Electronic Supplementary Material (ESI) for Materials Advances.
This journal is © The Royal Society of Chemistry 2023

Supplementary information

for

Enhanced biocidal activity of Pr^{3+} doped yttrium silicates by Tm^{3+} and Yb^{3+} co-doping

Patryk Fałat[a], Min Ying Tsang[a,b], Irena Maliszewska[c], Szymon J. Zelewski[d], Bartłomiej Cichy[e], Tymish Y. Ohulchanskyy[f], Marek Samoć[a], Marcin Nyk[a], and Dominika Wawrzyńczyk[a,]*

[a]*Institute of Advanced Materials, Faculty of Chemistry, Wroclaw University of Science and Technology, Wyb. Wyspianskiego 27, 50-370 Wroclaw, Poland.*
[b]*Institute of Functional Materials, Faculty of Chemistry, University of Vienna, Währinger Straße 42, A-1090 Wien, Austria.*
[c]*Department of Organic and Medicinal Chemistry, Faculty of Chemistry, Wroclaw University of Science and Technology, Wyb. Wyspianskiego 27, 50-370 Wroclaw, Poland.*
[d]*Department of Semiconductor Materials Engineering, Faculty of Fundamental Problems of Technology, Wroclaw University of Science and Technology, Wyb. Wyspianskiego 27, 50-370 Wroclaw, Poland.*
[e]*Institute of Low Temperature and Structure Research, Polish Academy of Sciences, Okolna 2, 50-422 Wroclaw, Poland.*
[f]*College of Physics and Optoelectronic Engineering, Shenzhen University, Nanhai Avenue 3688, Nanshan District, Shenzhen 518060, Guangdong, China.*

