

Supporting Information for

Enhancement of copper antiviral activity with glutathione treatment

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Determination of corrosion parameter $1/Z_{diff}$ based on EIS results

The EIS data were analyzed with an equivalent circuit shown in supplementary Figure S3 [20]. Capacitance components approach to zero as negligible at high frequency whereas those approach to infinity at low frequency. When we define the impedance at high frequency range (20 kHz) and at low frequency range (10 mHz) as Z_{high} and Z_{low} , respectively, these can be expressed as following equations;

$$Z_{high} = R_s$$

$$Z_{low} = 2R_c + R_s$$

where R_c and R_s indicate charge transfer resistance and the sum of electric resistance of electrolyte and EIS measurement system, respectively. When we consider the difference between these impedances as Z_{diff} , it can be written as follows;

$$Z_{diff} = Z_{low} - Z_{high} = 2R_c$$

Since the corrosion rate, I_{corr} is proportional to the reciprocal number of R_c , $1/Z_{diff}$ is employed as a corrosion parameter to monitor the change in the corrosion rate as

$$I_{corr} = k / R_c = k' / Z_{diff}$$

where k and k' are constants. EIS measurements were performed in triplicate.

Determination of oxide thickness based on the results of chronopotentiometric measurement

During the chronopotentiometric measurement, the copper cation in the oxide is reduced to the metal form, and calculated using the Faraday's law of electrolysis as follows [22]:

$$m_{\text{Cu-Cu(OH)}_2} = (M_{\text{Cu}} \times I \times t_{\text{Cu(OH)}_2}) / 2F$$

$$m_{\text{Cu-CuO}} = (M_{\text{Cu}} \times I \times t_{\text{CuO}}) / 2F$$

$$m_{\text{Cu-Cu}_2\text{O}} = (M_{\text{Cu}} \times I \times t_{\text{Cu}_2\text{O}}) / F$$

where $m_{\text{Cu-Cu(OH)}_2}$, $m_{\text{Cu-CuO}}$ and $m_{\text{Cu-Cu}_2\text{O}}$ indicate the weight (g) of reduced Cu in Cu(OH)_2 , CuO and Cu_2O layer, respectively. M_{Cu} , F , and I represent the molecular weight (g/mol) of Cu, Faraday constant (9.6485×10^4 C/mol), and current density (0.11 mA/cm²), respectively. $t_{\text{Cu(OH)}_2}$, t_{CuO} and $t_{\text{Cu}_2\text{O}}$ indicate the reduction time of Cu(OH)_2 , CuO and Cu_2O , respectively. The amounts (weight) of the oxides and hydroxide were calculated by the following equations:

$$m_{\text{Cu(OH)}_2} = m_{\text{Cu-Cu(OH)}_2} \times (M_{\text{Cu(OH)}_2} / M_{\text{Cu}})$$

$$m_{\text{CuO}} = m_{\text{Cu-CuO}} \times (M_{\text{CuO}} / M_{\text{Cu}})$$

$$m_{\text{Cu}_2\text{O}} = m_{\text{Cu-Cu}_2\text{O}} \times (M_{\text{Cu}_2\text{O}} / 2M_{\text{Cu}})$$

where $M_{\text{Cu(OH)}_2}$, M_{CuO} and $M_{\text{Cu}_2\text{O}}$ represent the molecular weight of Cu(OH)_2 , CuO and Cu_2O (97.6, 79.6, 143.1 g/mol), respectively. The thicknesses (cm) of the oxide layers were decided using the following equations:

$$y_{\text{Cu(OH)}_2} = m_{\text{Cu(OH)}_2} / (S \times d_{\text{Cu(OH)}_2})$$

$$y_{\text{CuO}} = m_{\text{CuO}} / (S \times d_{\text{CuO}})$$

$$y_{\text{Cu}_2\text{O}} = m_{\text{Cu}_2\text{O}} / (S \times d_{\text{Cu}_2\text{O}})$$

where S , $d_{\text{Cu(OH)}_2}$, d_{CuO} and $d_{\text{Cu}_2\text{O}}$ indicate the surface area of the working electrode (0.899 cm²) and the densities of Cu(OH)_2 , CuO and Cu_2O (3.37, 6.31 and 6.0 g/cm³), respectively.

