Sponge-cavity in vitro model for evaluating microbial penetration and shockwave-based disruption in simulated combat wounds

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Background:

Combat-related deep wounds with cavity formation frequently present a therapeutic challenge due to colonization by multidrug-resistant (MDR) bacteria and persistent biofilms. Extracorporeal shock wave therapy (ESWT) has shown promise in promoting tissue regeneration and disrupting biofilms [1], but its direct effects on microbial communities within complex wound environments are not fully elucidated. A standardized, imaging-compatible in vitro model is urgently needed to investigate these interactions and optimize therapeutic protocols.

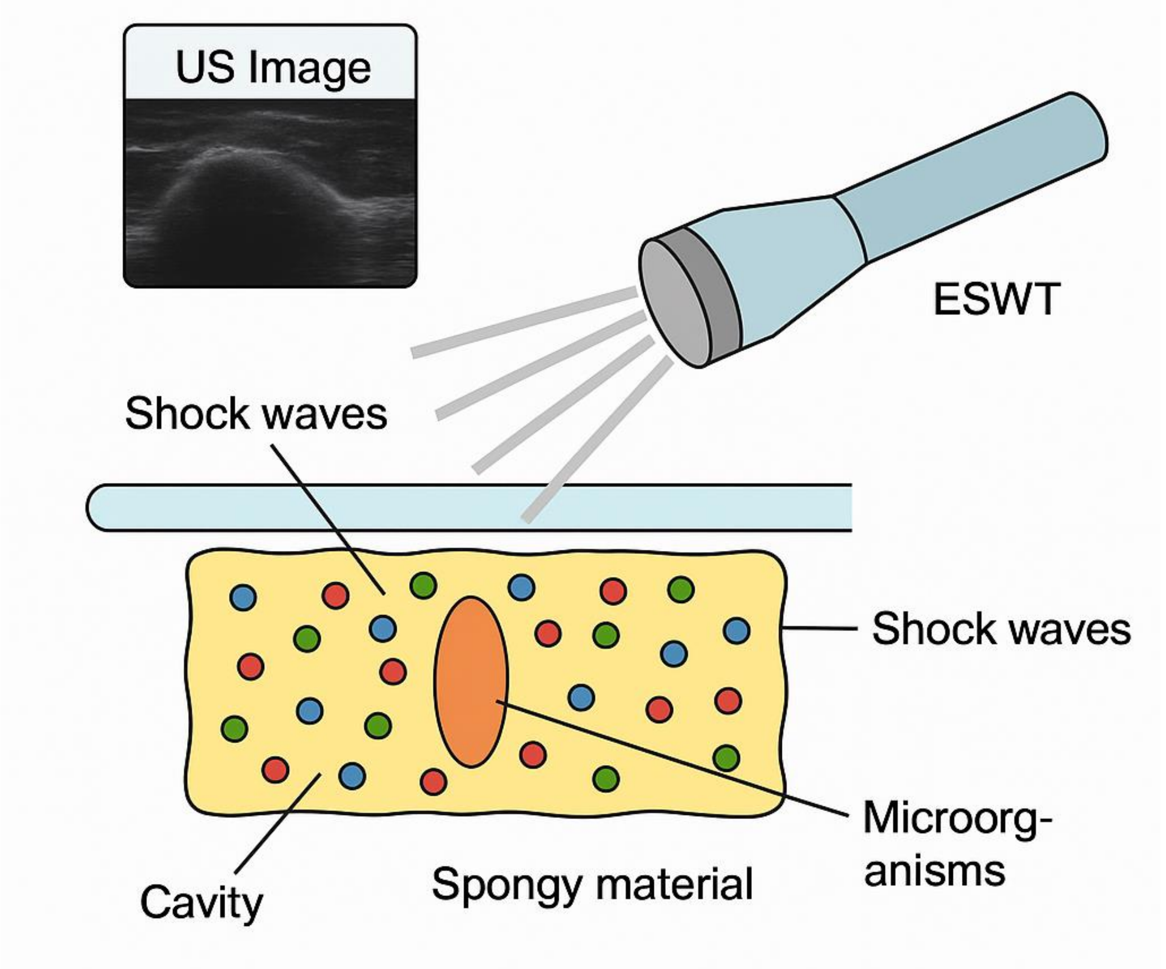
Objective:

To develop and validate a sponge-based in vitro wound model simulating deep, cavity-type wounds, enabling the systematic evaluation of ESWT's biofilm-disruptive and bactericidal potential, supported by ultrasound monitoring.

