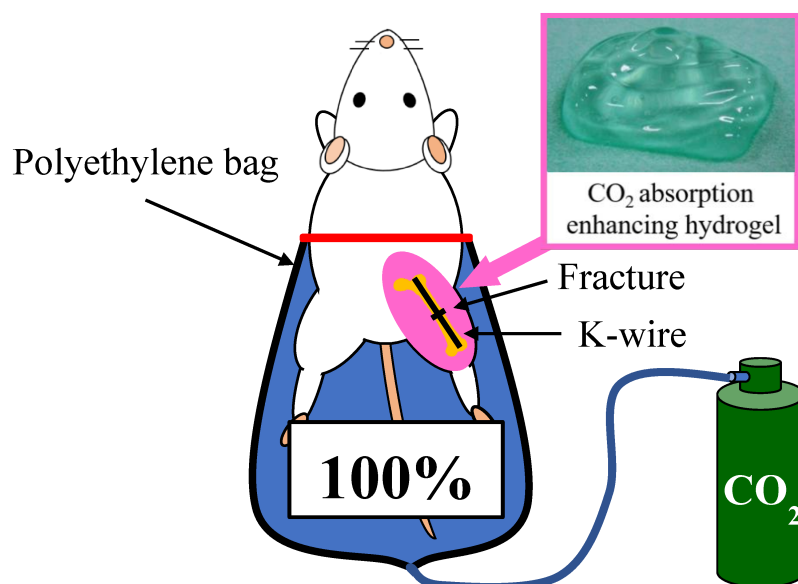


## Supplementary figure and tables



**Figure S1.** The method of transcutaneous CO<sub>2</sub> application in a rat femoral fracture model.

A standard femoral shaft fracture was created in the right femur and was fixed with an intramedullary Kirschner wire. After the fracture limb was shaved, CO<sub>2</sub> absorption-enhancing hydrogel was applied to the whole fractured limb, and both lower limbs were sealed in a polyethylene bag filled with 100% CO<sub>2</sub> for 20 minutes a day.

**Table S1.** Specific primers used for real-time PCR amplifications

Gene name	Primer sequence (5'-3')	
	Forward	Reverse
GAPDH	AAATGGTGAAGGTCGGTGTG	TGAAGGGGTCGTTGATGG
Collagen II	GGGCTCCCAGAACATCACCTACCA	TCGGCCCTCATCTCCACATCATTG
Collagen X	GGCAGCAGCACTATGACCCAA	ACAGGCCTACCCAAACGTGAGTCC
MMP-13	CCCTGGAGCCCTGATGTTT	CTCTGGTGTGTTTTGGGGTGCT
VEGF	TGCACTGGACCCTGGCTTTAC	CGGCAGTAGCTTCGCTGGTAG
TSP-1	GAGTGTCACTGCCAGAACTCA	GTCTGTACTGAAGAGCCCTCA
eNOS	GACCCTCACCGATACAACATAC	CATACAGGATAGTCGCCTTCAC
Runx2	GCGTCAACACCATCATTCTG	CAGACCAGCAGCACTCCATC
Osterix	AGCTCTTCTGACTGCCTGCCTA	TGGGTGCGCTGATGTTTGCT
TNF- $\alpha$	GCTCACAATGTCTGTGCTTAGAG	GCAGTAGCCACAGCTCCAG
RANKL	AGACACAGAAGCACTACCTGACTC	GGCCCCACAATGTGTTGTA
M-CSF	CGAGGTGTGCGGAGCACTGTA	CGATCAACTGCTGCAAAATCTG
ADAMTS 4	CGCTGACCGCCAATGCCAACTG	GCCCAAGGTGAGTGCTTCGTCTG

GAPDH; glyceraldehyde-3-phosphate dehydrogenase, MMP-13; matrix metalloproteinase-13, VEGF; vascular endothelial growth factor, TSP-1; thrombospondin-1, eNOS; endothelial nitric oxide synthase, Runx2; runt-related transcription factor 2, TNF- $\alpha$ ; tumor necrosis factor- $\alpha$ , RANKL; receptor activator of NF- $\kappa$ B ligand, M-CSF; macrophage colony-stimulating factor, ADAMTS 4; A disintegrin and metalloproteinase with thrombospondin motifs 4

**Table S2.** Summary of intra-rater and the inter-rater ICC reproducibility (n = 5 raters)

	Radiographic assessment	Histological assessment
	Determinations of bone union	Scoring of degree of fracture repair
Rater 1 intra-rater reliability	ICC 0.931 (95% CI 0.856–0.967)	ICC 0.980 (95% CI 0.970–0.988)
Rater 2 intra-rater reliability	ICC 0.925 (95% CI 0.844–0.964)	ICC 0.953 (95% CI 0.926–0.970)
Rater 3 intra-rater reliability	ICC 0.893 (95% CI 0.776–0.949)	ICC 0.967 (95% CI 0.948–0.979)
Rater 4 intra-rater reliability	ICC 0.931 (95% CI 0.856–0.967)	ICC 0.990 (95% CI 0.984–0.993)
Rater 5 intra-rater reliability	ICC 0.966 (95% CI 0.930–0.983)	ICC 0.973 (95% CI 0.957–0.982)
Inter-rater reliability	ICC 0.944 (95% CI 0.905–0.971)	ICC 0.950 (95% CI 0.931–0.966)

ICC; inter class correlation coefficient, CI; confidence intervals