

Assessment of bacterial adhesion to 3D-printed implant surfaces: an in vitro study

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Background

- FRI and PJI: most severe complications in orthopaedic and CMF surgery; biofilm infections that start with bacterial adhesion to the implant.
- 3D-printed patient-specific implants: as-printed surfaces carry layer lines, fused particles and high micro-roughness.
- Effect of material and post-processing on early adhesion: not systematically compared.

Aim

To compare *Staphylococcus aureus* adhesion on 3D-printed titanium in four post-processing states, on 3D-printed PEEK and on resorbable PLDLLA/β-TCP, and to relate adhesion to surface roughness and morphology.

Methods

Specimens: discs $\varnothing 6 \times 2$ mm, 7 groups (Table 1): LPBF titanium grade 2 — as built / sandblasted / + vibratory finished / + electropolished; MEX-printed PEEK; jetted PLDLLA/β-TCP; machined titanium grade 4 control

Surface: white-light profilometry (S_a , S_z ; $n = 4$) + SEM (5 kV)

Adhesion: *S. aureus* Mu 12 (clinical FRI isolate), $OD_{600} = 0.1 \approx 10^6$ CFU/mL, 300 μ L per disc, static, 36 °C, 6 h / 24 h

Quantification: PBS rinse → sonication (1 min, 40 kHz) → serial dilution → CFU count; 3 independent runs, 13 discs per group

Imaging: live/dead confocal microscopy (SYTO 9 / propidium iodide)

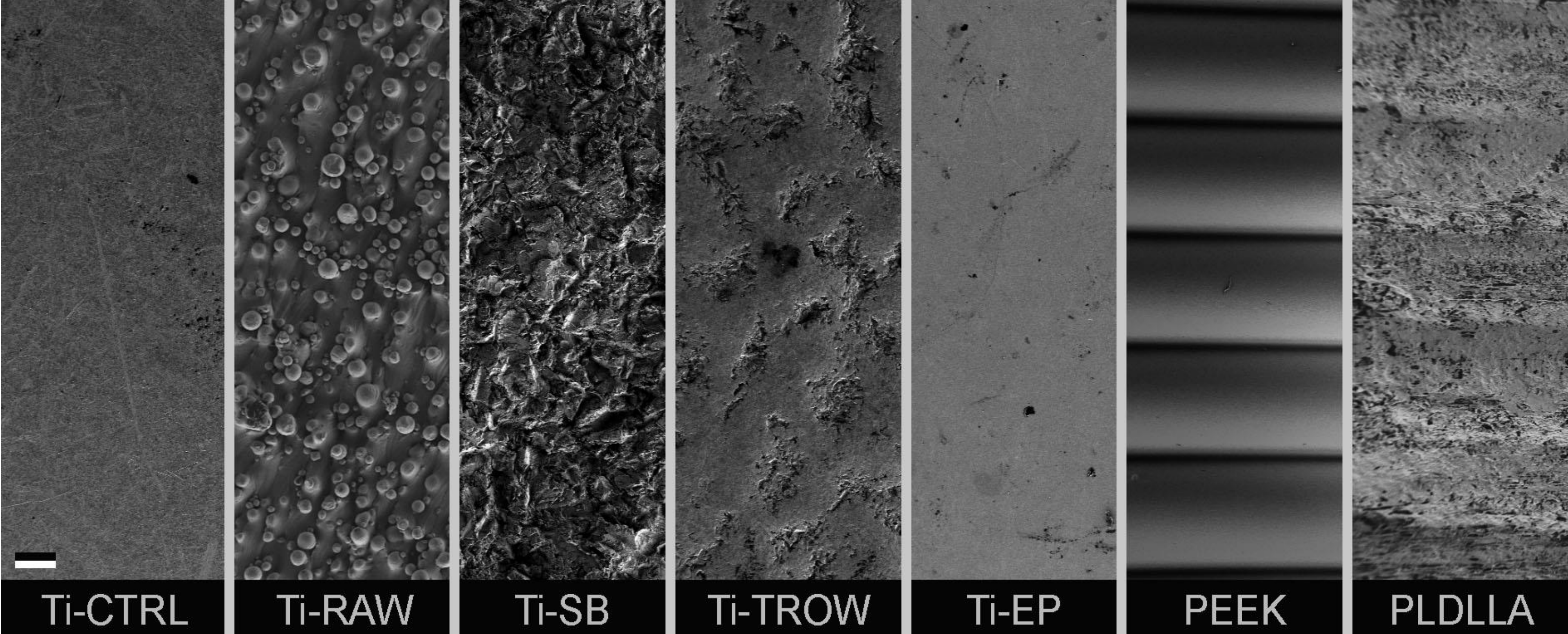
Statistics: Kruskal–Wallis + Dunn’s post hoc, Mann–Whitney, Spearman

