

Histological and Immunohistochemical Characterization of Osteoimmunological Processes in Scaffold-Guided Bone Regeneration in an Ovine Large Segmental Defect Model

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1 Supplementary Table 1. Details of experimental groups.

Details of experimental groups. mPCL-TCP = medical-grade polycaprolactone and tricalcium phosphate; mPCL-TCP-CaP = medical-grade polycaprolactone and tricalcium phosphate and calcium phosphate surface coating; PRP = Platelet-Rich-Plasma; ICGB = iliac crest bone graft; RIA system = Reamer-Irrigator-Aspirator system; rhBMP-7 = recombinant human bone morphogenic protein-7; CPF = cortico-periosteal flap; Allo-MSC = allogenic mesenchymal stem cells

Experimental Groups			
Scaffold	Bone Graft Material / Substitute	Tibial Defect Sizes	Time Points
mPCL-TCP	RIA	6 cm	6 hours 12 months 14 months 15 months 21 months 27 months
mPCL-TCP	ICGB (6 ml)	6 cm	2 months 6 months
mPCL-TCP	rhBMP-7 (2 mg) carried in PRP	6 cm	12 months
mPCL-TCP-CaP	CPF	3 cm 6 cm	12 months 12 months 15 months
mPCL-TCP	Allo-MSC	3 cm	36 months

2 Supplementary Table 2. Antibody markers used for immunohistochemistry.

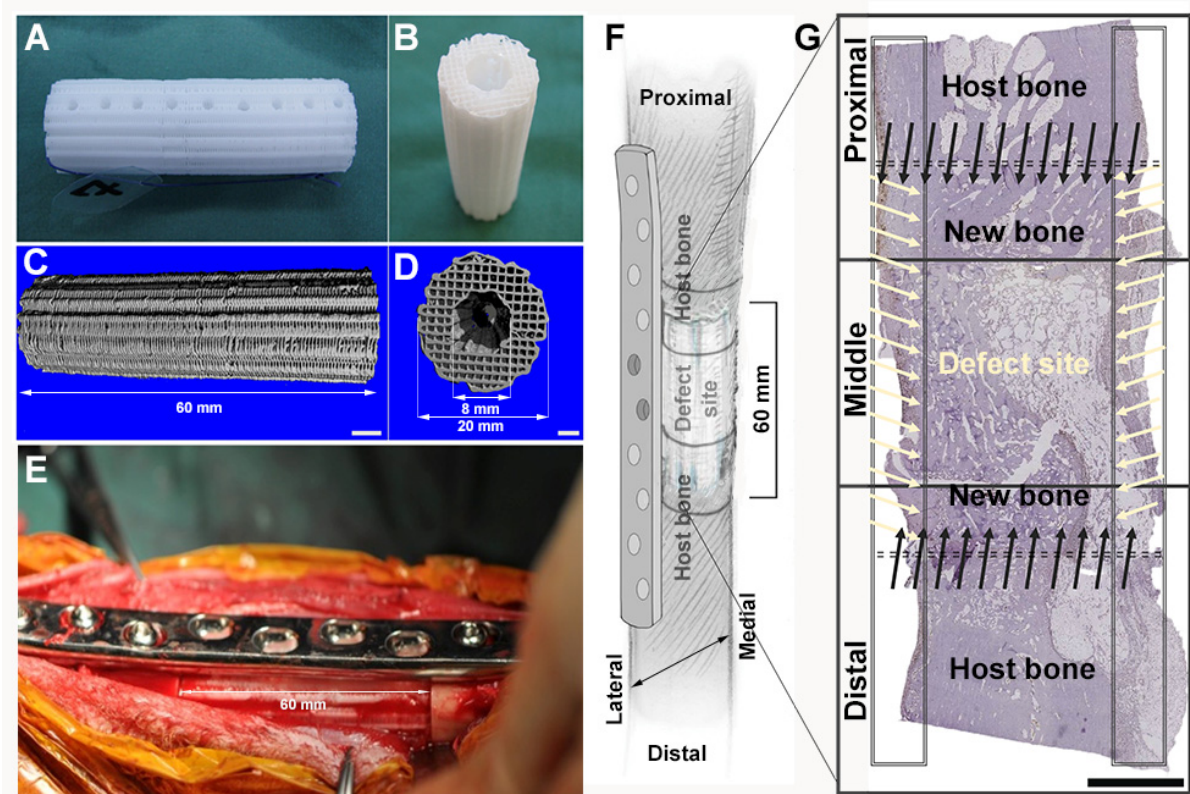
Verified primary antibody staining to detect inflammatory and blood vessel markers as well as bone ECM proteins in the preclinical critical-sized bone defect model. PK: Proteinase K. ab: abcam; SC: Santa Cruz;

