

## SUPPORTING INFORMATION

### **Perfluorocarbon/Gold-Loading for Non-Invasive in Vivo Assessment of Bone Fillers Using <sup>19</sup>F Magnetic Resonance Imaging and Computed Tomography**

Simone Mastrogiacomo,<sup>†,1</sup> Weiqiang Dou,<sup>‡,1</sup> Olga Koshkina<sup>§</sup>, Otto C. Boerman<sup>‡</sup>, John A. Jansen<sup>†</sup>, Arend Heerschap,<sup>‡,\*</sup> Mangala Srinivas,<sup>§,\*</sup> and X. Frank Walboomers<sup>†,\*</sup>

<sup>†</sup>Department of Biomaterials, Radboud University Medical Center, P.O. Box 9101, 6500 HB Nijmegen (309), The Netherlands

<sup>‡</sup>Department of Radiology and Nuclear Medicine, Radboud University Medical Center, Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands

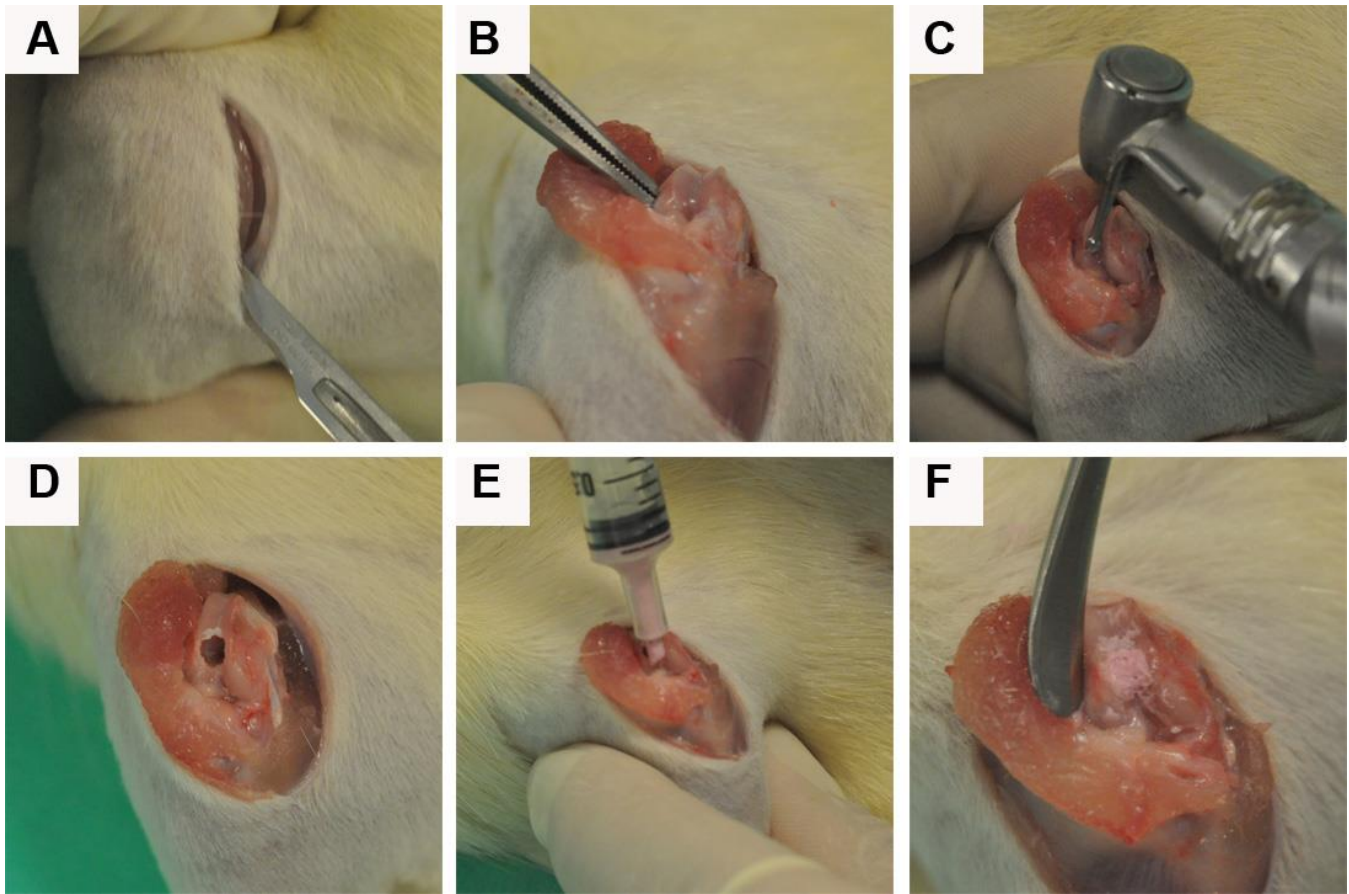
<sup>§</sup>Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences (RIMLS), Geert Grooteplein Zuid 28, 6525 GA Nijmegen, The Netherlands

