Effects of concentration-dependent human serum albumin coating on cellular response and bacterial inhibition *in vitro*

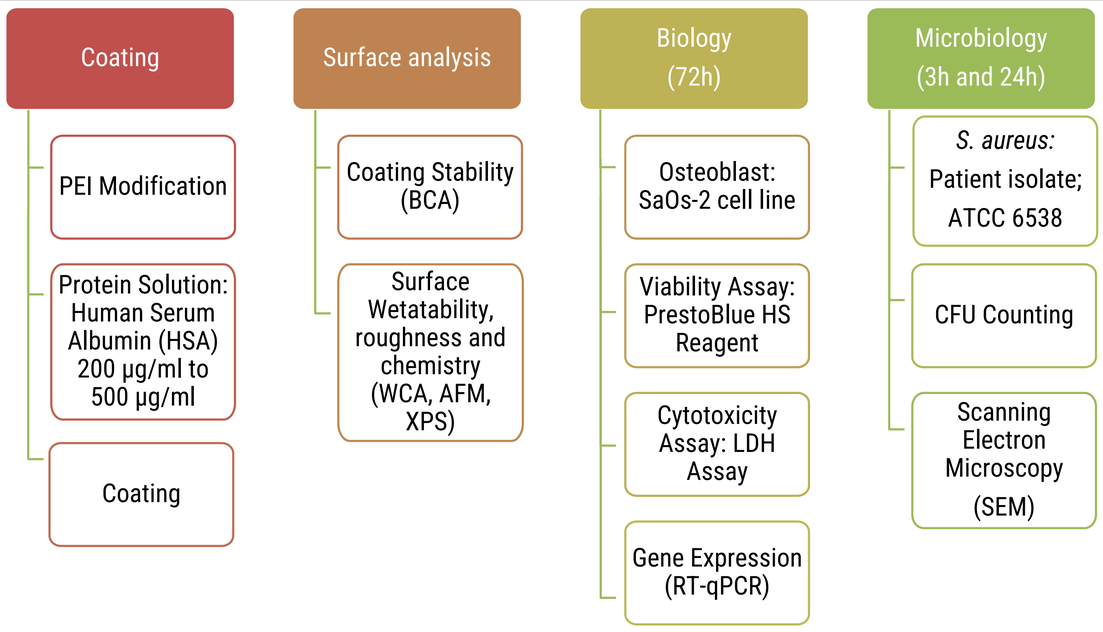
M. Azari (Speaker)[1], Z. Xi[2], O. Makarewicz[3,4], K. D. Jandt[2], B. Wildemann[1]

1 Clinic for Trauma-, Hand- and Reconstructive Surgery, Jena University Hospital, Jena, Germany
2 Otto Schott Institute of Materials Research, Friedrich Schiller University Jena, Germany
3 Institute of Infectious Diseases and Infection Control, Jena University Hospital, Jena, Germany
4 Integrated Research and Treatment Center - Center for Sepsis Control and Care (CSCC), Jena University Hospital, Jena, Germany

Background

- There are two major challenges that can arise after orthopedic implant placement: inadequate bone-implant integration and implant-associated infection [1].
- The application of human serum albumin (HSA) has shown initial promising results, supporting osteogenic responses and reducing microbial colonization [2].
- This study evaluated the effect of concentration-dependent HSA coatings on the Ti-6Al-4V alloy to identify an optimal balance between cellular compatibility and antibacterial activity.

