converted to the stress-strain curve based on the initial sample dimensions. The modulus was calculated from the linear slope based on the created stress-strain curves. Table S2 showed that the modulus of the Cy5.5@ECM and Cy5.5@ECMB composites was 21.95±1.08 MPa and 11.25±0.56 MPa, respectively.

Percentage graft shrinkage was calculated with respect to the initial size during implantation and the remaining size during explantation. As shown in Table S3, at 4 weeks, 35.79±2.87% and 23.56±2.61% of shrinkage were observed in the Cy5.5@ECM and Cy5.5@ECMB groups, respectively. At 8 weeks, 56.35±4.24% and 36.72±3.18% of shrinkage were present in the Cy5.5@ECM and Cy5.5@ECMB groups, respectively.

Fig. S1:



Fig. S1. Schematic drawing of the Cy5.5@ECMB composites preparation.

Fig. S2:



Fig. S2. Luminescent images (a) and Fluorescent pseudo-colored images (b) of the Cy5.5@ECMB composites with increasing conjugation ratios.

Table S1:

Theoretical	Treated dye	Unconjugated	Conjugated	Actual
ratio ^a	(nmol)	dye (nmol) ^b	dye (nmol) ^c	labeling ratio ^d
0.0	0.0	0.0	0.0	0.0
0.1	25	8.5	16.5	0.066
0.5	125	45.4	79.6	0.318
1.0	250	104.7	145.3	0.581
1.5	375	147.7	227.3	0.705
2.0	500	261.9	238.1	0.952

Table S1. Quantitation of NIR fluorophore labeling ratios on the ECM-based composites.

^{*a*} The theoretical and ^{*d*} actual conjugation ratios were calculated based on mole ratio between the dye and amino groups in the ECM-based composites. ^{*b*} The unconjugated dye amount was calculated by measuring free dye concentration in the rinsed solutions. ^{*c*} The conjugated dye amount was obtained by subtracting the unconjugated dye amount from the treated dye amount.

Fig. S3:



Fig. S3. Optical property and stability of Cy5.5 NHS ester in collagenase buffer at different time points.

Table S2:

Table S2.	The modulus	of the C	v5.5@ECM	and Cv5.5@	ECMB com	posites.
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Sample	Modulus (MPa)		
Cy5.5@ECM	$21.95{\pm}1.08^*$		
Cy5.5@ECMB	11.25±0.56		

*vs Cy5.5@ECMB (p< 0.05).

Table S3:

Time Shrinkage percentage (%)	4 weeks	8 weeks
Cy5.5@ECM	35.79±2.87*	56.35±4.24*
Cy5.5@ECMB	23.56±2.61	36.72±3.18

Table S3. Percentage graft shrinkage in the Cy5.5@ECM and Cy5.5@ECMB groups.

*vs Cy5.5@ECMB (p< 0.05).