** corresponding author: dominika.wawrzynczyk@pwr.edu.pl*

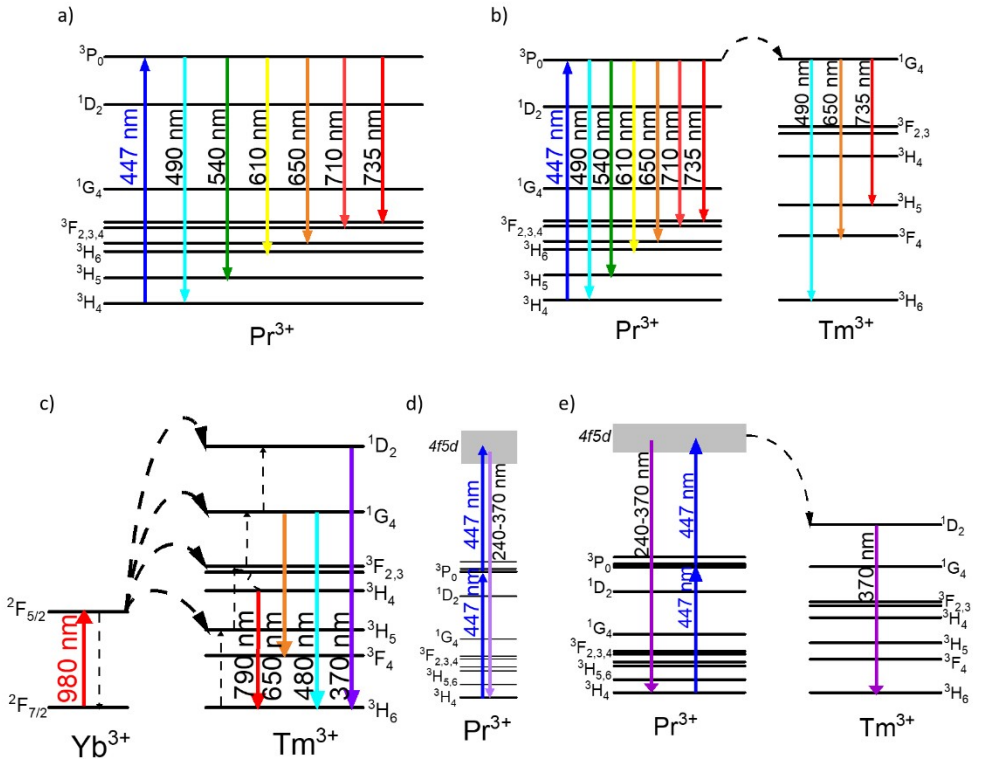

Figure S1. A schematic diagram of electronic levels and transitions corresponding to (a) down-conversion emission of Pr^{3+}ions upon 447 nm excitation, (b) down-conversion emission of Pr^{3+} and Tm^{3+}ions upon 447 nm excitation (c) NIR-to-UV up-conversion in a system bearing Pr^{3+}, Tm^{3+}, and Yb^{3+} ions upon 980 nm excitation, (d) VIS-to-UVC up-conversion of Pr^{3+} ions upon 447 nm excitation and (e) VIS-to-UV up-conversion in a system bearing Pr^{3+}, Tm^{3+}, and Yb^{3+}ions upon 447 nm excitation.

Materials and methods

Synthesis of lanthanide doped silicates

Y_2O_3 (99.99%), Yb_2O_3 (99.99%), Pr_2O_3 (99.99%), Tm_2O_3 (99.99%) and TEOS (99.999%) were purchased from Sigma Aldrich. EtOH (99.9%) and HNO_3 (65%) were purchased from Avantor Performance Materials Poland S.A. All chemicals were used as received, with no purification.

Table S1. Amounts of Y_2O_3, Pr_2O_3, Yb_2O_3 and Tm_2O_3 used for precursors syntheses.

$Y_2Si_2O_7$	**Y_2O_3**	**Pr_2O_3**	**Yb_2O_3**	**Tm_2O_3**
undoped		-	-	-
1.2%Pr	4.38 mmol	0.05 mmol	-	-
1.2% Pr, 0.5% Tm, 5% Yb	4.08 mmol	0.05 mmol	0.22 mmol	0.02 mmol

Crystal structure characterization

Table S2. Calculated cell parameters for studied un-doped and doped silicates.

	$Y_2Si_2O_7$		$Y_2Si_2O_7:Pr^{3+}$		$Y_2Si_2O_7:Pr^{3+},Tm^{3+},Yb^{3+}$
	mp-581644	mp-561551	mp-581644a	mp-581644b	mp-581644
Unit cell	P-1	P1 21/m1	P-1	P-1	P-1
a (Å)	6.60208	5.04427	6.53164	6.61358	6.60242
b (Å)	6.63591	8.08721	6.76527	6.66074	6.66096
c (Å)	36.07779	7.3275	36.28017	35.15834	36.10046
α (deg)	94.71056	90	94.98871	94.64206	94.53239
β (deg)	90.60159	108.5303	90.98531	90.72639	90.61315
γ (deg)	92.00661	90	91.81551	92.04543	92.10380