Antibody	Positive control	Reactivity	Raised in	Clonality	Cat. no.	Antigen retrieval	Blocking	Dilution	Incubation	DAB
Inflammation/Osteoclastic activity markers										
Nitric oxide synthase (iNOS)	Sheep spleen	Mouse	Rabbit	Polyclonal	ab15323	PK 5 min	2% BSA	1:100	60 min	3 min
Mannose receptor (MR)	Sheep liver	Human, Mouse, Rat	Rabbit	Polyclonal	ab64693	PK 5 min	2% BSA	1:500	60 min	45 sec
Arginase-1 (ARG-1)	Sheep spleen	Human, Mouse, Rat	Rabbit	Polyclonal	Proteintech 16001-1-AP	PK 5 min	2% BSA	1:100	60 min	5 min
Cluster of differentiation 68 (CD68)	Sheep spleen	Mouse, Rat	Rabbit	Polyclonal	ab125212	PK 5 min	2% BSA	1:300	60 min	1:30 min
Interferon regulatory factor 5 (IRF5)	Sheep spleen	Human, Mouse, Rat, Human, Mouse, Rat	Mouse	Monoclonal	sc-56714	PK 5 min	2% BSA	1:100	60 min	5 min
			Rabbit	Polyclonal	Proteintech 10547-1AP	PK 5 min	2% BSA	1:100	60 min	3 min
Cluster of differentiation (CD3)	Sheep spleen	Human	Mouse	Monoclonal	ab17143	PK 5 min	2% BSA	1:100	60 min	5 min
Cluster of differentiation 45 (CD45)	Sheep spleen	Human, Mouse, Rat	Rabbit	Polyclonal	ab10558	PK 5 min	2% BSA	1:100	60 min	5 min
Vascularization markers										
Vascular endothelial growth factor (VEGF)	Sheep tibia	Human, Mouse, Rat	Rabbit	Polyclonal	SC152	PK 5 min	2% BSA	1:500	60 min	1:30 min

Cluster of differentiation 31 (CD31) (PECAM-1 (M-20))	Sheep tibia	Human, Mouse, Rat	Goat	Polyclonal	SC1506-R	PK 5min	2% BSA	1:1000	60 min	1 min
von Willebrand factor (vWF)	Sheep tibia	Human	Rabbit	Polyclonal	IR527	PK 5min	2% BSA	Ready to use	60 min	1:30 min
Alpha smooth muscle actin (α -SMA)	Heart	Human, Mouse, Rabbit, Sheep, Pig, Rat,	Mouse	Monoclonal	ab7817	PK 5 min	2% BSA	1:500	60 min	2 min
Angiopoietin-1 (ANG1)	Sheep tibia	Human, Mouse, Rat	Rabbit	Polyclonal	ab102015	PK 5 min	2% BSA	1:100	60 min	1 min
Noggin (NOG)	Sheep tibia	Human, Mouse, Rat	Rabbit	Polyclonal	SC25656	PK 5 min	2% BSA	1:100	60 min	5 min
NOTCH 1	Sheep tibia	Human, Mouse, Rat	Rabbit	Polyclonal	SC6014-R	PK 5 min	2% BSA	1:100	60 min	5 min
Extracellular matrix markers										
Collagen type I (COL I)	Sheep tibia	Human	Rabbit	Polyclonal	ab34710	PK 5min	2% BSA	1:1000	60 min	2 min
		Human	Rabbit	Monoclonal	ab138492			1:500		30 sec
Collagen type II (COL II)	Sheep tibia	Avian, Bovine, broad species, Chicken, Fish, Goat, Human, Mouse, Ovine, Rabbit, Rat, Shark, Zebrafish, Xenopus	Mouse	Monoclonal	DSHB II-II6B3	PK 5min	2% BSA	1:100	60 min	40 sec
Bone morphogenetic protein 2 (BMP2)	Sheep tibia	Human, Mouse, Rat,	Mouse	Monoclonal	SC137087	PK 5 min	2% BSA	1:50	60 min	overnight