Table 1 Materials, manufacturing and post-processing

Group	Material / manufacturing	Post-processing
Ti-CTRL	cp titanium grade 4, machined	Vibratory finishing
Ti-RAW	cp titanium grade 2, LPBF	As built
Ti-SB	cp titanium grade 2, LPBF	Alumina sandblasting
Ti-TROW	cp titanium grade 2, LPBF	Sandblasting + vibratory finishing
Ti-EP	cp titanium grade 2, LPBF	Sandblasting + vibratory + electropolishing
PEEK	PEEK filament, material extrusion	Drill press + plug cutter
PLDLLA/β-TCP	PLDLLA 70:30 + 30 wt% β-TCP, jetting	None

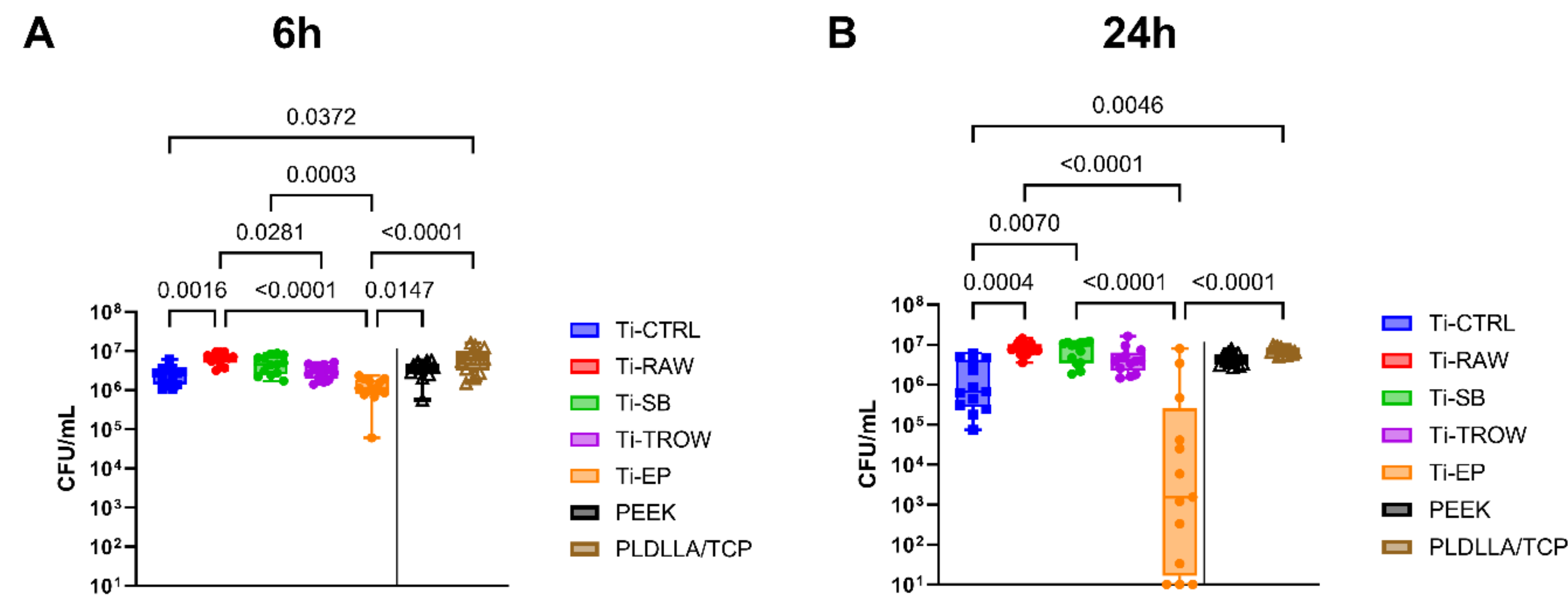
Results

Figure 1 Surface morphology (SEM, scale bar 100 μ m).



