

# Optimization of an Injectable, Resorbable, Bioactive Cement Able to Release the Anti-Osteoclastogenic Biomolecule ICOS-Fc for the Treatment of Osteoporotic Vertebral Compression Fractures

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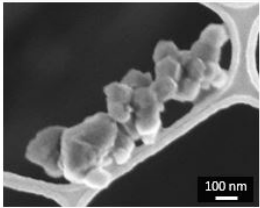
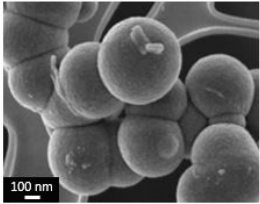
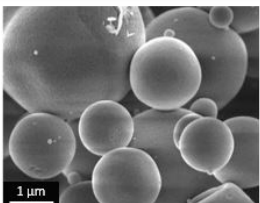
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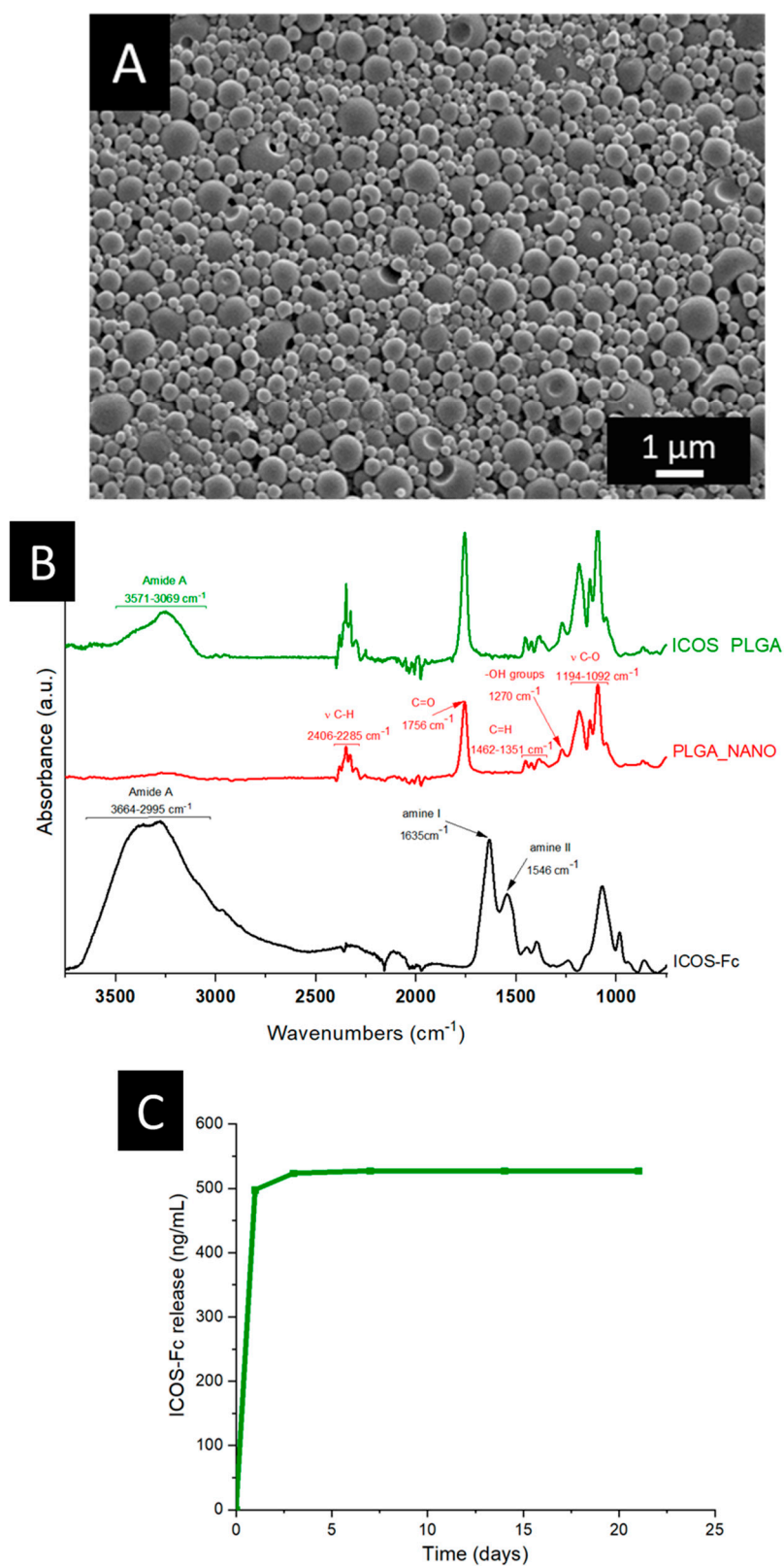
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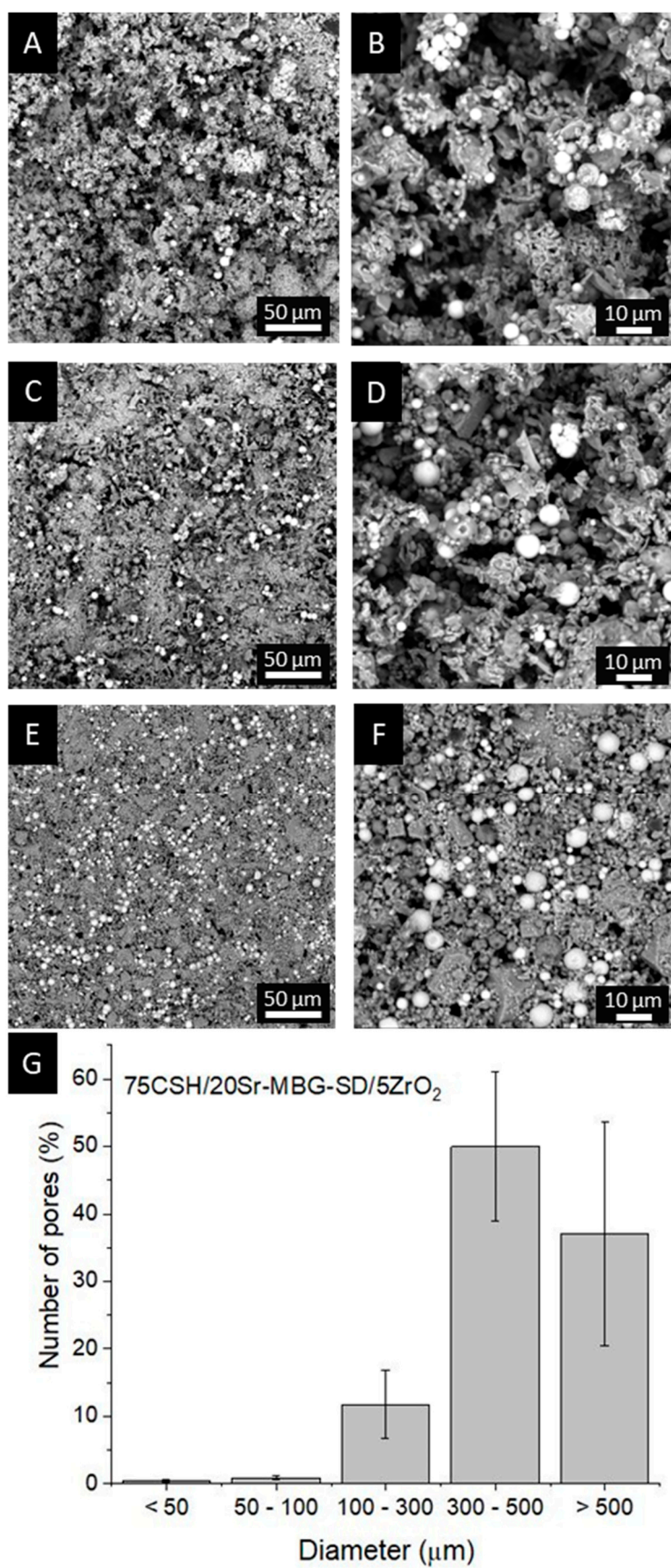
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	<b>Density</b>		<b>Size</b>		<b>Morphology</b>
<b>ZrO<sub>2</sub></b> (commercial available)	5.7 cm <sup>3</sup> /g		100-250 nm		
	<b>Specific Surface Area (SSA)</b>	<b>Pore Volume</b>	<b>Density</b>	<b>Size</b>	<b>Morphology</b>
<b>Sr-MBG-SG</b>	650 m <sup>2</sup> /g	0.7 cm <sup>3</sup> /g	0.8 cm <sup>3</sup> /g	100-500 nm	
<b>Sr-MBG -SD</b>	100 m <sup>2</sup> /g	0.1 cm <sup>3</sup> /g	2.2 cm <sup>3</sup> /g	1-5 μm	