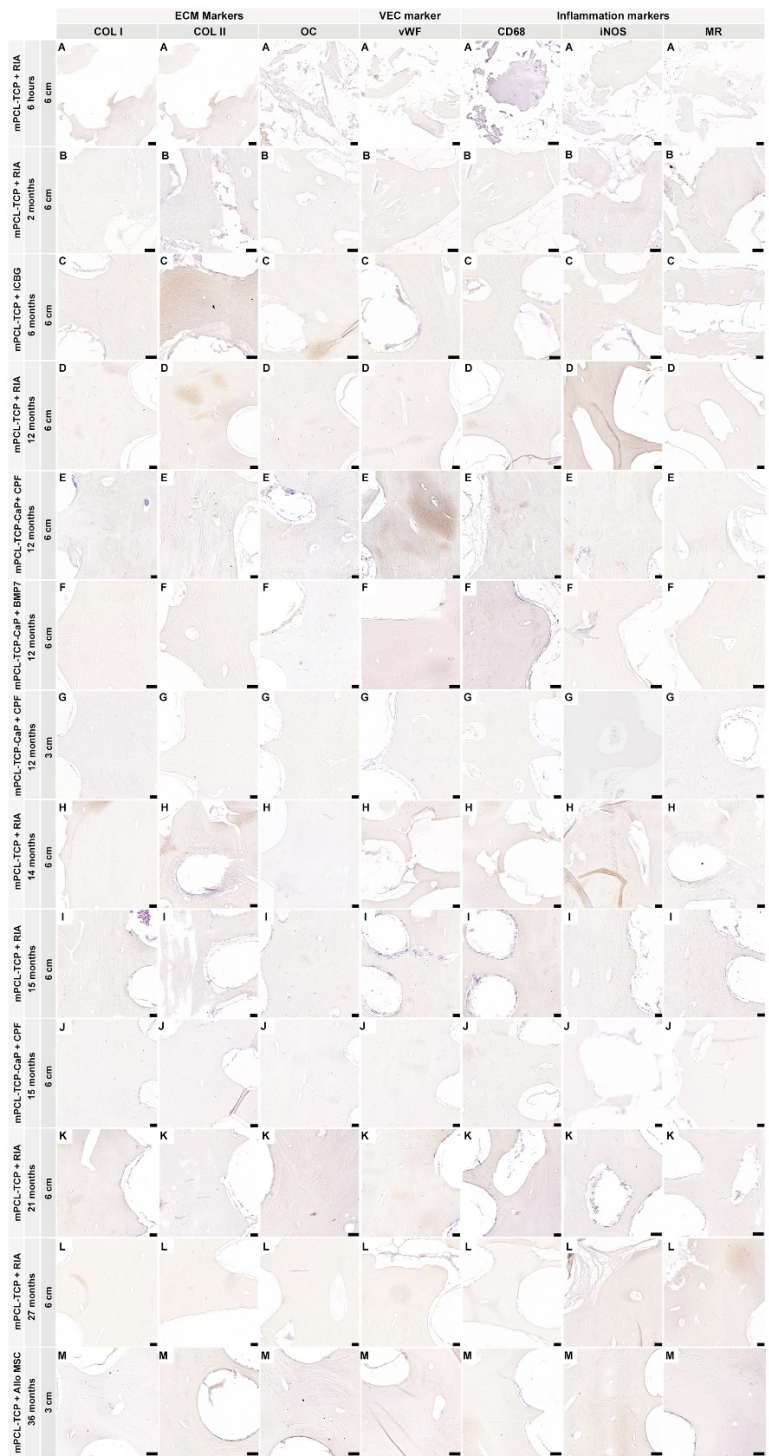
Osteoprotegerin (OPG)	Sheep tibia	Human	Rabbit	Polyclonal	ab73400	PK 5 min	2% BSA	1:100	60 min	30 sec
Alkaline Phosphatase (ALP)	Sheep tibia	Human, Mouse, Rat,	Mouse	Monoclonal	sc166261	PK 5 min	2% BSA	1:500	60 min	5 min
Sclerostin (SCL)	Sheep tibia	Human, Mouse, Rat, Sheep,	Rabbit	Polyclonal	ab63097	PK 5 min	2% BSA	1:100	60 min	1 min
Osteomodulin (OMD)	Sheep tibia	Human, Mouse	Rabbit	Polyclonal	ab154249	PK 5 min	2% BSA	1:500	60 min	1 min
Osteonectin (ON)	Sheep tibia	Bovine, Dog, Monkey, Rabbit, Cow, Human, Pig, Rat	Mouse	Monoclonal	DSHB AON-1	- PK 5 min	2% BSA	1:100	60 min	2 min
Osteopontin (OPN)	Sheep tibia	Dog, Mouse, Rat, Human, Pig,	Rabbit	Polyclonal	ab8448	PK 5 min	2% BSA	1:100	60 min	40 sec
Osteocalcin (OC)	Sheep tibia	Cow, Goat, Pig, Dog, Human, Rat, Rabbit, Sheep	Mouse	Monoclonal	ab13418	PK 5 min	2% BSA	1:500	60 min	30 sec