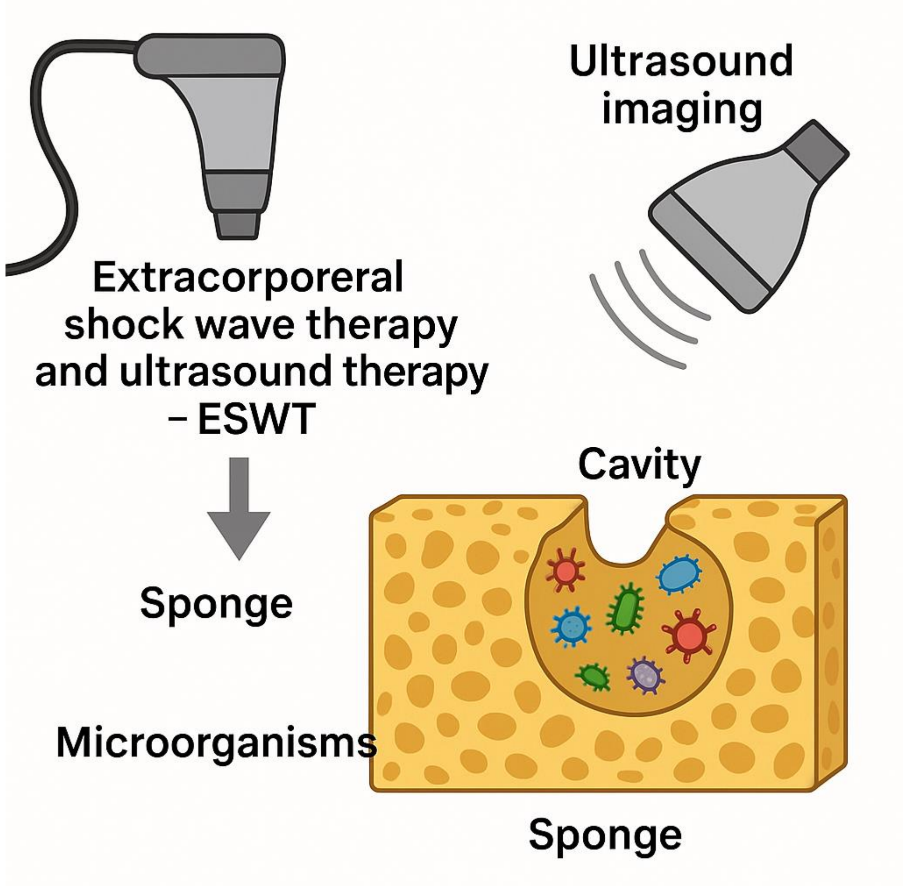
Methods:

A sterile, porous sponge matrix composed of medical-grade polyvinyl alcohol (PVA) or gelatin composites will be engineered with cavity-like channels to mimic wound morphology. These cavities will be inoculated with common wound pathogens including *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Following incubation to allow for biofilm formation, ESWT will be applied using radial or focused modalities across varying energy flux densities (0.1–0.5 mJ/mm²) and pulse counts (500–3000). Structural effects and shockwave penetration will be monitored using ultrasound imaging. Analytical endpoints will include:

- Viable bacterial counts (CFU/ml)
- Biofilm biomass (crystal violet assay)
- Confocal microscopy for structural analysis



Sponge-Cavity Model for Evaluating Microbial Penetration under ESWT an US Monitoring



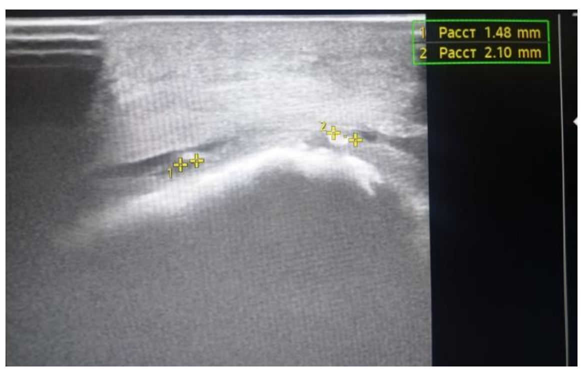
3D-fabricated, multilayer sponge–hydrogel wound phantom enabling niche-specific biofilms and ultrasound-guided ESWT dose mapping; modular ports for inoculation and sampling

Wound Stratification and Therapeutic Strategy:

Acute Infected Wounds (e.g., burns, abrasions, eyelid and gum wounds):
Chronic Trophic Wounds (vascular or neuropathic origin): consider + probiotics or regenerative injectables
Postoperative Wounds: Alternate or combine blue and yellow light to prevent infection and support tissue recovery
Other Trauma Wounds: Use personalized strategy based on load-bearing, tissue perfusion, and stage of healing

Case report: *Tibial Stump Wound with Fluid Collector*

Clinical Issue: A 6x4 mm hyperechogenic exostosis located on the anterior surface of a tibial stump was observed at a site of maximum prosthetic load. Surrounding this exostosis is a fluid-filled cavity approximately 50x40 mm in size and up to 8 mm in depth, with visible fibrotic fusions and hyperchogenic inclusions (1–3 mm). These findings suggest inflammation, possible infection, and risk for chronic irritation.
Proposal: Following drainage of the cavity, localized ESWT therapy can be applied to the load-bearing area to:
•Decrease inflammation and reduce fluid accumulation
•Stimulate tissue repair and prepare the surface for prosthetic adaptation
•Minimize risk of septic progression and persistent fibrosis



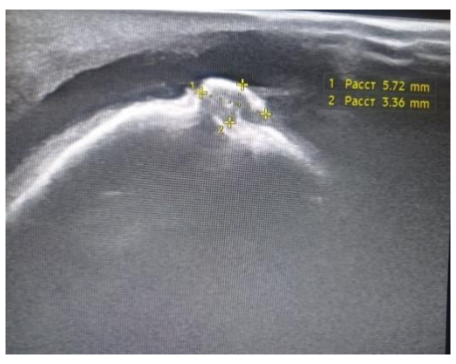


Figure: Ultrasound showing fluid-filled cavity with hyperchogenic inclusions and surrounding exostosis

Figure Wound on the surface of the stump

Future Outlook

Validation: Benchmark sponge-cavity results against *ex vivo* or *in vivo* wound models.
Advanced Readouts: Add live/dead staining and ultrasound elastography for biofilm vitality and disruption.
Beyond ESWT: Use model to test probiotics, regenerative injectables, and antimicrobial biomaterials.
Toward PPPM: Enable imaging-guided, personalized wound protocols for infection control and regeneration.

Conclusions

This model represents a novel translational platform for evaluating anti-infective strategies in wound care. The sponge-cavity model is:
Configurable: mimics various wound depths and densities
Compatible with ESWT and US imaging
Novel as a modular microbial testing system
It has high potential for use in military medicine, infection control research, and antimicrobial adjuvant therapy development.

References

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