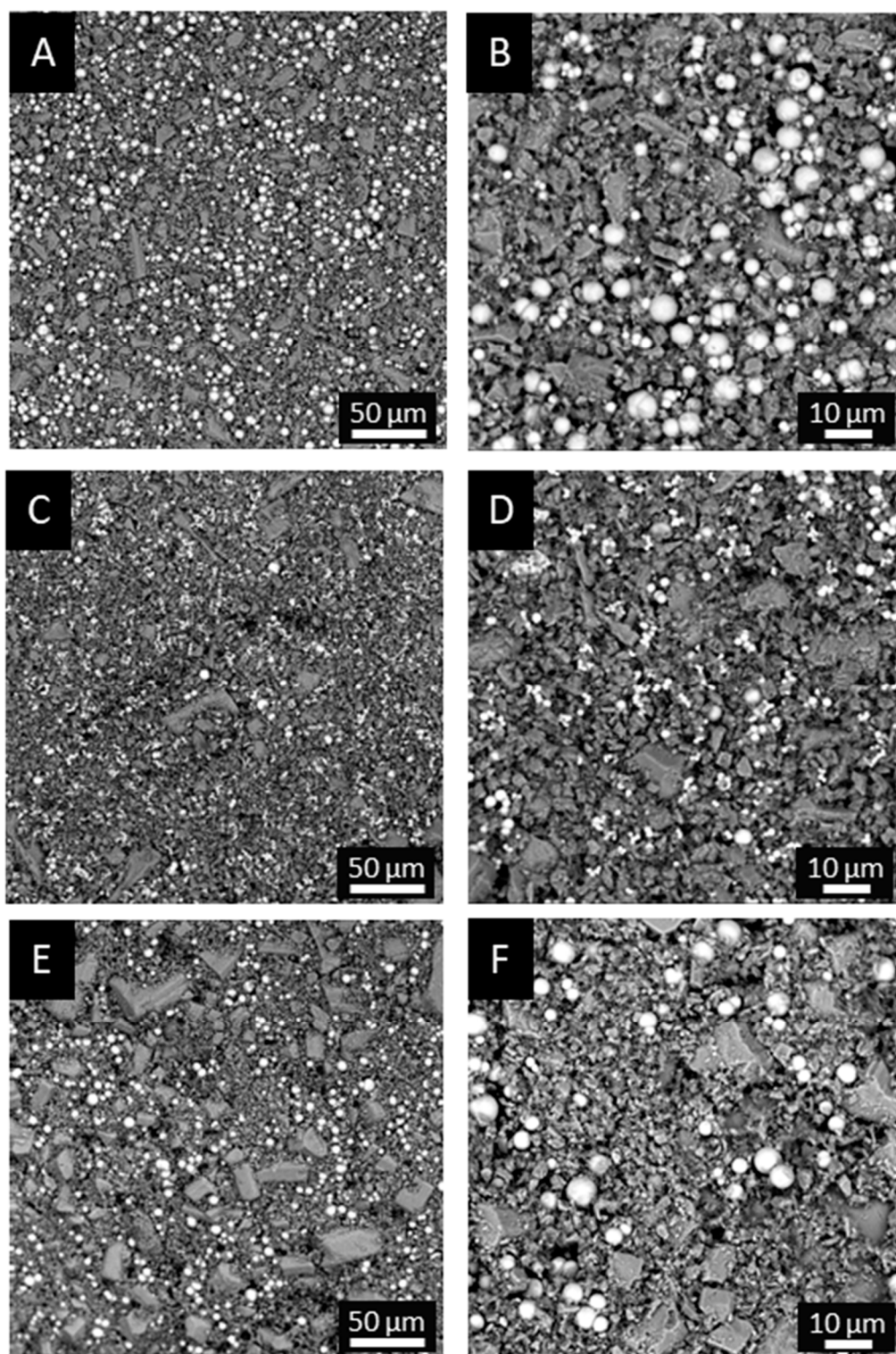
**Figure S1.** Characteristics and morphology of the commercially available ZrO<sub>2</sub> nanoparticles exploited to impart radiopacity to the cement as well as of the two types of Sr-MBGs separately included within the formulation to impart bioactive and pro-osteogenic features.