Table S1. Chemical compositions of testing materials (wt.%)

	Cu	Ni	Fe	Mn	Pb	Zn	Sn	P	Si	C	Al	S
C1020	>99.99	—	—	—	—	—	—	—	—	—	—	—
C5191	Rem.	—	0.003	—	0.002	0.001	6.55	0.096	—	—	—	—
C7150	Rem.	30.11	0.28	0.770	0.001	0.001	—	—	—	—	—	—
C2680	65.0	—	0.00	—	0.00	Rem.	—	—	—	—	—	—
Constantan	Rem.	44.6	—	0.98	—	—	—	—	—	—	—	—
MONEL400	Rem.	64.7	0.03	1.56	—	—	—	—	0.34	0.004	0.001	0.001

Table S2. The statistical analysis applied to the pair of the results in different treatment solutions and materials obtained by antiviral assay.

Figs.	Material/Treatment soln. A	Material/Treatment soln. B	Analytical Method	p values
1(a)	C1020+4mM GSH in 99%EtOH	C1020+99%EtOH	Parallelism	p < 0.001 for slope
	C1020+4mM GSH in 99%EtOH	C1020	Parallelism	p < 0.001 for slope
	C1020+4mM GSH in 99%EtOH	Glass+4mM GSH in 99%EtOH	Parallelism	p < 0.001 for intercept
	C1020+4mM GSH in 99%EtOH	Glass+99%EtOH	Parallelism	p < 0.001 for intercept
1(b)	C1020+4mM GSH in 99%EtOH	C1020+4mM GSH in 95%EtOH	Parallelism	p<0.001 for slope
	C1020+4mM GSH in 99%EtOH	C1020+4mM GSH in 80%EtOH	Parallelism	p<0.001 for slope
	C1020	C1020+4mM GSH in 95%EtOH	Parallelism	p<0.001 for slope
	C1020	C1020+4mM GSH in 80%EtOH	Parallelism	p<0.05 for slope
2	C1020+4mM GSH in 99%EtOH	C1020	Parallelism	p<0.001 for slope
	C5191+4mM GSH in 99%EtOH	C5191	Parallelism	p<0.001 for slope
	Constantan+4mM GSH in 99%EtOH	Constantan	Parallelism	p<0.001 for slope
	C2680+4mM GSH in 99%EtOH	C2680	Parallelism	p < 0.001 for intercept
	MONEL+4mM GSH in 99%EtOH	MONEL	Parallelism	p < 0.001 for intercept
	C7150+4mM GSH in 99%EtOH	C7150	NA	
S4	C1020+4mM GSH in 99%EtOH	C1020+2mM GSH in 99%EtOH	Parallelism	p<0.001 for slope
	C1020+4mM GSH in 99%EtOH	C1020+1mM GSH in 99%EtOH	Parallelism	p<0.001 for slope
	C1020	C1020+2mM GSH in 99%EtOH	Parallelism	p < 0.001 for intercept
	C1020	C1020+1mM GSH in 99%EtOH	Parallelism	p < 0.001 for intercept
S5	C1020+4mM GSH in 99%EtOH @5min	C1020@5min	t-test	p < 0.01
	C1020+4mM GSH in 99%EtOH @10min	C1020@10min	t-test	p < 0.01

C1020, C5191, C7150, C2680, Constantan, MONEL: testing materials. See Table S1.

GSH: glutathione

EtOH: ethanol

Parallelism: Parallelism test for 2 regression lines.

NA: Not applicable.

t-test: Student's t-test.

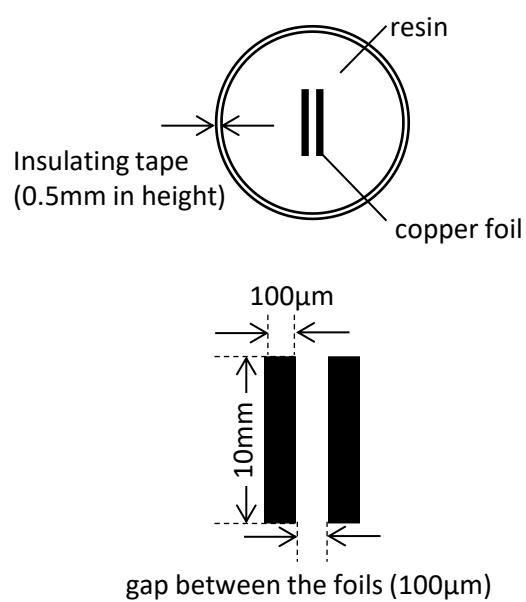


Fig.S1 Schematic explanation of the specimen for electrochemical impedance measurement

