

Article

# Hydroxyapatite Thin Films of Marine Origin as Sustainable Candidates for Dental Implants

Gabriela Dorcioman <sup>1</sup>, Valentina Grumezescu <sup>1</sup>, George E. Stan <sup>2</sup>, Mariana Carmen Chifiriuc <sup>3,4,5</sup>, Gratiela Pircalabioru Gradisteanu <sup>4,6,\*</sup>, Florin Miculescu <sup>7</sup>, Elena Matei <sup>2</sup>, Gianina Popescu-Pelin <sup>1</sup>, Irina Zgura <sup>2</sup>, Valentin Craciun <sup>1</sup>, Faik Nüzhet Oktar <sup>8,9</sup> and Liviu Duta <sup>1,\*</sup>

<sup>1</sup> Lasers Department, National Institute for Lasers, Plasma and Radiation Physics, 077125 Magurele, Romania

<sup>2</sup> National Institute of Materials Physics, 077125 Magurele, Romania

<sup>3</sup> Department of Microbiology, Faculty of Biology, University of Bucharest, 060101 Bucharest, Romania

<sup>4</sup> Earth, Environmental and Life Sciences Division, Research Institute of the University of Bucharest (ICUB), 060101 Bucharest, Romania

<sup>5</sup> Romanian Academy, 010071 Bucharest, Romania

<sup>6</sup> Academy of Romanian Scientists, 051157 Bucharest, Romania

<sup>7</sup> Faculty of Materials Science and Engineering, Politehnica University of Bucharest, 060042 Bucharest, Romania

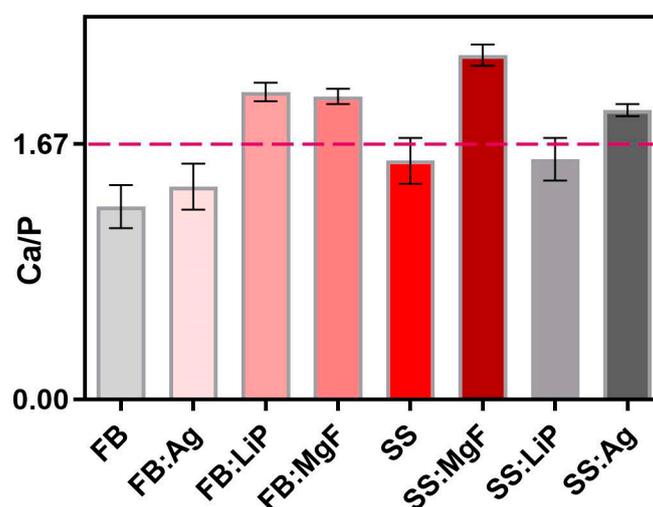
<sup>8</sup> Department of Bioengineering, Faculty of Engineering, University of Marmara, 34722 Istanbul, Turkey

<sup>9</sup> Advanced Nanomaterials Research Laboratory (ANRL), University of Marmara, 34722 Istanbul, Turkey

\* Correspondence: gratiela.gradisteanu@icub.unibuc.ro (G.P.G.); liviu.duta@inflpr.ro (L.D.); Tel.: +40-(0)7-2766-8664 (G.P.); +40-(0)2-1457-4450 (ext. 2023) (L.D.)

