

Supplementary data

Figure S1

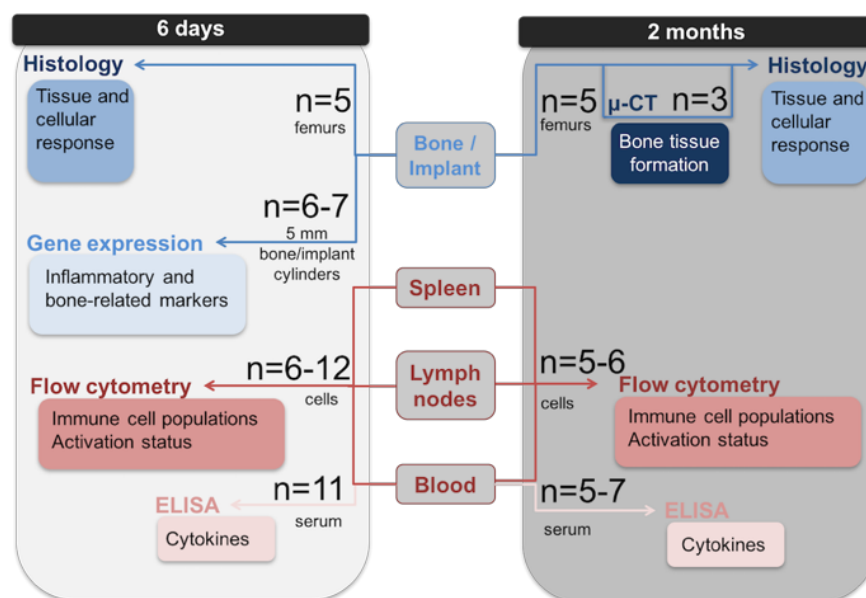


Fig. S1. Experimental design diagram: bone defects were performed in Wistar rats and immune response and bone remodelling were evaluated at 6 days and 8 weeks post-injury. The methods applied in the evaluation of each sample type are indicated as well as the number of animals studied per condition (non-operated, empty defect and with Fg-3D scaffold).