Spectroscopic studies

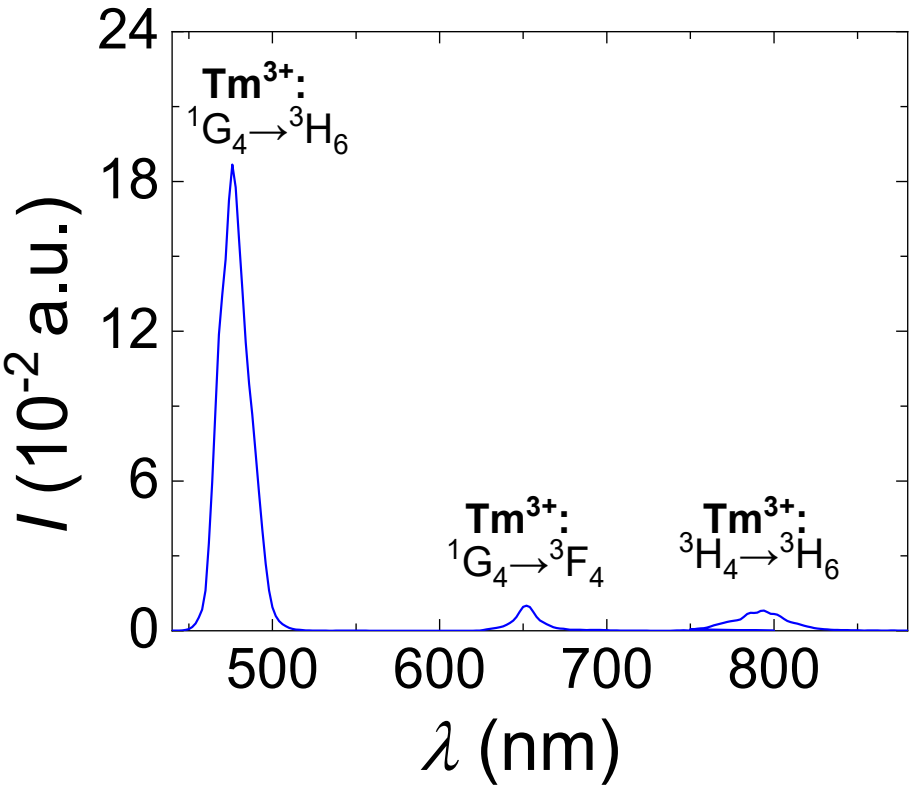

Figure S2. NIR-to-VIS up-conversion spectra of $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+} powders upon 980 nm CW laser excitation.

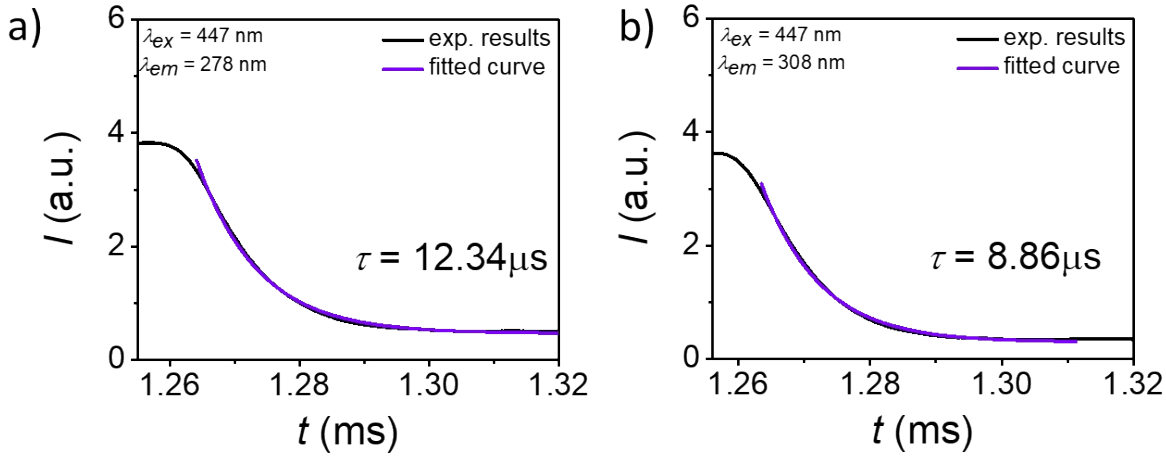

Figure S3. VIS-to-UVC UC photoluminescence time decay curves for $Y_2Si_2O_7$:Pr^{3+} powders upon 447 nm excitation measured at 278 nm (a) and 308 nm (b). Purple lines represent single exponential decay fitting lines.

Bacterial inactivation studies

Materials and methods

Four microbial species were used in this study: *Acinetobacter baumannii* (PCM 8740), *Staphylococcus aureus* (PCM 2024), *Bacillus cereus* (PCM 2021), and *Candida albicans* (ATCC 10231). One colony of each test organism was inoculated in 5 mL of Mueller-Hinton Broth (Oxoid). In the case of *C. albicans*, this medium was supplemented with 1% glucose. The suspensions were incubated for 24 h in the dark at 37 °C. After this time, each culture was centrifuged separately (5 min/6000 rpm) and the obtained pellet was suspended in 5 mL of phosphate buffered saline (PBS) sterile PBS to give an inoculum of approximately 1-2 x 10^6 colony-forming units (CFU/mL).

All experiments were preceded by examining: (*1*) the effect of light irradiation on the viability of planktonic cultures of *A. baumannii, S. aureus, B. cereus, and C. albicans* and (*2*) dark cytotoxicity of the tested materials.

Studies of the effect of light irradiation on the viability of the microorganisms were carried out by transferring 100 µL of a standardized cell suspension into each well of a microtiter plate and irradiating with laser light (447 nm, 650 mW) for 5, 7 and 10 minutes and the cell viability was studied using BacTiter-Glo™ test.