<sup>1,\*</sup> These authors contributed equally to this work

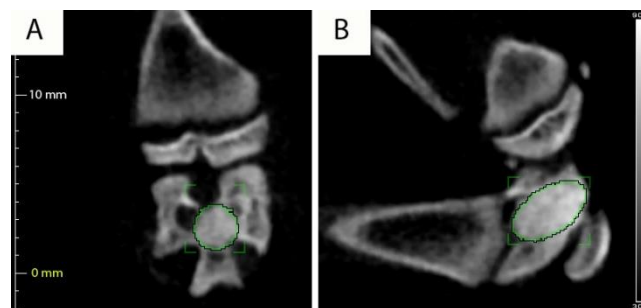
\*Corresponding author:

Dr. X. Frank Walboomers, Department of Biomaterials, Radboud University Medical Center, P.O. Box 9101, 6500 HB Nijmegen (309), The Netherlands.

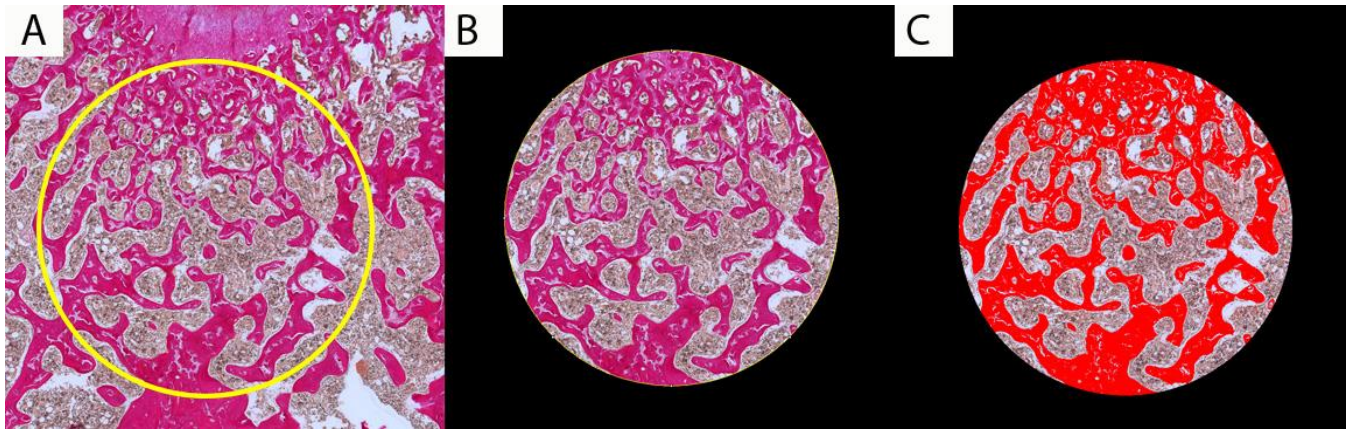
Email: frank.walboomers@radboudumc.nl



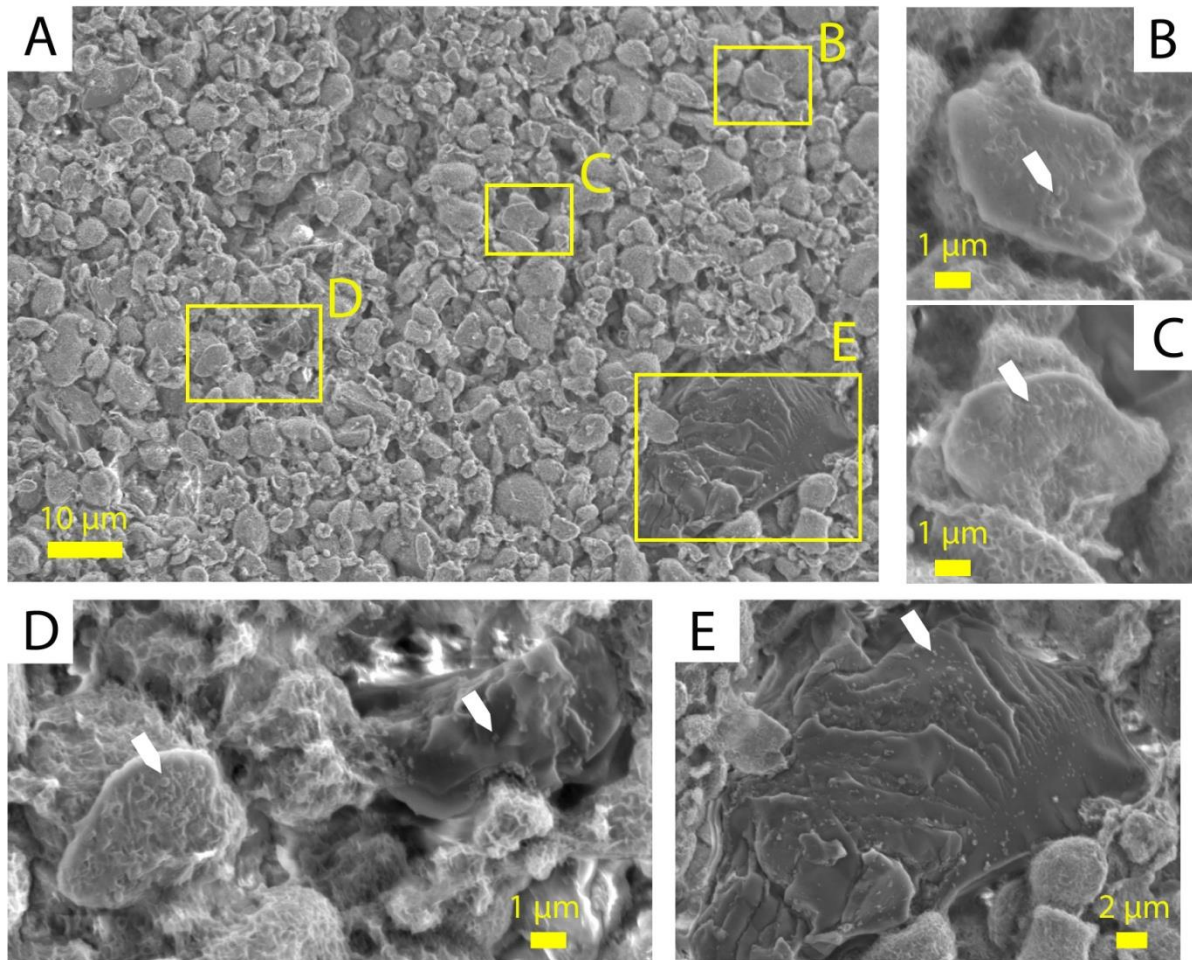
**Figure S1.** Femoral condyle defect, *in vivo* surgical procedure. A longitudinal incision was performed on the medial surface of the knee (a). After patella dislocation, the condyle is exposed (b). With a dental drill a cylindrical defect (2.5 mm in diameter) it is performed along the direction of the femur, (c) and (d). The CPC with or without contrast agents was used to fill the defect. In (e) the CPC/PFCE/gold composition is injected in the condyle defect. By using a spatula the shape of the cement was adapted to the anatomical shape (f), and thereafter the wound was closed in layers, using resorbable stitches.



**Figure S2.** CT representation of a rat condyle filled with CPC. The coronal direction in (a) and the sagittal direction in (b) are shown. By using IRW, a constant volume of interest (VOI) including the implanted material was drawn. Based on this VOI, the relative signal intensity in percentage was calculated.



**Figure S3.** Bone formation quantification based on the images from histology. In (a) the starting EVG stained picture of one section and the selected ROI (i.e. circle of 2.5 mm in diameter), in (b) the region outside of the circle is excluded, in (c) the selected area after threshold adjustment. By using ImageJ software, the total area (in  $\mu\text{m}^2$ ) of the circle is calculated. The total bone formation is expressed as ratio between the bone area and the total selected area (in percentage).