Figure S2

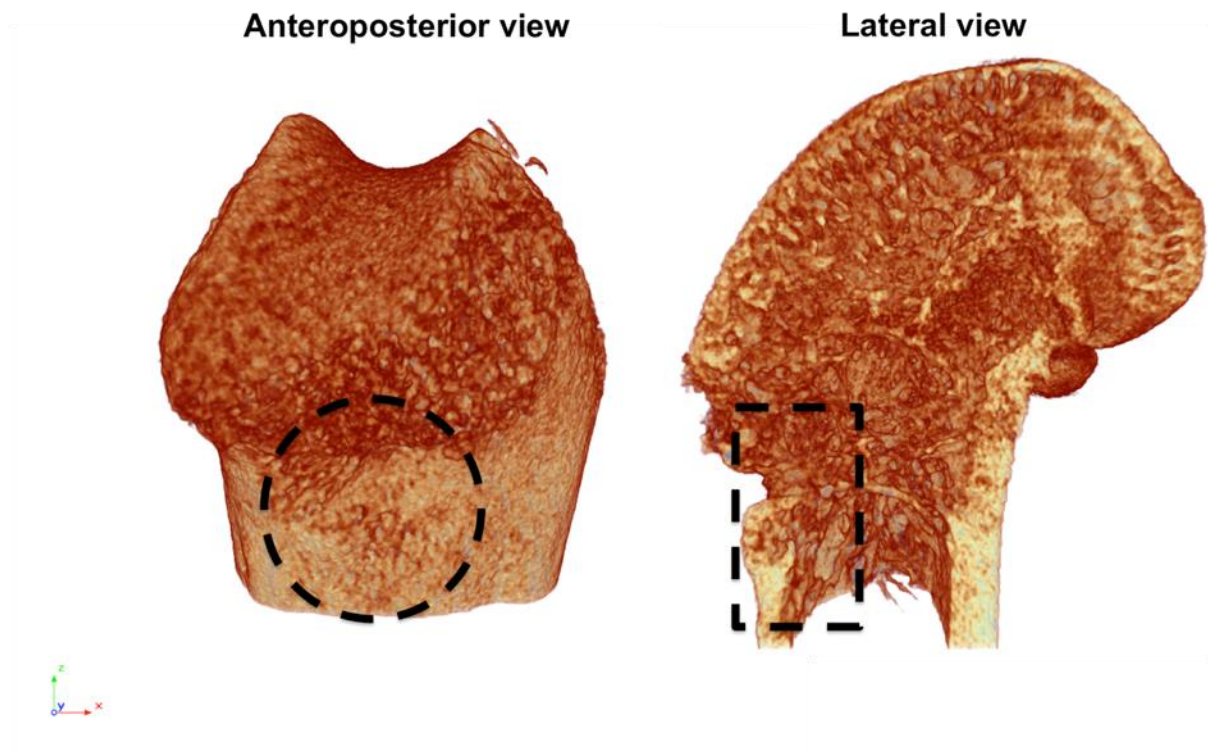


Fig. S2. Cylindrical VOI with 3 mm in diameter and 2 mm in deepness used for microCT analysis of bone defect region at 8 weeks post-implantation.

Figure S3

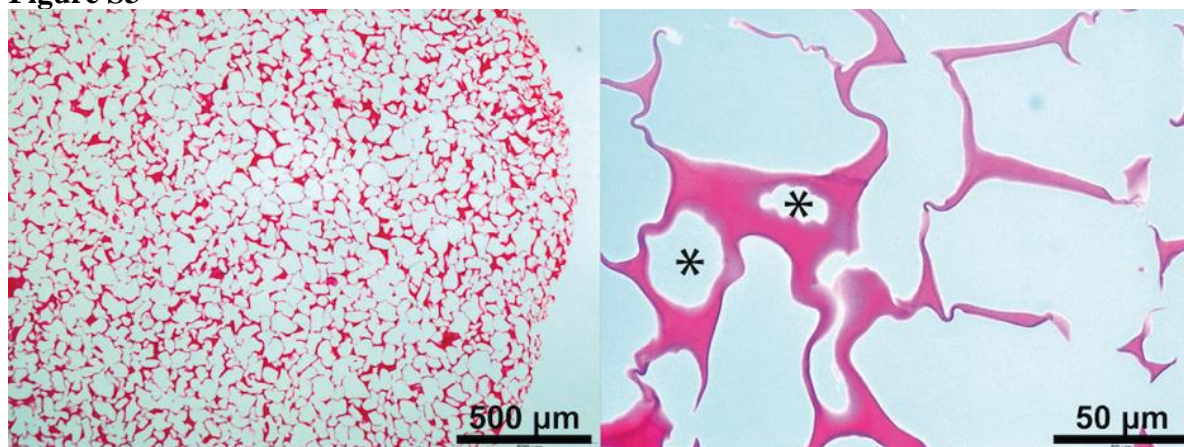


Fig. S3 Light microscopy images of Fg-3D scaffolds after H&E staining. Interconnecting pores are indicated with asterisks.