The determination of dark cytotoxicity of the tested materials was performed by adding 100 µl of a standardized cell suspension and 5, 15 or 30 mg/well of the test phosphor to a well of a microtiter plate. The plate was then incubated at 37 ºC in dark and the cell viability was monitored after 1, 4 and 8 h using BacTiter-Glo™ test.

All experiments were preceded by examining the effect of light irradiation on the viability of planktonic cultures of *A. baumannii, S. aureus, B. cereus, and C. albicans*. Studies of the effect of light irradiation on the viability of the test microorganisms were carried out by transferring 100 µL of a standardized cell suspension into each well of a microtiter plate and irradiating with laser light (447 nm, 650 mW) for 5, 7 and 10 minutes and the cell viability was studied using BacTiter-Glo™ test. The study of the effect of photoinactivation of pathogenic cells in planktonic cultures was investigated by adding 30 mg or 15 mg of un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+} to each well containing bacterial suspension. Then the microorganisms were irradiated with laser light for 5 and 7 minutes. After irradiation, the test phosphor was removed and the viability of the cells was determined by the BacTiter-Glo™ test. This microbial cell viability assay determines the number of viable cells in culture from the quantification of ATP present in living cells. The test is based on the measurement of the

luminescent signal which is proportional to the amount of ATP and is directly proportional to the number of viable cells in the culture. The ATP detection assay is based on the properties of a thermostable luciferase (Ultra-Glo™ Recombinant Luciferase). All tests were performed in duplicate.

The reduction in planktonic cell viability was calculated using the following formula:

$$\frac{(LI - LB) - (LT - LB)\ x\ 100\%}{LI - LB}$$

LI- luminescence intensity of initial sample (microbial standardized suspension before irradiation)

LB- luminescence intensity of background sample (no cells)

LT- luminescence intensity of the tested sample (microbial standardized suspension after irradiation)

Results

The studies of photo-inactivation of *S. aureus*, *B. cereus*, *A. baumannii*, and *C. albicans* were started by determining the effect of light irradiation on viability of the tested microorganisms. It was established that the laser irradiation time up to 7 minutes did not cause significant lethality of the microbial cells (the mortality rate did not exceed 15±3%; data not shown). The dark cytotoxicity of the studied powder materials against *Bacillus cereus* (PCM 2021), *Acinetobacter baumannii* (PCM 8740), *Staphylococcus aureus* (PCM 2024), and *Candida albicans* (ATCC 10231) was shown in Table S3. As can be seen in this table, the dark cytotoxicity of the phosphors against planktonic and biofilm cultures was insignificant and in most cases the cell mortality did not exceed 10-12%.

Table S3. The effect of un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+} on the viability of microorganisms in dark (dark cytotoxicity).

Powder materials	*B. cereus*					
	Planktonic			Biofilm		
	Time of incubation [h]					
	1	4	8	1	4	8
	Viability [%]					
un-doped $Y_2Si_2O_7$;						
5 mg/well	96±3	93±2	91±3	98±3	95±3	93±3
15 mg/well	92±3	91±2	89±3	96±3	95±2	95±2
30 mg/well	90±3	89±2	86±3	91±2	90±3	90±3