1. **Supplementary figure 1. Schematic overview of the histologic sectioning methodology used in our model of the ovine segmental tibial defect.**



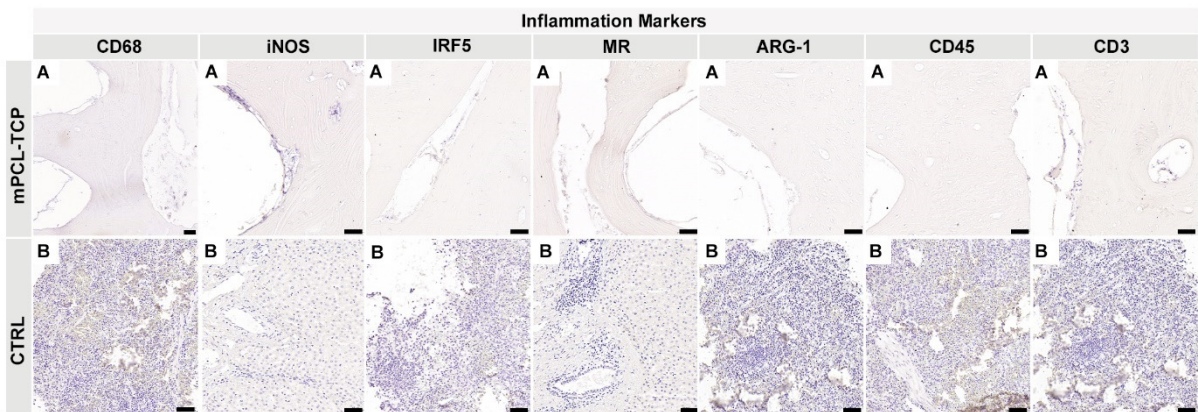
Supplementary figure 1. (A-D) Longitudinal and radial view, and dimensions of the mPCL-TCP scaffold. (F-G) Standardized transverse sectioning planes for the immunohistological specimens are proximal host bone and defect site interface, middle: defect site only; distal host bone and defect site interface. Reproduced and modified from Henkel *et al.* [20] and Medeiros Savi *et al.* [78]. Scale bar: 5 mm.

2. **Supplementary figure 2. Immunohistochemical analysis of protein expression in SGBR throughout the entire bone regeneration period, negative controls.**



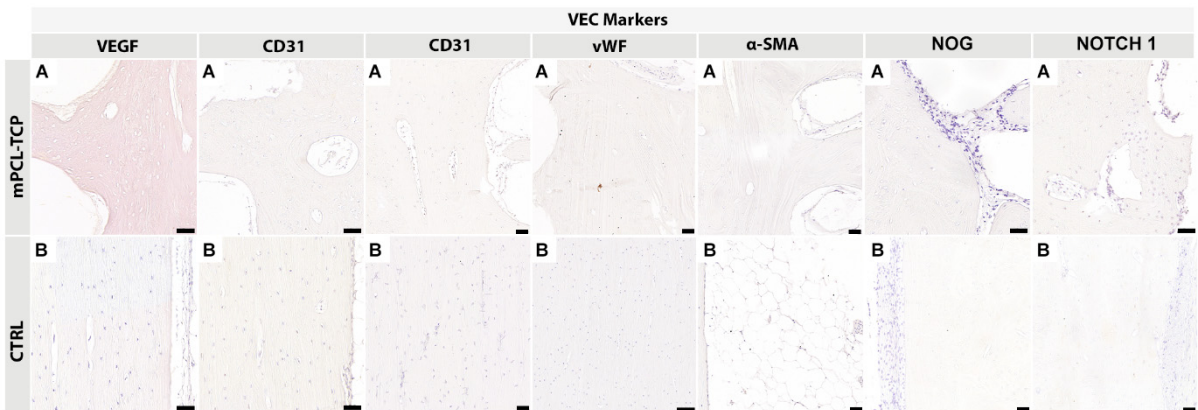
Supplementary figure 3. Immunohistochemical analysis of protein expression in SGBR throughout the entire bone regeneration period. Negative controls for Collagen type I (Col I), Collagen type II (Coll II); Osteocalcin (OC); von Willebrand Factor (vWF); cluster of differentiation 68 (CD68); inducible nitric oxidase synthesis (iNOS); Mannose receptor (MR). Scale bars: 50µm