Figure S4

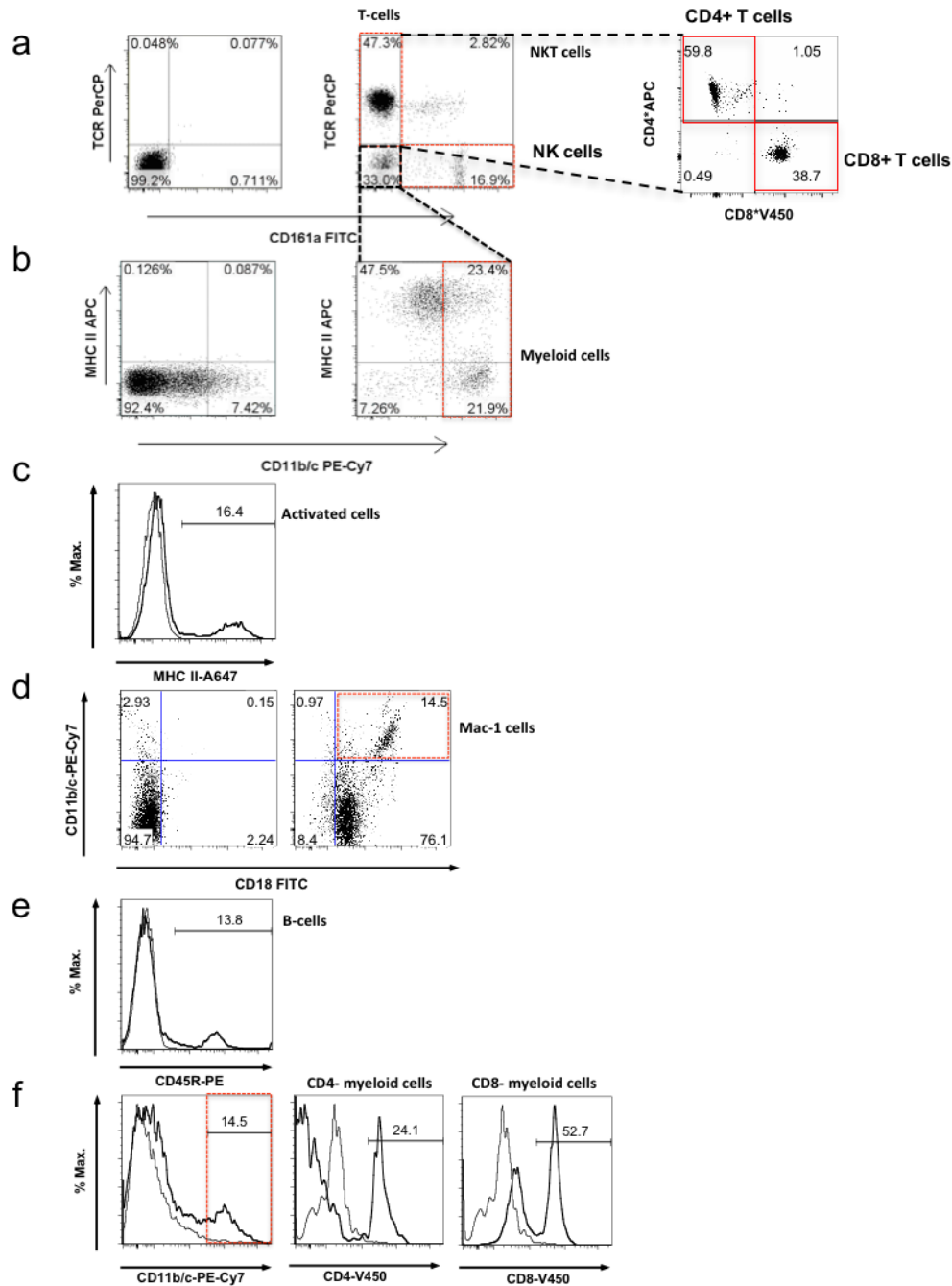


Fig. S4. Representative plots of the flow cytometry analysis illustrating the gates and surface markers used to evaluate the different immune populations: T cells, NK cells, NKT cells and the subsets CD4⁺ T cells and CD8⁺ T cells (a), myeloid cells (b), activated cells (c), Mac-1⁺ cells (d), B cells (e) and CD4⁺ and CD8⁺ myeloid cells (f).