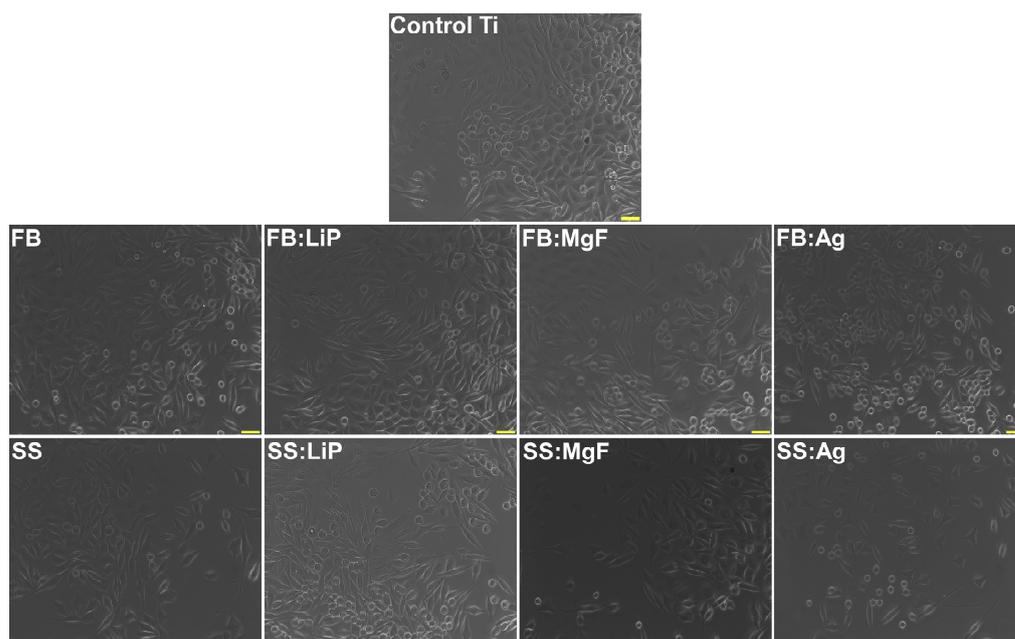
## 1. Preliminary *in vitro* testing

### 1.1 Apatite-forming ability

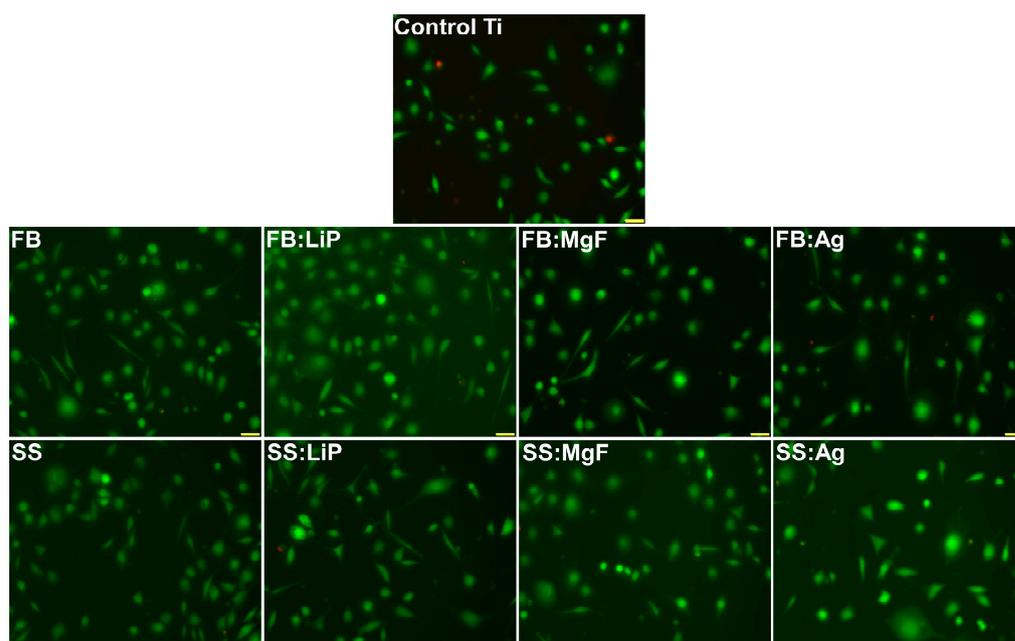


**Figure S1.** The values of the Ca/P ratio corresponding to the simple and doped FB si SS structures after immersion in SBF for 30 days. Note: dashed line represents the theoretical value of stoichiometric HA (~1.67).