Methods & Characterization

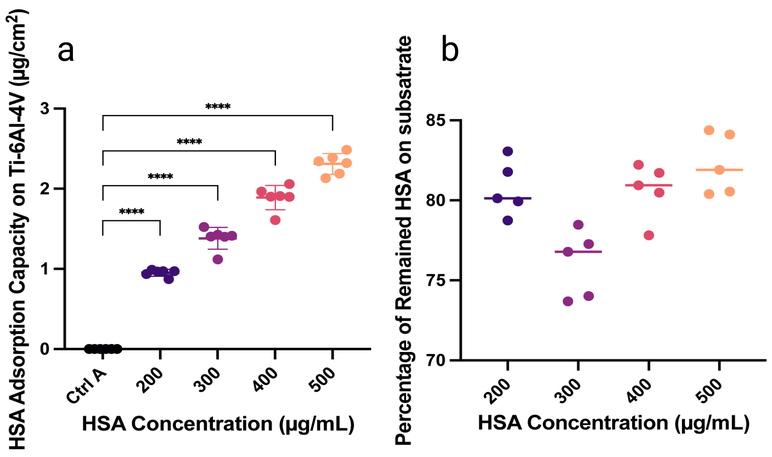


Statistics: One-way ANOVA with Kruskal-Wallis test with Dunn's multiple comparisons test, was used for comparison (*p < 0.05 indicated significance).

Results

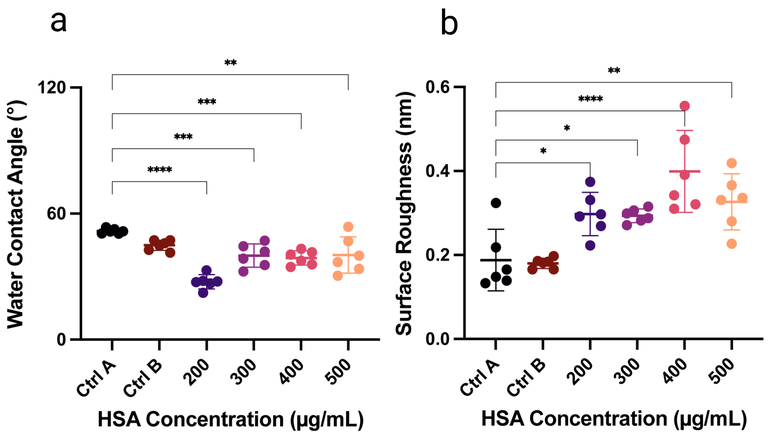
1. HSA loading & Stability

Figure 1. (a) Adsorbed human serum albumin (HSA) on Ti-6Al-4V surfaces (n=6). (b) Retention of adsorbed HSA in DMEM at 37 °C over 3 days (n=5). For all the group, at least 70-75% retained after 3 days in DMEM.



2. Surface Roughness & Wetting

Figure 2. (a) Static water contact angle. Hydrophilicity decreased after HSA coating, but still within the cell-favorable range, and (b) surface roughness Ra. Although Ra has significance increase, but they're all below 1nm, indicating extremely smooth surfaces. Ti-6Al-4V (Ctrl A), PEI–functionalized Ti-6Al-4V (Ctrl B) (n=6).



3. Surface Chemistry

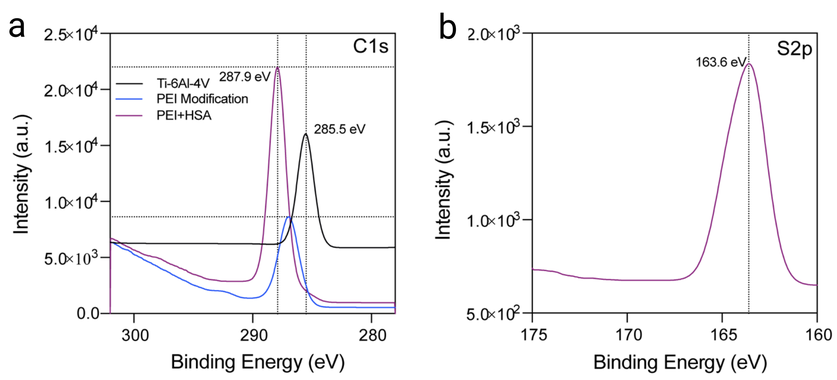


Figure 3. XPS analysis of Ti-6Al-4V surfaces before and after PEI/HSA modification. (a) N1s spectra; the PEI-modified surface shows a dominant peak at ~398.7 eV (amine N), which shifts to ~399.7 eV (amide N) after HSA coating. (b) A weak S2p signal is detected on the HSA-coated surface, close to the instrumental detection limit.

XPS was conducted by Prof. Dr. Andrey Turchanin research group.