**Figure S2.** ICOS\_PLGA nanoparticles characterization: (A) FE-SEM images of ICOS\_PLGA nanoparticles (25,000 $\times$ ). (B) ATR-FTIR spectra of ICOS-Fc (black line), unloaded PLGA nanoparticles (red line) and ICOS\_PLGA carriers (green line). (C) Cumulative amount of released ICOS-Fc from PLGA nanoparticles as a function of time determined by means of Micro BCA Protein Assay.

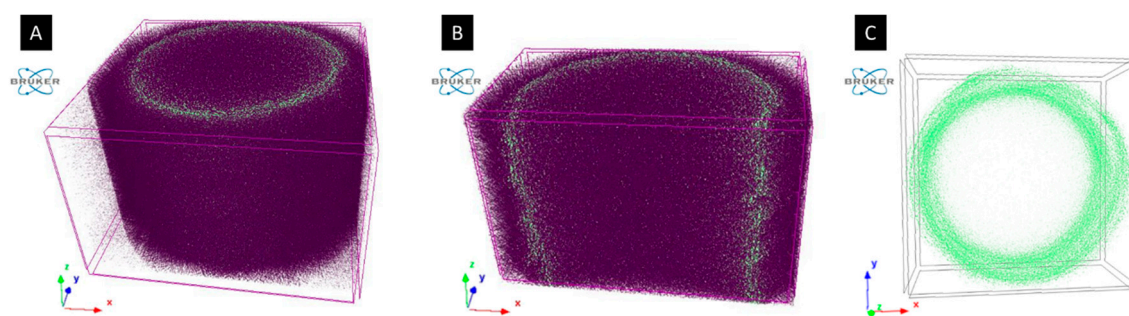


**Figure S3.** SEM images of cement formulations containing Sr-MBG-SD acquired at 1,000 $\times$  and 3,000 $\times$ : (A-B) 75CSH/20Sr-MBG-SD/5ZrO<sub>2</sub>, (C-D) 80CSH/15Sr-MBG-SD/5ZrO<sub>2</sub>, (E-F) 85CSH/10Sr-MBG-SD/5ZrO<sub>2</sub>. (G) Pore size distribution of the 75CSH/20Sr-MBG-SD/5ZrO<sub>2</sub> formulation.