Figure S5

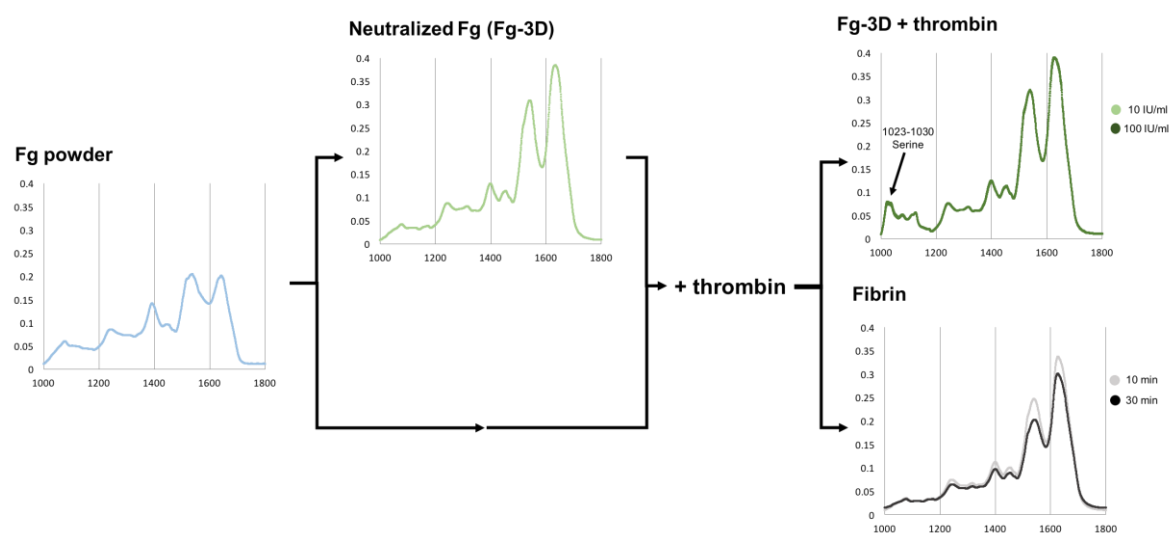


Fig. S5. ATR-FTIR spectra of Fg powder, Fg-3D before and after incubation with thrombin solution at 10 or 100 IU/ml, 40 mM CaCl₂ and 37°C.

Supplementary Table 1. List of primers used for quantitative RT-PCR analysis

Gene	Accession	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
GAPDH	[NM_017008.4]	CTGGAGAAACCTGCCAAGTA	TACTCCTTGGAGGCCATGTA
IFN- γ	[NM_138880.2]	GGCCATCAGCAACAACATA	GACAGCTTTGTGCTGGATCT
IL-1 β	[NM_031512.2]	GACAAGCAACGACAAAATCC	ACCGCTTTTCCATCTTCTTC
IL-2	[NM_053836.1]	AGCGTGTGTTGGATTTGACT	TCTCCTCAGAAATTCCACCA
IL-4	[NM_201270.1]	TCCGTGCTTGAAGAACAAGT	CCAGGAAGTCTTTCAGTGTGT
IL-6	[NM_012589.2]	AGCCAGAGTCATTTCAGAGCA	AGTTGGATGGTCTTGGTCCT
IL-8 (MIP-2)	[NM_053647.1]	TGAAGTTTGTCTCAACCCTGA	GGTGCAGTTCGTTTCTTTCT
IL-10	[NM_012854.2]	GACGCTGTCATCGATTCTC	TTCATGGCCTTGATAGACACC
Osteocalcin	[NM_013414.1]	AGGGCAGTAAGGTGGTGAAT	CTAAACGGTGGTGCCATAGA
TNF- α	[NM_012675.3]	TCTACTGAACCTCGGGGTGA	CCACCAGTTGGTTGTCTTTG
TGF- β 1	[NM_021578.2]	CGGACTACTACGCCAAAGAA	CCCGAATGTCTGACGTATTG
VEGFa	[NM_031836.3]	CAATGATGAAGCCCTGGA	CTATGCTGCAGGAAGCTCAT

Supplementary Table 2. Coefficients of variation for the most important biological parameters assessed in this study

Coefficient of variation (%)

Methodology	Molecule analyzed	Animal group		
		NO	Empty	Fg-3D
Gene expression	OC	79,4	96,4	54,3
	VEGF	35,7	89,1	47,8
	TGF-b	81,7	95	75,5
	IL-1b	53,4	115,6	24,1
	IL-6	87	133,3	65,9
	IL-8	45,4	177,8	34
Flow Cytometry	Myeloid cells - BI	14,4	81	61,3
	Myeloid cells - Sp	26,2	46,6	34,6
	Myeloid cells - LN	12,2	66,6	58
	B cells - BI	13,5	34,8	51,7
	B cells - Sp	12,2	11,4	30,7
	B cells - LN	25,3	38,3	33,4
	T cells - BI	13,6	7,5	13,2
	T cells - Sp	15,74	26	11,4
	T cells - LN	10	19,6	8
ELISA	TGF-b	98,1	65,5	58,6
	IL-1b	26,4	43,6	28,9