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

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# Supplementary Table 1

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

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| --- | --- |
| 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 |

# Supplementary Table 2

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;

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 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  Rabbit | Monoclonal  Polyclonal | sc-56714  Proteintech 10547-1AP | PK 5 min  PK 5 min | 2% BSA  2% BSA | 1:100  1:100 | 60 min  60 min | 5 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, Pig, Rabbit, Rat, Sheep, | 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  Human | Rabbit  Rabbit | Polyclonal Monoclonal | ab34710  ab138492 | PK 5min | 2% BSA | 1:1000  1:500 | 60 min | 2 min  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, Cow, Dog, Human, Monkey, Pig, Rabbit, Rat | Mouse | Monoclonal | DSHB - AON-1 | PK 5 min | 2% BSA | 1:100 | 60 min | 2 min |
| Osteopontin (OPN) | Sheep tibia | Dog, Human, Mouse, Pig, Rat, | Rabbit | Polyclonal | ab8448 | PK 5 min | 2% BSA | 1:100 | 60 min | 40 sec |
| Osteocalcin (OC) | Sheep tibia | Cow, Dog, Goat, Human, Pig, Rat, Rabbit, Sheep | Mouse | Monoclonal | ab13418 | PK 5 min | 2% BSA | 1:500 | 60 min | 30 sec |

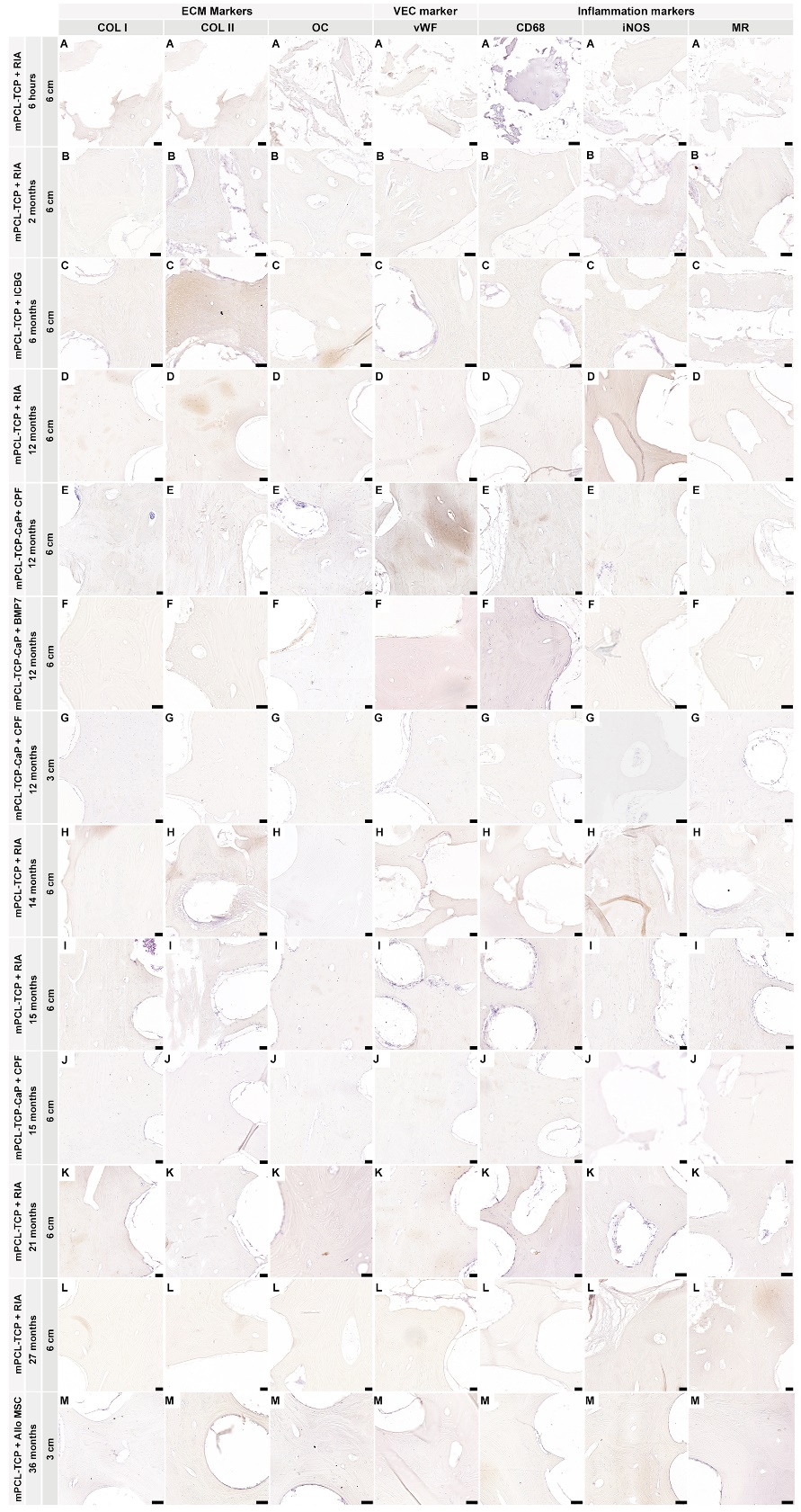
# Supplementary figure 1. Schematic overview of the histologic sectioning methodology used in our model of the ovine segmental tibial defect.

A close-up of several different types of plastic tubes

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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*. [27]. Scale bar: 5 mm.

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



Supplementary figure 2. 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

# Supplementary figure 3. Inflammation markers negative controls.

A close-up of a microscope

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Supplementary figure 3. 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

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

A close-up of a microscope slide

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Supplementary figure 4. 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: 50µm

Supplementary figure 5. Extracellular matrix markers negative controls.

A collage of images of cells

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Supplementary figure 5. 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: 50µm