$Y_2Si_2O_7:Pr^{3+}$,						
5 mg/well	86±2	86±2	82±2	96±2	93±2	90±3
15 mg/well	84±2	84±2	80±3	93±2	91±2	91±2
30 mg/well	80±2	80±2	80±3	90±2	90±2	90±2
$Y_2Si_2O_7:Pr^{3+},Tm^{3+},Yb^{3+}$						
5 mg/well	83±3	81±3	80±3	94±3	91±3	88±3
15 mg/well	80±3	79±3	76±3	89±3	86±3	82±3
30 mg/well	75±3	70±3	70±3	86±3	83±3	80±3
Powder materials	*A. baumannii*					
	Planktonic			Biofilm		
	Time of incubation [h]					
	1	4	8	1	4	8
	Viability [%]					
un-doped $Y_2Si_2O_7$;						
5 mg/well	96±2	94±2	94±2	97±3	97±3	95±3
15 mg/well	92±2	91±2	90±2	93±3	94±3	93±3
30 mg/well	90±3	88±2	86±2	93±2	91±2	90±2
$Y_2Si_2O_7:Pr^{3+}$,						
5 mg/well	91±2	90±2	90±2	93±2	93±2	93±3
15 mg/well	86±3	86±3	86±3	90±2	90±2	90±2
30 mg/well	84±2	84±2	82±2	86±2	85±3	86±3
$Y_2Si_2O_7:Pr^{3+},Tm^{3+},Yb^{3+}$						
5 mg/well	91±2	91±2	90±2	90±3	90±3	90±3
15 mg/well	88±2	85±2	86±2	90±2	87±2	96±2
30 mg/well	86±2	82±2	81±2	89±3	86±2	85±2
Powder materials	*S. aureus*					
	Planktonic			Biofilm		
	Time of incubation [h]					
	1	4	8	1	4	8
	Viability [%]					
un-doped $Y_2Si_2O_7$;						
5 mg/well	93±3	90±3	90±3	96±2	95±2	96±2
15 mg/well	91±2	90±2	88±3	93±2	96±2	94±2
30 mg/well	90±2	87±2	86±2	92±2	91±3	92±3
$Y_2Si_2O_7:Pr^{3+}$,						
5 mg/well	90±2	88±2	86±2	92±2	91±2	91±3
15 mg/well	83±2	83±2	84±3	92±2	92±3	92±2
30 mg/well	82±2	80±2	80±2	90±2	90±3	90±3
$Y_2Si_2O_7:Pr^{3+},Tm^{3+},Yb^{3+}$						
5 mg/well	88±2	86±2	84±2	90±2	90±3	88±2
15 mg/well	84±2	82±2	81±2	90±2	90±2	83±2
30 mg/well	82±2	80±3	80±2	88±2	85±2	80±2
Powder materials	*C. albicans*					
	Planktonic			Biofilm		
	Time of incubation [h]					
	1	4	8	1	4	8
	Viability [%]					
un-doped $Y_2Si_2O_7$;						
5 mg/well	94±2	92±2	91±2	96±2	96±3	94±3
15 mg/well	91±2	87±2	82±2	93±3	91±3	92±3
30 mg/well	90±2	87±2	86±2	92±2	92±2	93±3
$Y_2Si_2O_7:Pr^{3+}$,						
5 mg/well	90±2	86±2	86±2	91±2	90±2	90±2
15 mg/well	90±2	86±3	85±2	91±2	90±3	90±3
30 mg/well	89±2	86±2	86±2	90±2	86±2	88±3
$Y_2Si_2O_7:Pr^{3+},Tm^{3+},Yb^{3+}$						
5 mg/well	89±2	86±2	82±2	90±2	90±3	90±2
15 mg/well	86±2	83±2	80±2	88±2	86±2	86±2
30 mg/well	82±2	80±2	80±3	86±2	86±2	85±3

The control (100% viability) consisted of microbial suspensions or biofilms incubated in the dark without the addition of the tested silicates.
*Viability of biofilms was assessed by the MTT test.

The experiments on photo-eradication of pathogens were carried out for two doses of light *i.e.* 250 J/cm^2 and 350 J/cm^2 which corresponded to 5 and 7 minutes of exposure of cells to 447 nm laser light (650 mW), respectively. As can be seen in Figure S4a and b, all tested samples, after excitation with laser light, showed significant cytotoxic activity against planktonic culture of *A. baumannii* and mortality rate was dependent on the type and concentration of sample and the exposure time. When 30 mg of material per well was used in the experiments, the five-minute exposure to laser light resulted in a reduction in the number of bacterial cells by 85.7±2.6, 83.7±2.6 and 94.2±2.8 for un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4a). Longer time of light treatment (7 min) caused a higher cell mortality of pathogenic cells amounting to 88.8±2.7, 86.5±2.6 and 98.4±3.0 for un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Figure S4a).
As depicted in Figure S4b, the five-minute exposure of the tested phosphors to laser light in the presence of 15 mg/well of the investigated materials resulted in a reduction in the number of bacterial cells by 53.0±1.6, 29.5±0.9, and 64.5±1.9 for un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively. The 7 min light treatment caused a higher cell mortality of *A. baumannii* cells amounting to 63.0±1.9, 39.0±1.2 and 71.0±2.1 for un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4b).
The effect of photo-inactivation of planktonic cells of *B. cereus* is shown in Figure S4c and d. The obtained results confirmed that mortality rate of pathogenic bacteria depended on the type and concentration of sample, as well as the irradiation time. When the bacterial cells were exposed to the laser light for 5 minutes in the presence of 30 mg/well of the materials, the mortality rate was 65.3±2.0, 42.1±1.3 and 84.8±2.5 for un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4c). The seven-minute exposure of bacteria to laser light resulted in a reduction in the number of cells by 68.5±2.1, 56.1±1.7 and 95.1±2.9 for un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+}, and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4c). The five-minute exposure of the studied planktonic culture to laser light in the presence of 15 mg/well of un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+} resulted in a reduction in the number of bacterial cells by 53.0±1.6, 40.5±1.2 and 76.5±2.3, respectively (Fig. S4d). Longer times of light treatment (7 min) caused a higher cell mortality of the studied bacterial