3. **Supplementary figure 3. Inflammation markers negative controls.**



Supplementary figure 4. Inflammation markers negative controls for Cluster of differentiation 68 (CD68), Nitric oxide synthase (iNOS), interferon regulatory factor 5 (IRF5), mannose receptor (MR), Arginase-1 (ARG-1), cluster of differentiation 45 (CD45) and cluster of differentiation 3 (CD3). Scale bars: 50µm

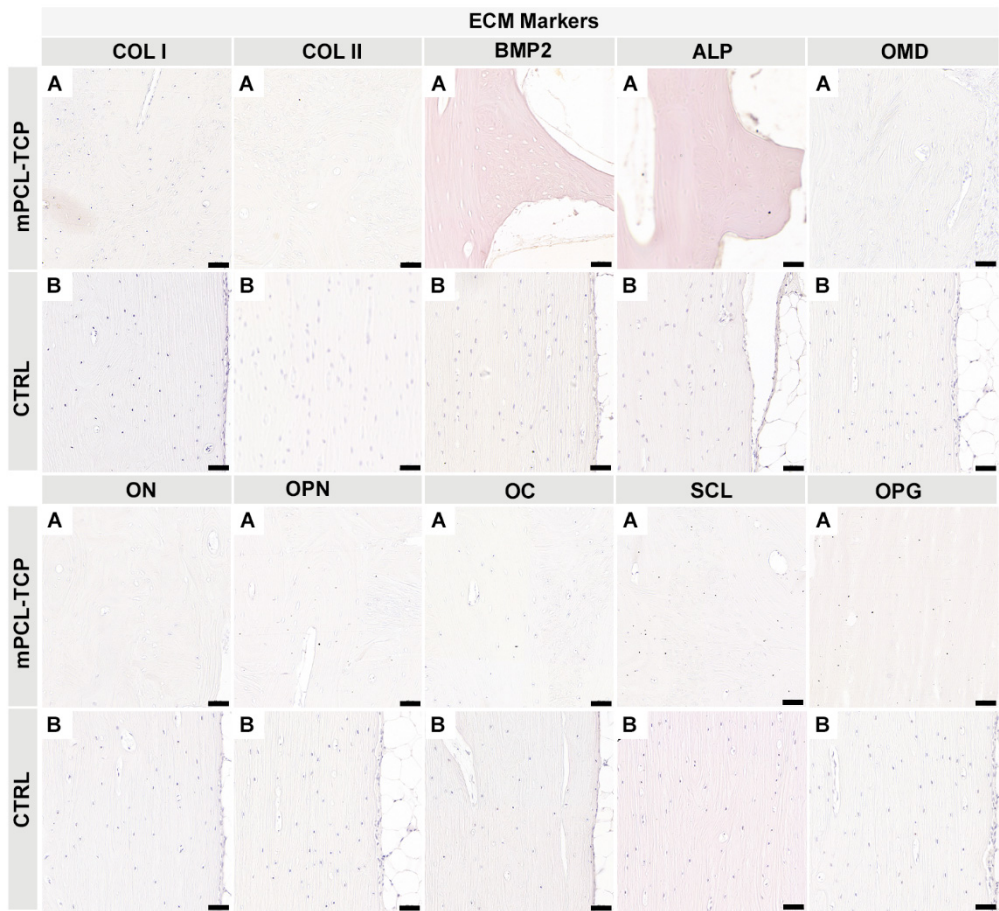
4. **Supplementary Figure 4. Vascular endothelial cellular markers negative controls.**



Supplementary figure 5. Vascular endothelial cellular markers negative controls for Vascular Endothelial Factor (VEGF); Angiopoietin (ANG1); Cluster of differentiation 31 (CD31); von

Willebrand Factor (vWF); Anti smooth Muscle Actin (α -SMA); Noggin (NOG). Scale bars: 50 μ m

5. **Supplementary figure 5. Extracellular matrix markers negative controls.**



Supplementary figure 6. Extracellular matrix markers negative controls for: Collagen type I (Col I), Collagen type II (Coll II); Bone Morphogenic Growth Factor 2 (BMP2); Alkaline Phosphatase (ALP); Osteomodulin (OMD); Osteonectin (ON); Osteopontin (OPN); Osteocalcin (OC); Sclerostin (SCL) and Osteoprotegerin (OPG); Isotypes controls for NOG and NOTCH were used from another tissue sample, however done at the same time. Scale bars: 50 μ m