4. Cell Viability, Cytotoxicity and Gene Expression

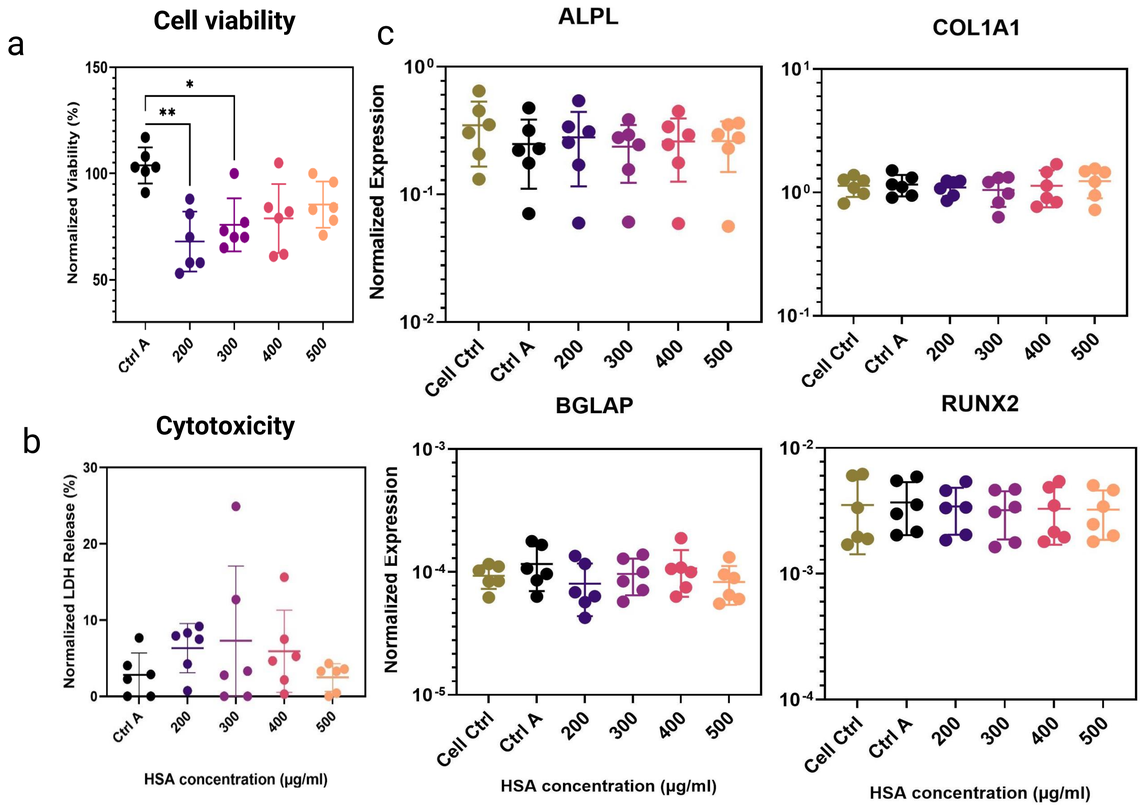


Figure 4. Cell biology (a) Cell viability assessed with PrestoBlue™ HS Reagent and (b) cytotoxicity evaluation by LDH release after 72h on different HSA-coated disks (200–500 µg/ml) normalized to cell control (glass), polished Ti-6Al-4V (Ctrl A). (C) Normalized expression of *ALPL*, *COL1A1*, *BGLAP* (OCN) and *RUNX2* after 72h incubation on the disks, cell control (glass) and polished Ti-6Al-4V (Ctrl A) (n=6).