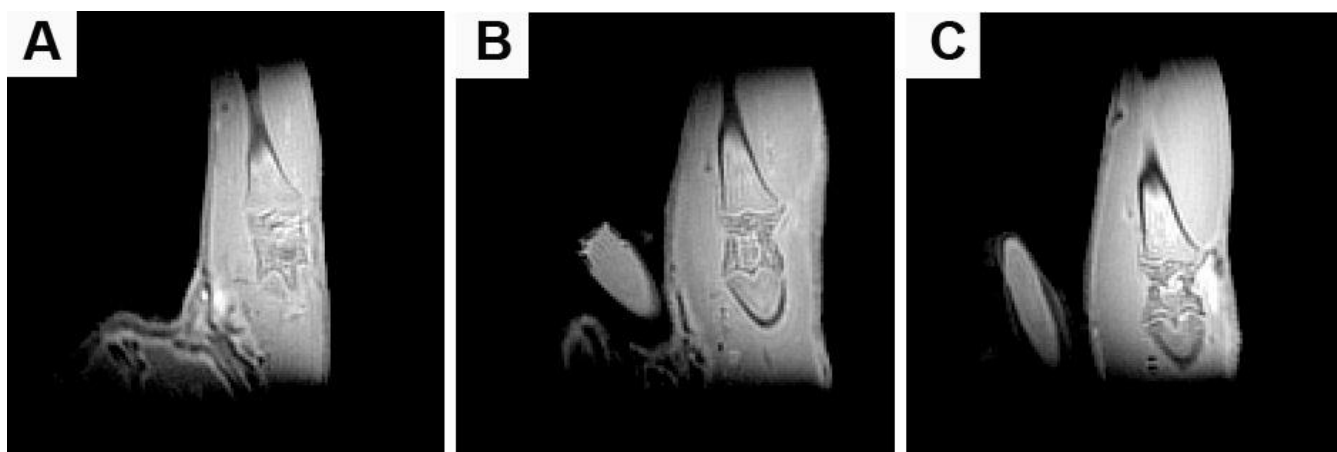
**Figure S4.** Scanning electron micrographs of (A) an overview of the surface of CPC/PFCE/gold composite (magnification X2500). Yellow rectangles indicate (B, C) agglomerates of PLGA/PFCE, (D) agglomerates of PLGA/PFCE and cryo-grinded PLGA particle, (E) cryo-grinded PLGA particle. For figure B, C, D magnification is X15000; for Figure E magnification is X5000. White arrows indicate singular PLGA/PFCE nanoparticles attached to the overall agglomerate. Note that in (E) individual PLGA/PFCE nanoparticles resulted attached also to the cryo-grinded PLGA particles.





	CPC	CPC/PFC-Gold
S1	++	+
S2	++	+++
S3	+	++
<b>Total</b>	++	++

**Figure S5.** Cohesion test. Pictures from the CPC and the CPC/PFCE/gold pastes are shown after 24 hours incubation in PBS at 37 °C. On the top three samples from the CPC composite, while on the bottom the same amount of samples from the CPC/PFCE/gold composite. In the table are reported the score from the cohesion test. Score was attributed as follows: +++ = the paste broke in 1 single piece, ++ = the paste broke in > 2 pieces and <3 pieces, + = the paste broke in > 3 pieces.



**Figure S6.** <sup>1</sup>H ZTE images (coronal view) of an example rat condyle directly after surgery (A), at 4 week (B) and at 8 weeks (C).

**Table S1.** Randomization scheme of the experimental groups

No defect	Empty defect	CPC	CPC/PFCE/gold
Rat 5 - Right leg	Rat 7 - Right leg	Rat 10 - Left leg	Rat 8 - Left leg
Rat 9 - Right leg	Rat 6 - Left leg	Rat 11 - Left leg	Rat 4 - Right leg
Rat 12 - Right leg	Rat 6 - Right leg	Rat 4 - Left leg	Rat 14 - Left leg
Rat 13 - Left leg	Rat 1 - Left leg	Rat 15 - Left leg	Rat 5 - Right leg
Rat 15 - Right leg	Rat 16 - Right leg	Rat 8 - Right leg	Rat 7 - Left leg
Rat 2 -Left leg	Rat 9 - Left leg	Rat 11 - Right leg	Rat 10 - Right leg
Rat 3 - Right leg	Rat 3 - Left leg	Rat 14 - Right leg	Rat 16 - left leg
Rat 12 - Left leg	Rat 13 - Right leg	Rat 1 - Right leg	Rat 2 - Right leg