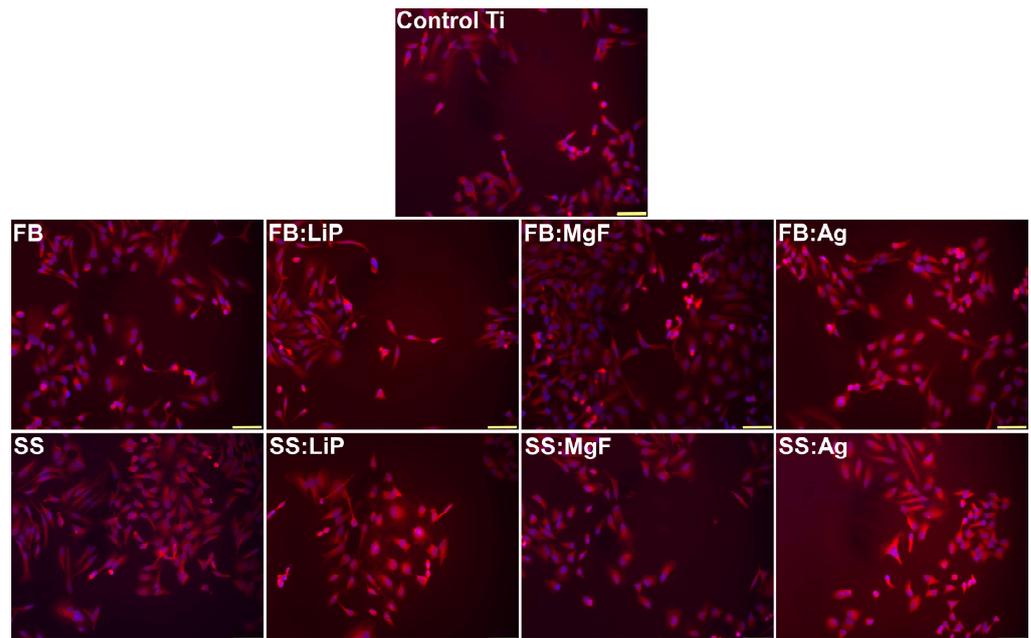
## 2. Cytocompatibility Assays



**Figure S2.** Contrast phase microscopy images of control Ti and simple and doped FB and SS thin films' biocompatibility, tested on osteoblast cells (G292). Magnification bar: 100 μm.

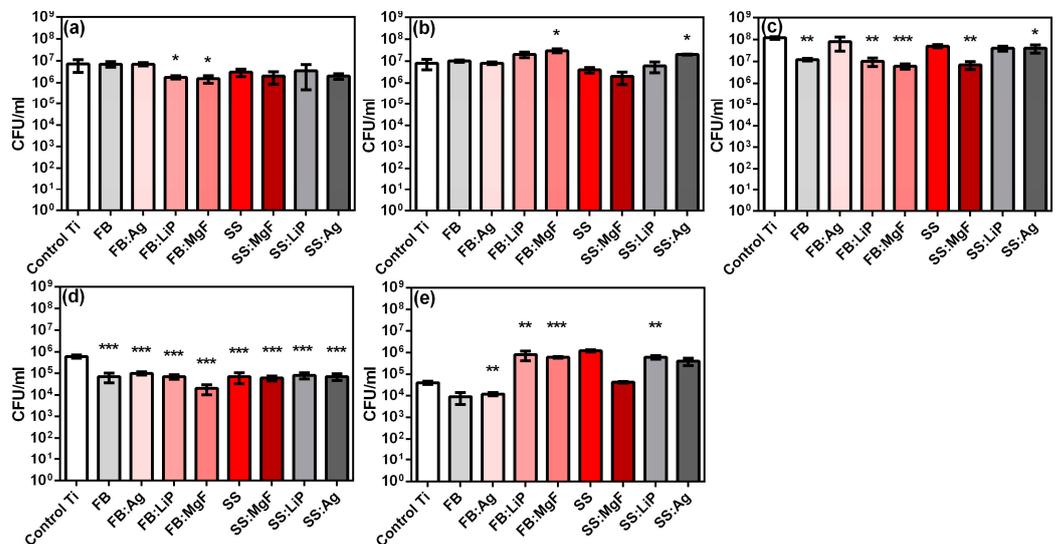


**Figure S3.** Fluorescence microscopy images of "Live/Dead" test, performed with NCTC L929 fibroblast cells on control Ti and simple and doped FB and SS thin films. Magnification bar: 100 μm.