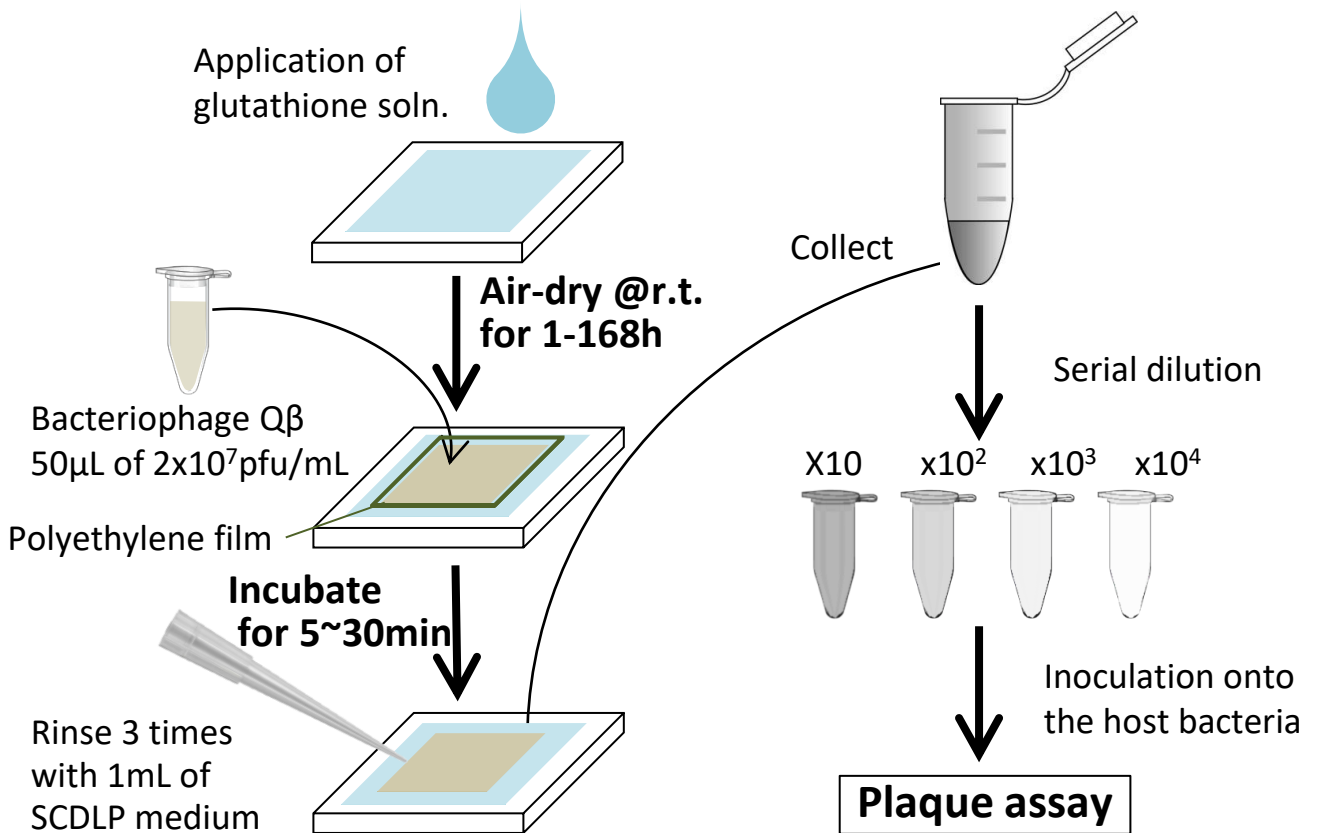


Fig.S2 Schematic illustration of antiviral assay using bacteriophage Q β

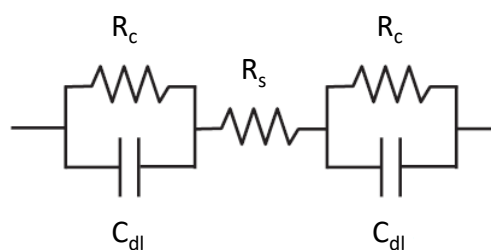


Fig. S3 Equivalent circuit model used for data analysis of electrochemical impedance spectroscopy