cells amounting to 63.5±1.9 51.3±1.5 and 85.6±2.6 for undoped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4d).

As shown in Figure S4e and f, the reduction in the number of the tested pathogenic yeast cells was also dependent on the type and concentration of sample and the exposure time. After a 5-minute exposure of the *C. albicans* suspension culture to laser light (in the presence of 30 mg/well of the synthesized lanthanide-doped phosphors), the cell death rate was 80.4±2.4 19.7±0.6 and 77.5±2.3 for un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4e). Extending the exposure time to 7 minutes resulted in the significantly higher cell eradication, amounting to 81.1±2.4, 31.9±1.0 and 81.0±2.4 for undoped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4e). When 15 mg of each compound per well was used in eradication experiment, the five-minute exposure to laser light resulted in a reduction in the number of planktonic culture of *C. albicans* by 33.5±0.6, 18.7±0.6 and 75.8±2.3 for undoped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4f). Longer times of light treatment (7 min) caused a slightly higher cell mortality amounting to 39.0±1.2, 21.0±0.9 and 80.9±2.4 for un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7Pr^{3+}$ and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4f).

The effect of photo-inactivation of planktonic cells of *S. aureus* is presented in Figure S4g and h. The obtained results confirmed that mortality rate of pathogenic coccus depended on the type and concentration of sample, and irradiation time. When the pathogenic cells were exposed to the laser light for 5 minutes (in the presence of 30 mg/well of the ceramics), the mortality rate was 38.5±1.2, 22.5±0.7 and 80.3±2.4 for un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4g). The seven-minute exposure of bacteria to laser light resulted in a reduction in the number of cells by 45.5±1.4, 27.5±0.8 and 86.0±2.6 for un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4g). As can be seen in Figure S4h, the five-minute irradiation of the studied planktonic culture with laser light in the presence of 15 mg/well of the investigated materials reduced the number of this pathogenic cells by 35.5±1.1, 22.0±0.7 and 76.3±2.3 for undoped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+}, and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively. Prolonging the time of microorganism irradiation with laser light to 7 minutes (under the same experimental conditions) caused a reduction in the number of the viable cells by 40.0±1.2, 20.0±0.6 and 80.3±2.4for un-doped $Y_2Si_2O_7$, $Y_2Si_2O_7$:Pr^{3+} and $Y_2Si_2O_7$:Pr^{3+},Tm^{3+},Yb^{3+}, respectively (Fig. S4h).