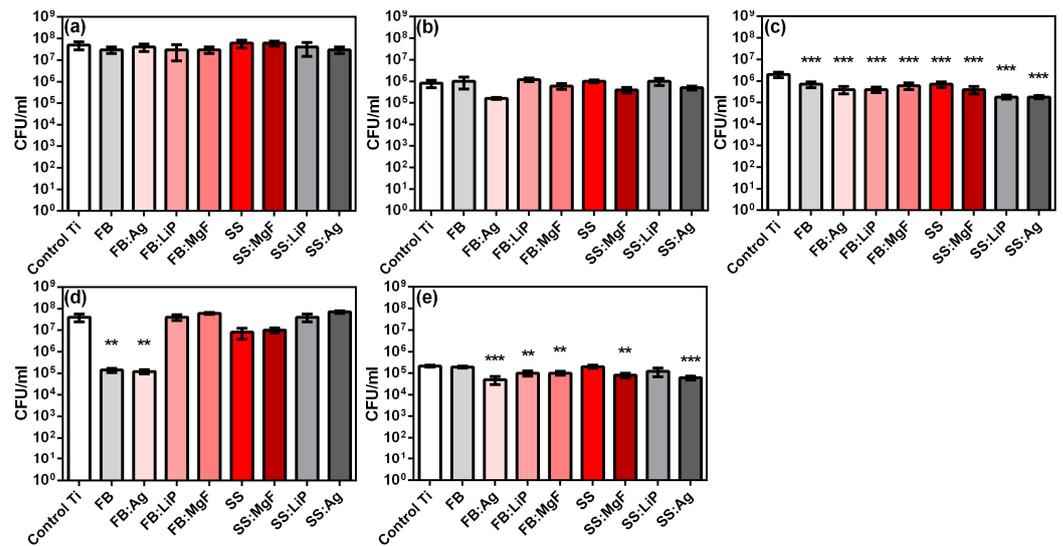


**Figure S4.** Fluorescence microscopy images acquired for control Ti and simple and doped FB and SS thin films using epithelial cells (HeLa). Magnification bar: 100  $\mu$ m.