R_s : the sum of the electric resistance of electrolyte and the EIS system used for the measurement

R_c : charge transfer resistance

C_{dl} : double-layer capacitance

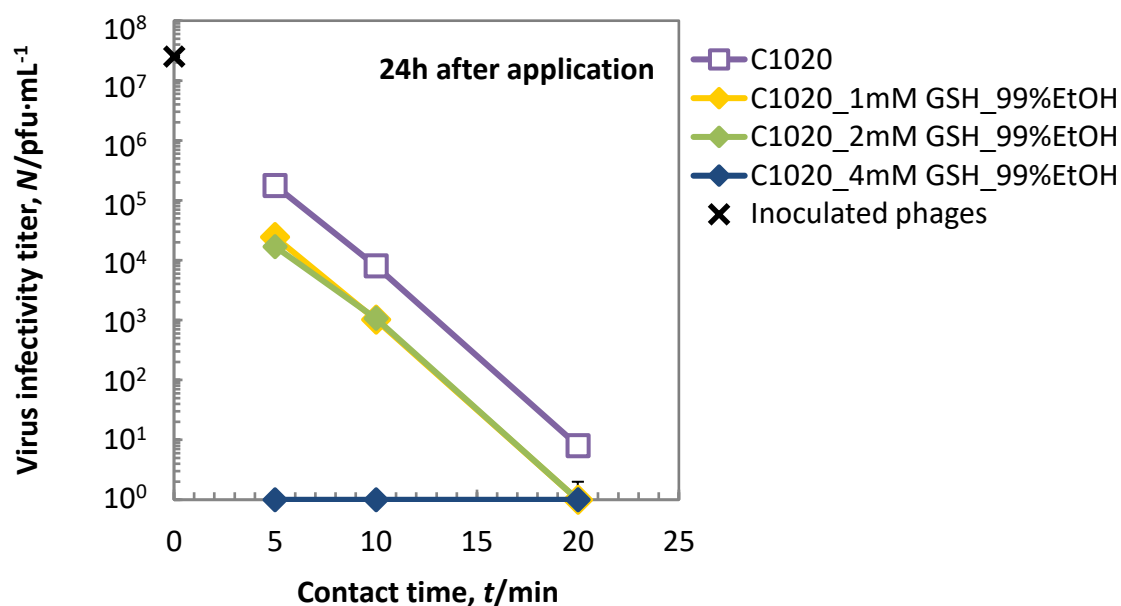


Fig.S4 Effect of the concentrations of GSH on antiviral activity of C1020 ($n=2$, mean \pm s.d.).

By parallelism tests of 2 regression lines, C1020 treated with 4mM GSH in 99vol.% EtOH has a statistically different slope from that treated with 2 mM or 1 mM GSH in 99vol.% EtOH ($p<0.001$). Non-treated C1020 has a statistically different intercept from that treated with 2 mM or 1 mM GSH in 99vol.% EtOH ($p<0.001$).