5. Bacterial Inhibition

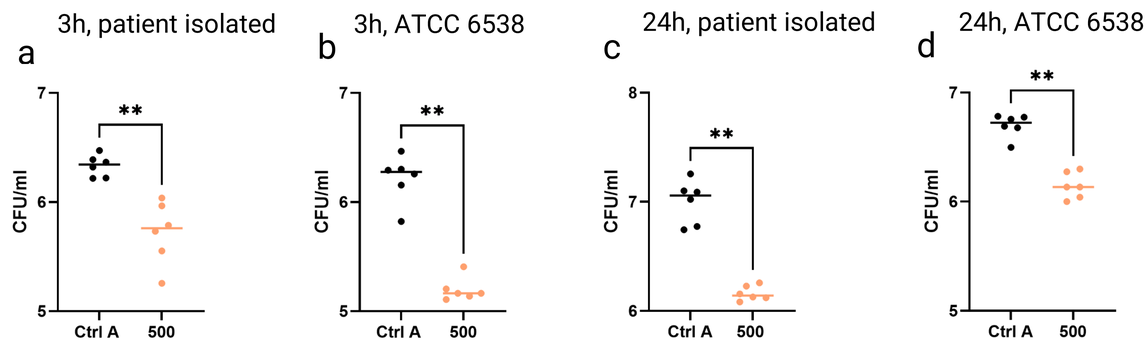


Figure 5. Quantification of bacterial growth by CFU counting after 3h incubation of patient isolated (a) and ATCC strain (b), same as counting after 24h incubation of patient isolated (c) and ATCC (d) Significant reduction in bacterial counts on the 500 µg/ml HSA was achieved compared to polished Ti-6Al-4V (Ctrl A).

Figure 6. SEM images of patient isolated *S. aureus* on Ti-6Al-4V control group (a) and the 500 µg/ml HSA (b).

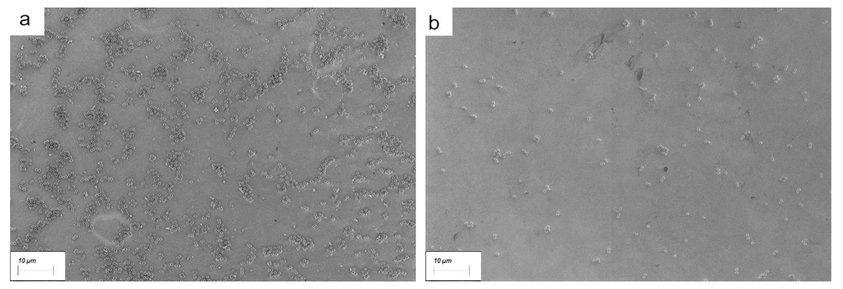
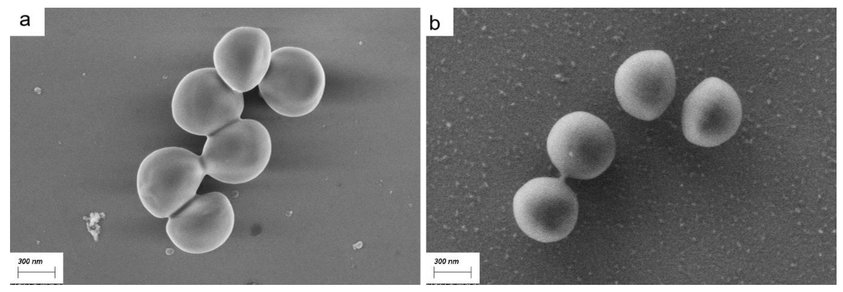


Figure 7. SEM images showing bacterial adhesion behavior on different surfaces: patient isolated strain on the polished Ti-6Al-4V (a) in contrast to bacteria on the 500 µg/ml HSA (b).



Conclusion

1. HSA coating on PEI-Ti-6Al-4V remained in place after 3 days (at least 70-75%),
2. Hydrophilic, amide-rich surface confirmed: WCA stays in the cell-favorable range,
3. Viability of osteoblasts on higher loading 400 and 500 µg/mL disks comparable to Ti-6Al-4V control and no altered expression of osteogenic genes,
4. 500 µg/ml HSA coated samples decreased *S. aureus* adhesion confirmed by CFU and SEM images,
5. 500 µg/ml HSA coatings, can reduce bacterial adhesion while maintaining osteoblasts cell integrity and osteogenic gene expression,
6. Further improvement/optimization necessary for better antibacterial effect.

[1] Misir A. Current developments in orthopaedic implant technology. J Orthop Surg Res. 2025 Oct 28;20(1):927. doi: 10.1186/s13018-025-06279-w. PMID: 41152964; PMCID: PMC12560316.

[2] Kinnari, T.J., et al., Bacterial adherence to titanium surface coated with human serum albumin. Otology & Neurotology, 2005. 26(3): p. 380-384..

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