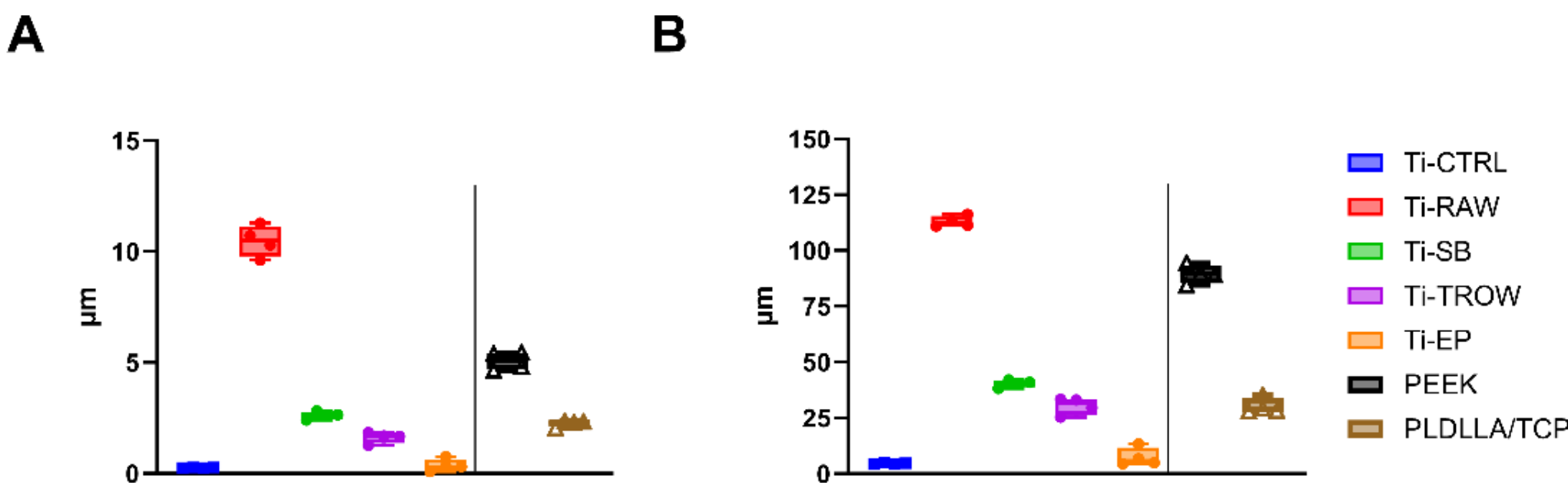
Post-processing smooths the printed surface step by step. As-printed titanium: partially fused powder particles (20–50 μ m) → electropolished titanium: featureless. PEEK: ~ 200 μ m layer lines; PLDLLA/β-TCP: porous, cracked surface.

Figure 2 Bacterial adhesion after 6 h (A) and 24 h (B).



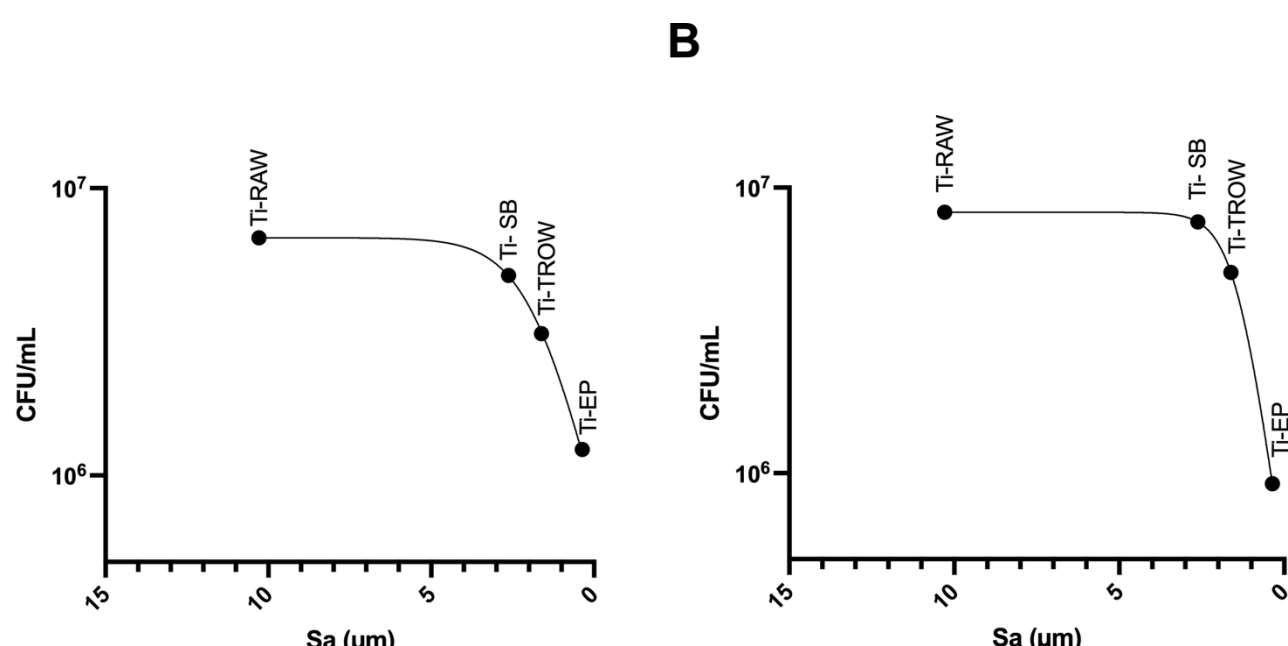
Electropolished titanium shows the lowest bacterial adhesion. Ti-EP $\approx 10^6$ CFU/mL vs $\approx 10^7$ on as-printed titanium (≈ 1 log less); at 6 h significantly below all groups except the machined control, at 24 h below all groups ($p < 0.0001$). PEEK and PLDLLA/β-TCP were colonized as heavily as as-printed titanium.