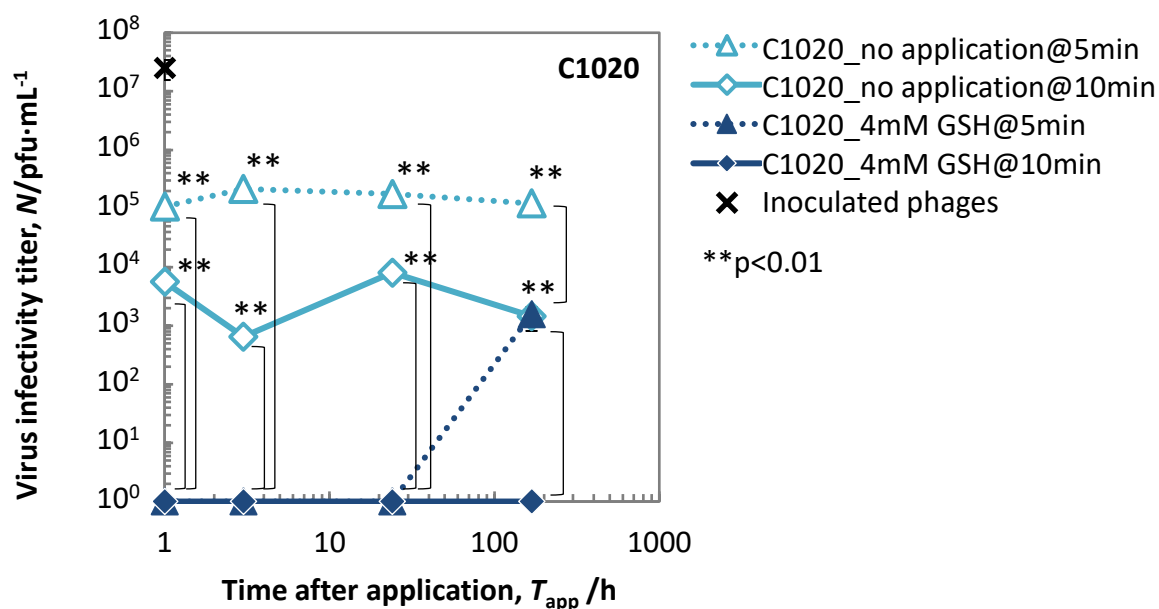


Fig.S5 Effect of the leaving time after GSH treatment on the enhancement of antiviral activity of C1020 ($n=2$, mean \pm s.d.)

The virus infectivity titer after 5 or 10 min of contact to the specimen surface was plotted against the time after GSH treatment. Statistical significance ($p < 0.01$) was observed between the pair of the values with/without GSH treatment of a certain leaving time (1, 3, 24, and 168 h) at the same contact time as 5 or 10 min by Student's t -test.