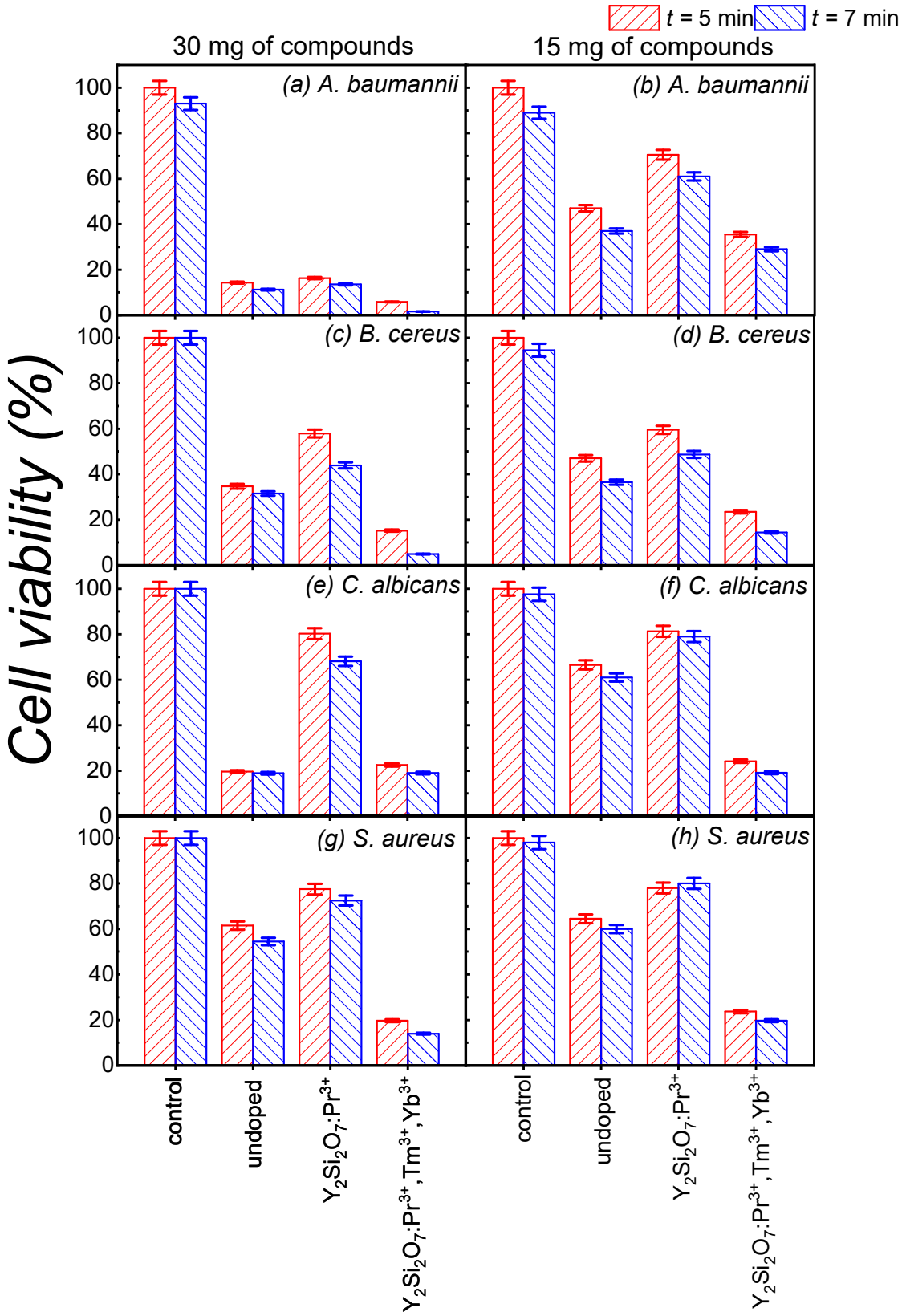

Figure S4. The effect of 447 nm CW laser irradiation time (5 min or 7 min) on the viability of (a,b) *A. baumannii*, (c,d) *B. cereus* (e,f) *C. albicans* and (g,h) *S. aureus* in the presence of 30 mg (left column) or 15 mg (right column) of the investigated silicates, respectively.