Figure 3 Surface roughness: S_a (A) and S_z (B), $n = 4$.



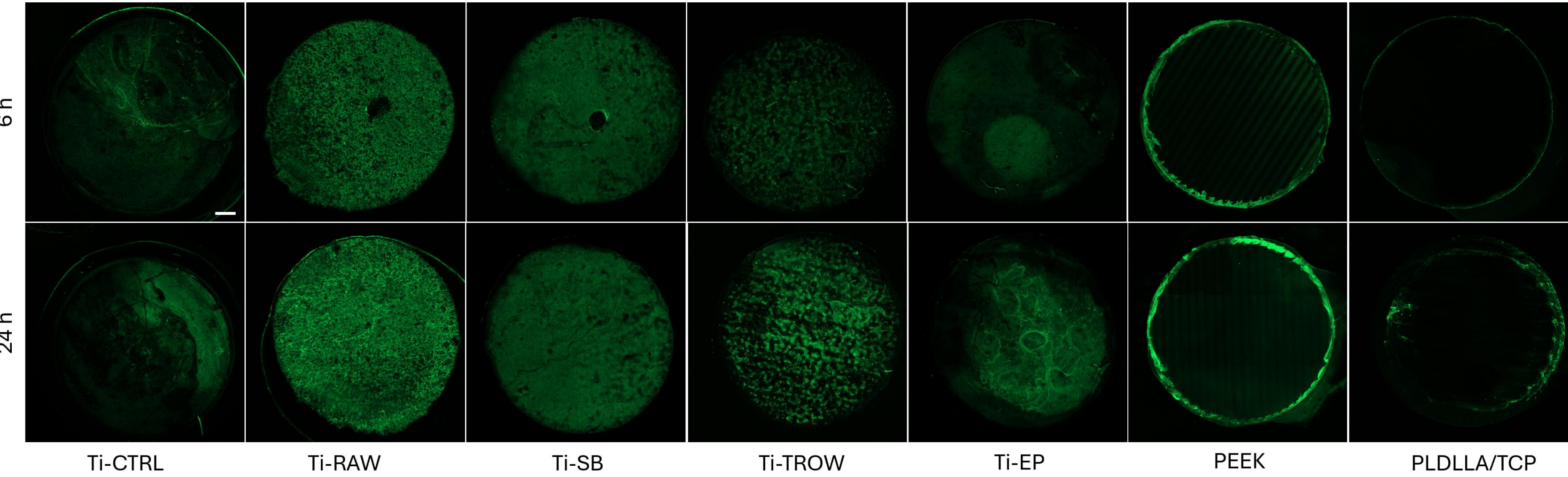
Every finishing step lowers roughness. $S_a \approx 10$ μ m (as built) → 2–3 μ m (sandblasted) → 1–2 μ m (vibratory) → ≈ 0.5 μ m (electropolished); $S_z > 100$ μ m → 10–15 μ m.

Figure 4 S_a versus adhesion at 6 h (A) and 24 h (B).



Rougher surface = more bacteria. Lower S_a → fewer adherent bacteria across the titanium groups, at 6 h and 24 h (Spearman $r = 1$, $p = 0.0417$).

Figure 5 Live/dead confocal microscopy at 6 h and 24 h.



Imaging confirms the counts. Dense bacteria on rough titanium (Ti-RAW, Ti-SB), sparse on Ti-EP. PEEK and PLDLLA/β-TCP autofluorescence → not interpretable.

Conclusions

- Electropolished titanium showed significantly lower *S. aureus* adhesion than as-printed titanium, mechanically finished titanium, PEEK and PLDLLA/β-TCP
- Higher titanium surface roughness correlated with increased bacterial colonisation → surface topography is a critical factor in early bacterial adhesion.
- The high adhesion on untreated surfaces is not an inherent limitation of 3D printing, but a consequence of the as-printed surface state: **post-processing can counteract it** without undermining the benefits of customised implant design.