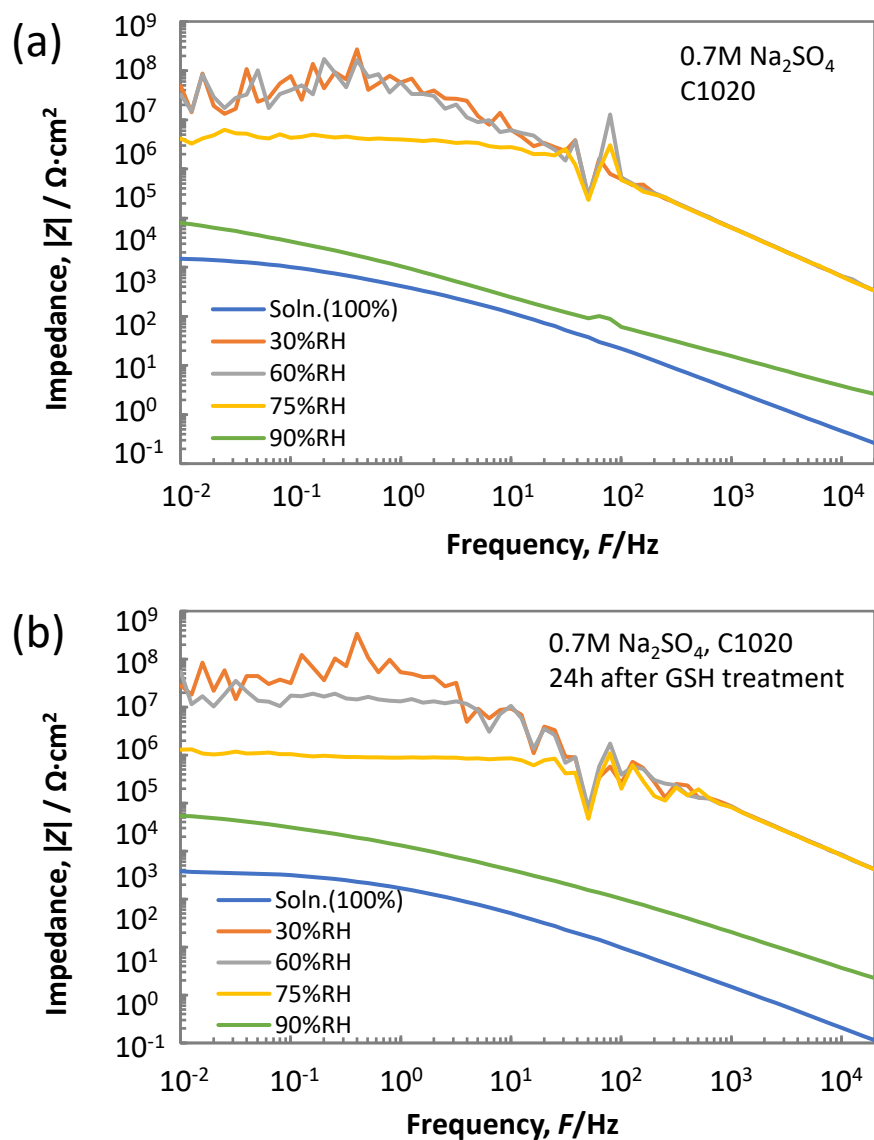


Fig.S6 Typical examples for the results of electrochemical impedance measurement on C1020 under controlled humidity.