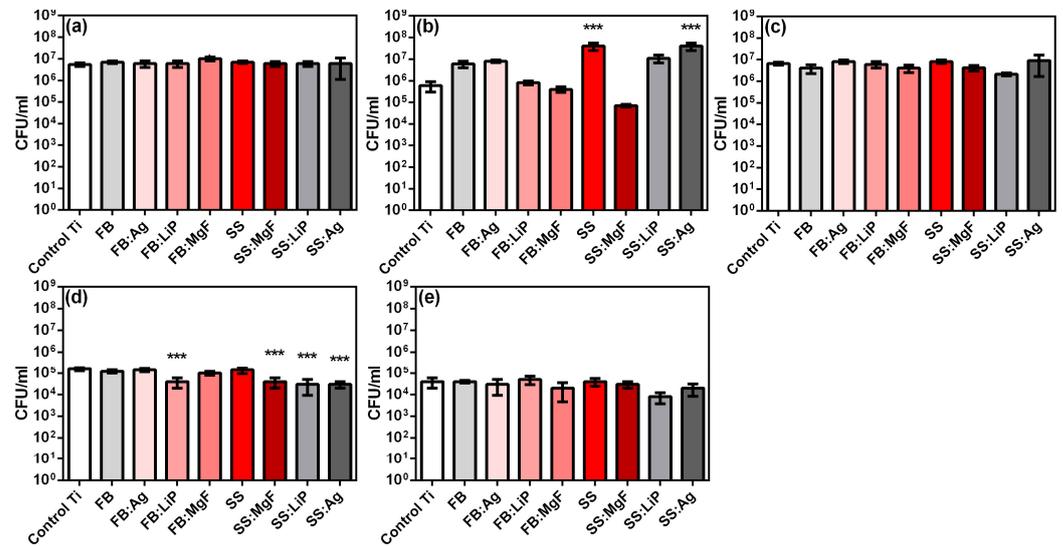
### 3. Antimicrobial Activity



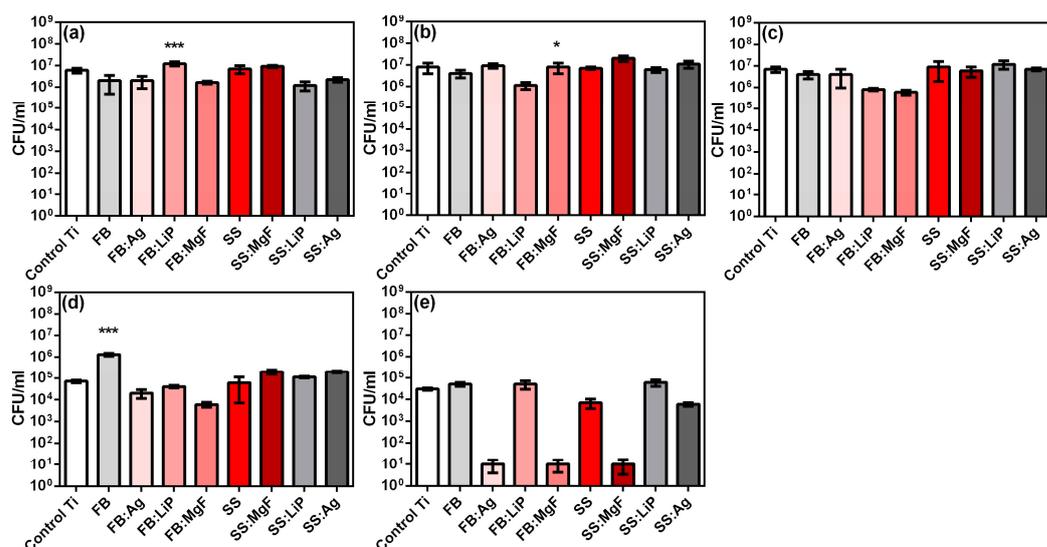
**Figure S5.** Number of microbial viable cells recovered from the biofilms developing on control Ti and simple and doped FB and SS thin films at T<sub>0</sub> (a), 2 h (b), 4 h (c), 24 h (d), and 48 h (e) using the *E. coli* strain (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.0001$ ).



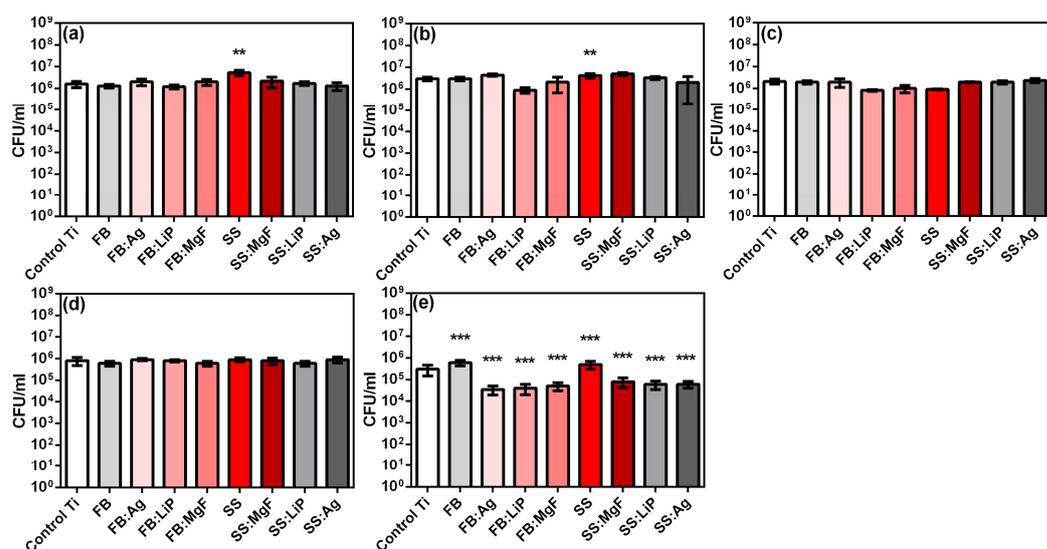
**Figure S6.** Number of microbial viable cells recovered from the biofilms developing on control Ti and simple and doped FB and SS thin films at  $T_0$  (a), 2 h (b), 4 h (c), 24 h (d), and 48 h (e) using the *P. aeruginosa* strain (\*\* $p < 0.01$ ; \*\*\* $p < 0.0001$ ).



**Figure S7.** Number of microbial viable cells recovered from the biofilms developing on control Ti and simple and doped FB and SS thin films at  $T_0$  (a), 2 h (b), 4 h (c), 24 h (d), and 48 h (e) using the *S. aureus* strain (\*\*\*) $p < 0.0001$ ).



**Figure S8.** Number of microbial viable cells recovered from the biofilms developing on control Ti and simple and doped FB and SS thin films at  $T_0$  (a), 2 h (b), 4 h (c), 24 h (d), and 48 h (e) using the *E. faecalis* strain (\* $p < 0.05$ ; \*\*\* $p < 0.0001$ ).



**Figure S9.** Number of microbial viable cells recovered from the biofilms developing on control Ti and simple and doped FB and SS thin films at  $T_0$  (a), 2 h (b), 4 h (c), 24 h (d), and 48 h (e) using the *C. albicans* strain (\*\* $p < 0.01$ ; \*\*\* $p < 0.0001$ ).