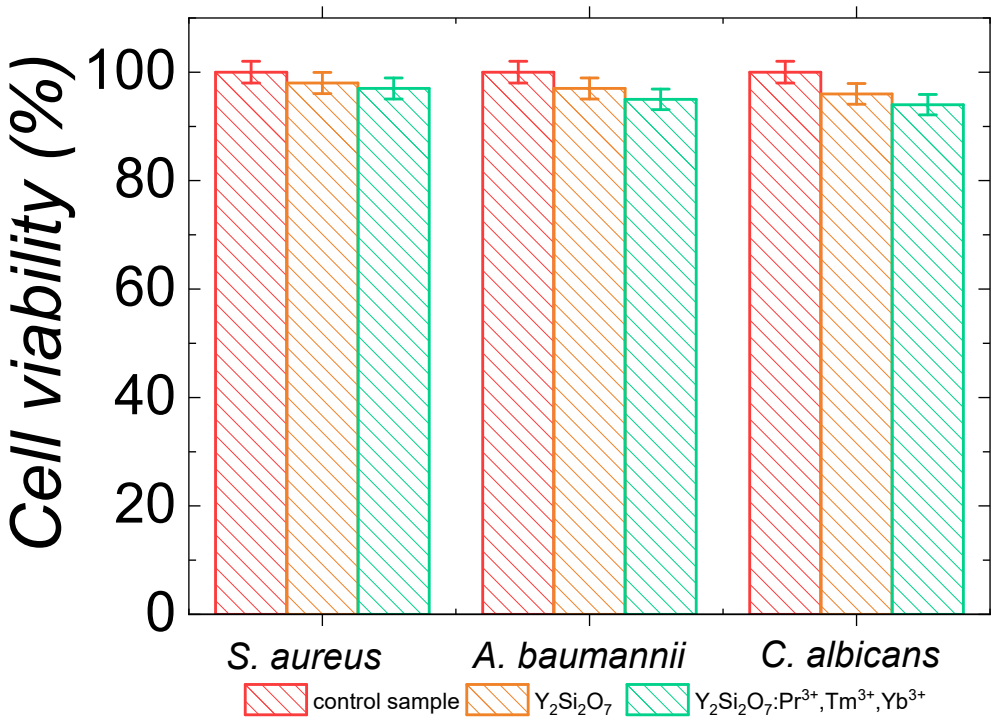

Figure S5. Viability of biofilm (*S. aureus*; *A. baumannii* and *C. albicans*) cells after irradiation with 980 nm laser light (~500 mW/cm^2) in the presence of un-doped $Y_2Si_2O_7$ and $Y_2Si_2O_7:Pr^{3+},Tm^{3+},Yb^{3+}$ phosphor, and without them (control). 100% represents viability of non-irradiated cells.

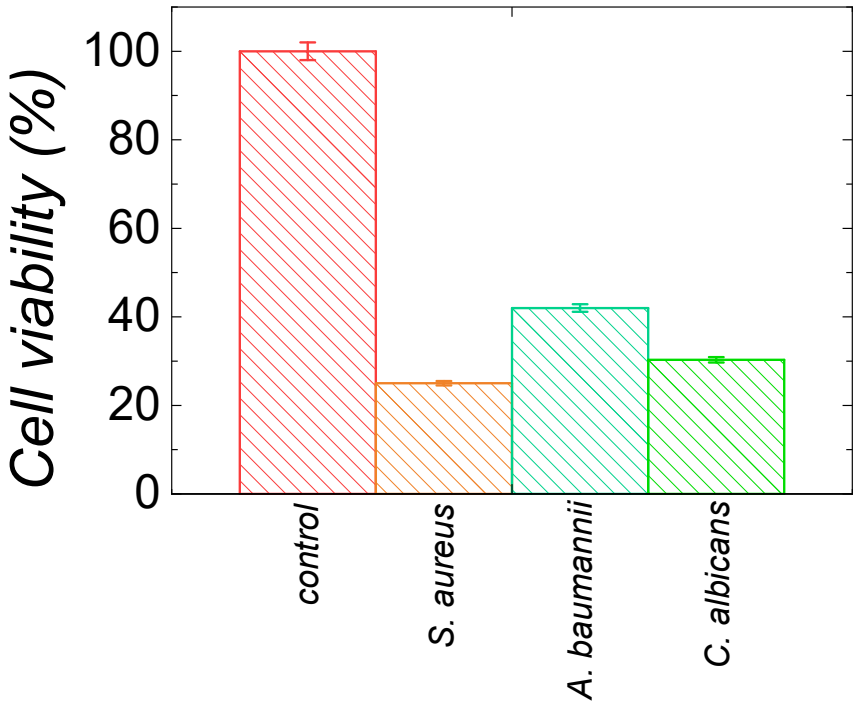

Figure S6. Viability of biofilm (S. aureus; A. baumannii and C. albicans) cells after irradiation with UV emitting lamp.