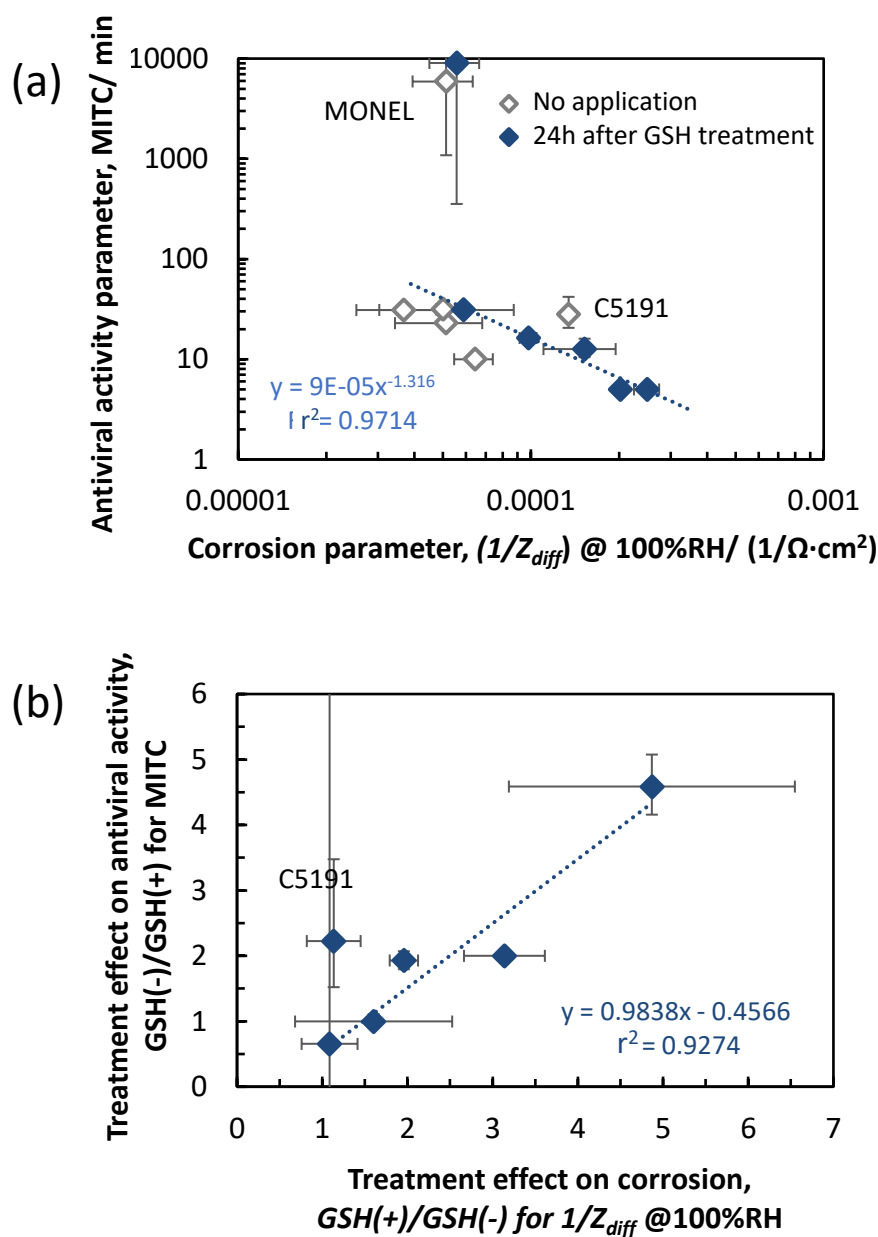


Fig.S7 Correlation between MICT, a parameter of antiviral activity and $1/Z_{diff}$, a parameter of corrosion rate for copper and its alloys (a), and the correlation of enhancement level in antiviral activity and in corrosion rate by GSH treatment described by the ratio of GSH(-)/GSH(+) for MICT and GSH(+)/GSH(-) for $1/Z_{diff}$ (b) GSH(+) and GSH(-) indicate with/without GSH treatment. The regression line shown in (a) was calculated for 5 data with GSH treatment except MONEL. The regression line shown in (b) was calculated for 5 data except C5191. The error bars in X and Y axes indicate standard deviations and 95% confidential intervals, respectively.

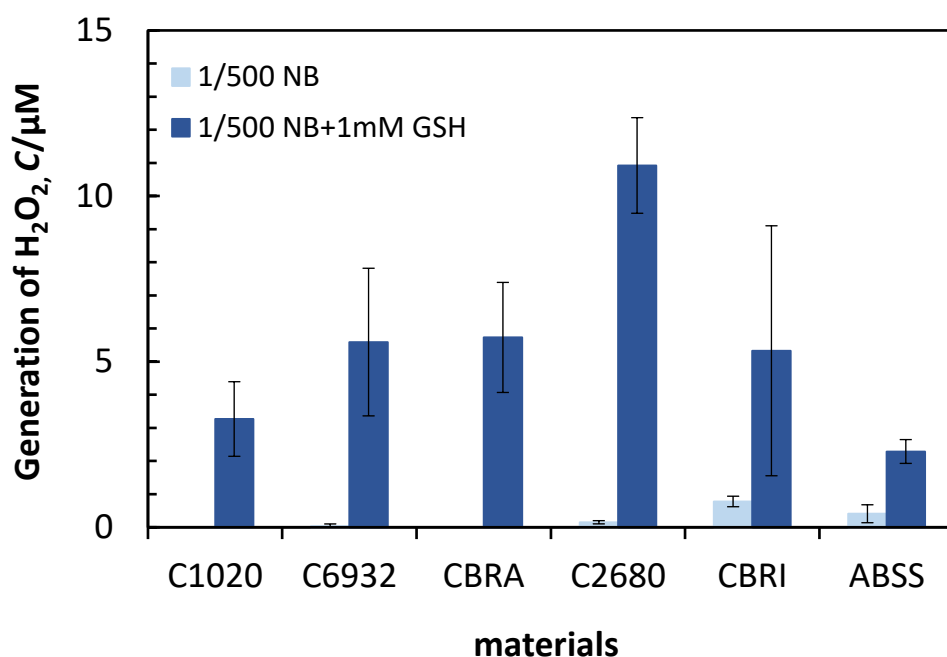


Fig. S8 H_2O_2 generation after 24 h of immersion into 1/500 NB with/without 1mM GSH (n=3, mean \pm s.d.).

NB: Nutrient Broth, GSH: glutathione, CBRA: CLEANBRASS® (Cu-27.4wt%Zn-2.0wt%Ni-0.5wt%Sn), CBRI: CLEANBRIGHT® (Cu-34.7wt%Zn-10.9wt%Ni-0.4wt%Sn), ABSS: antibacterial stainless steel NISSAM3 (Fe-18.1wt%Cr-9.4wt%Ni-3.8wt%Cu-1.4wt%Mn-0.6wt%Si). Re-arranged data in ref. 29.

Five hundred times dilution of NB is specified as a suspension medium for antibacterial tests by ISO22196:2011 *Measurement of antibacterial activity on plastics and other non-porous surfaces*. The specimen having 0.95 cm² of an exposure area was immersed into 5 mL of 1/500 NB with/without 1 mM GSH for 24h. Then, the H_2O_2 in the supernatant was measured using Hydrogen Peroxide Assay Kit (CL-204, National Diagnostics, Atlanta, USA) following the protocols supplied. The H_2O_2 generation was decided as the difference in concentrations with/without addition of catalase (0.5 mg/mL, from bovine liver, for biochemistry, FUJIFILM Wako Pure Chemical).