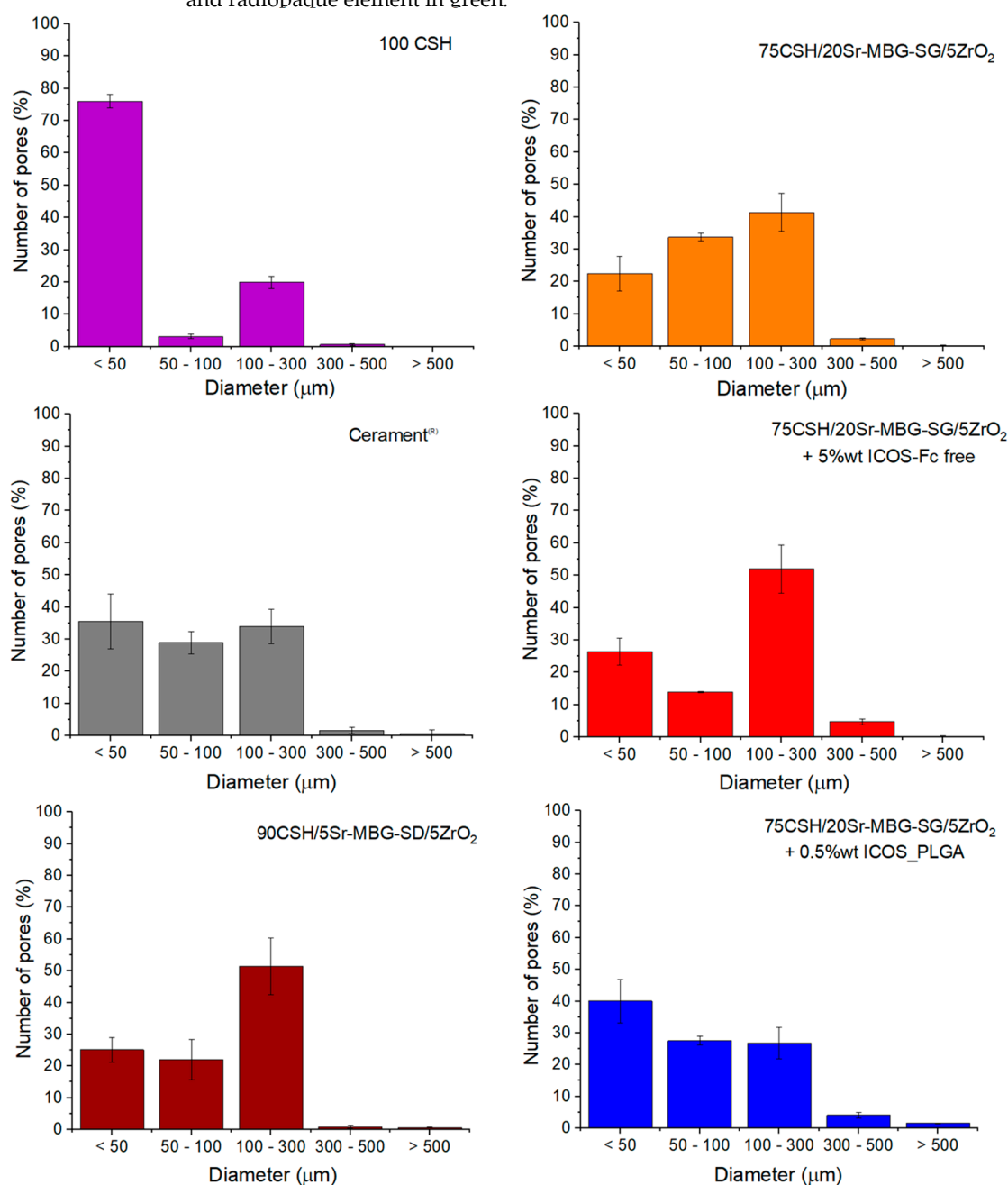


**Figure S4.** SEM images of cement formulations containing Sr-MBG-SG acquired at 1,000× and 3,000×: (A-B) 75CSH/20Sr-MBG-SG/5ZrO<sub>2</sub>, (C-D) 80CSH/15Sr-MBG-SG/5ZrO<sub>2</sub> and (E-F) 85CSH/10Sr-MBG-SG/5ZrO<sub>2</sub>.

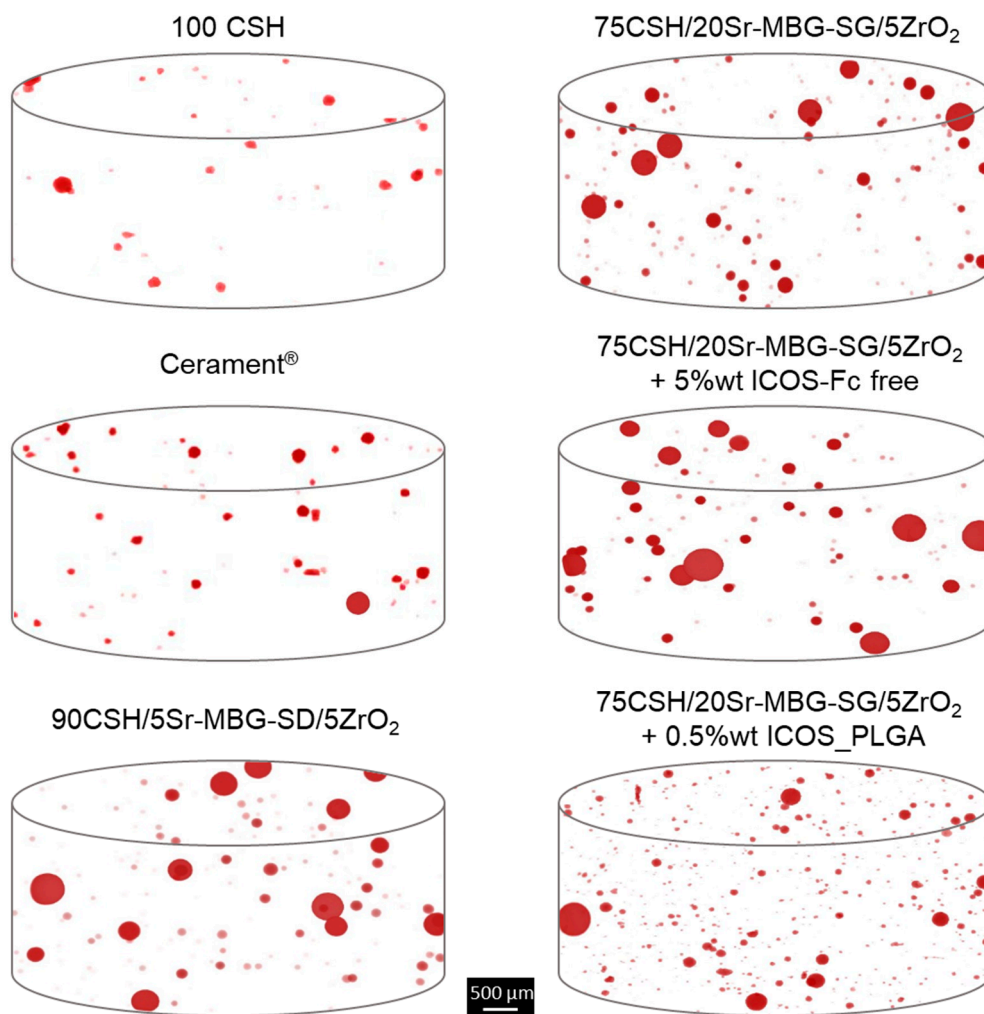




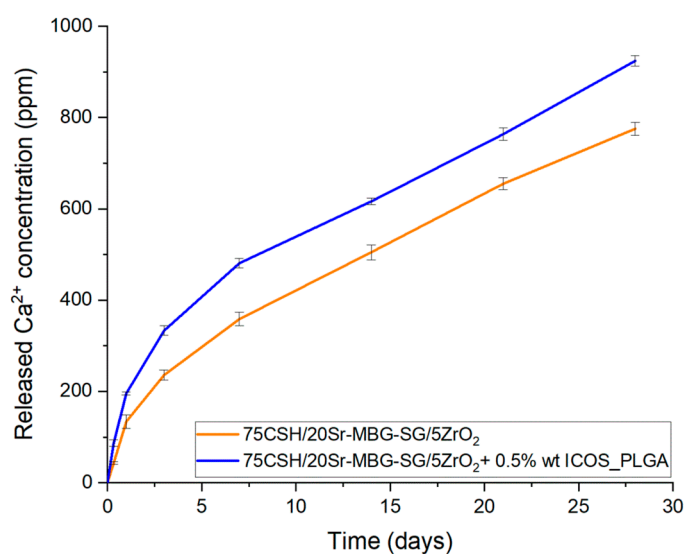
**Figure S5.** Radiopacity distribution of Cerament® visualized by means of CTVox software: (A) total volume, (B) cross-section and (C) only the radiopaque phase. Colors legend: CSD matrix in purple, and radiopaque element in green.



**Figure S6.** Pore size distributions of the investigated cements calculated through individual 3D analyses.



**Figure S7.** Qualitative representations of the pore distribution throughout the cement volumes obtained by using the Micro-CT software.



**Figure S8.** Calcium ion release kinetics from the optimized composite cement formulation with (orange line) and without (blue line) ICOS\_PLGA.