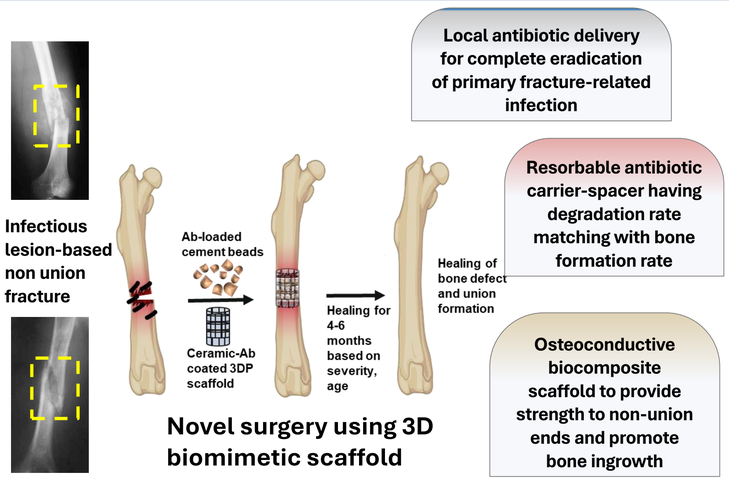
# Advanced bioceramic-3D scaffold intervention for managing infectious non-union bone trauma


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## Abstract



## Problem Statement

Infectious fractures account for nearly 5% of hip and femur fractures with occult infection progress to chronic necrosis and non-union. The standard debridement, antibiotic therapy, and implant retention approach often fails to fully clear infection, lacks mechanical support, and may compromise renal function. Current strategies using calcium sulfate carriers, Ilizarov fixation, and the Masquelet technique are infection-prone and limit patient mobility.

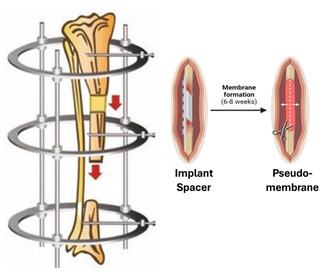



Figure 1. Ilizarov fixation, and the Masquelet technique

## Our Approach

To address this, we propose a one-step surgical approach using a self-resorbing, biomimetic 3D-printed scaffold integrated with antibacterial and osteoinductive agents and an osteoconductive biphasic bioceramic, OsteoCarrier (OC). The study aims to fabricate a bone mimetic scaffold and compare the physicochemical and biological performance of the indigenous OC with commercial calcium sulfate (CS) carriers.

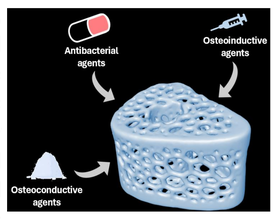



Figure 2. Schematic of biomimetic scaffold

## Scaffold Design and Printing Optimization

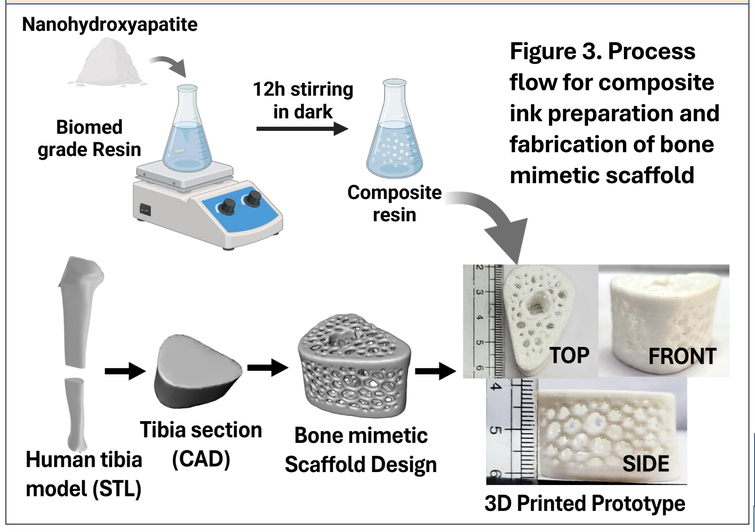
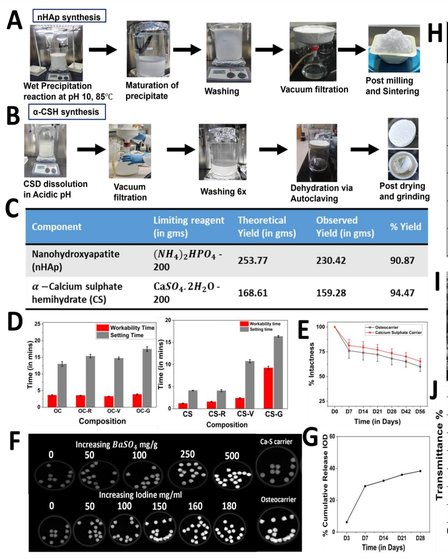



Figure 3. Process flow for composite ink preparation and fabrication of bone mimetic scaffold

## Bioceramic Characterization for Scaffold Pore Filling




C

| Component | Limiting reagent (in gms) | Theoretical Yield (in gms) | Observed Yield (in gms) | % Yield |
|---|---|---|---|---|
| Nanohydroxyapatite (nHAp) | $(NH_4)_2HPO_4$ - 200 | 253.77 | 230.42 | 90.87 |
| $\alpha$ –Calcium sulphate hemihydrate (CS) | $CaSO_4.2H_2O$ - 200 | 168.61 | 159.28 | 94.47 |

H

| Element | OC extract (ppm) | Exposure (µg/day) | USP <232> PDE (µg/day) |
|---|---|---|---|
| Cr | 0.12 | 0.0036 | 1100 |
| Ni | <LOD | ~0 | 20 |
| Cu | <LOD | ~0 | 300 |
| As | <LOD | ~0 | 15 |
| Cd | <LOD | ~0 | 2 |
| Pb | <LOD | ~0 | 5 |
| Al | <LOD | ~0 | 25 |

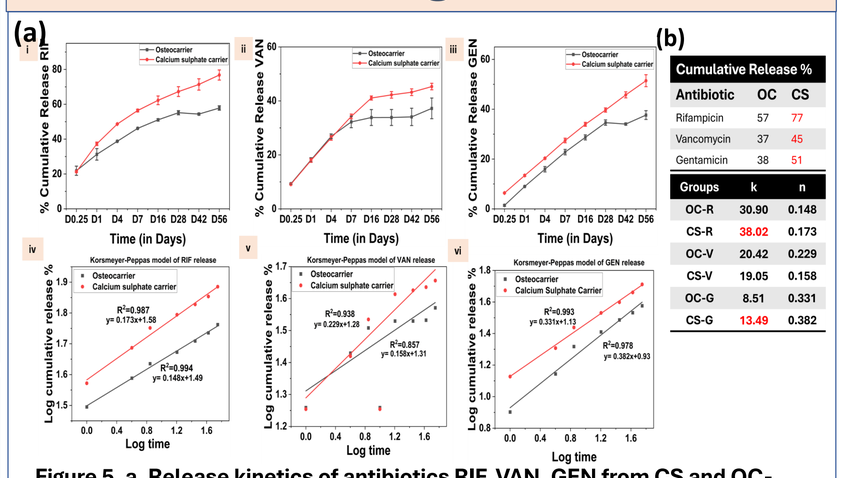



Figure 4. Material Synthesis (A) nHAp (B) CSH (C)% Yield calculation; (D) Setting and Working Time of OC and CS Carrier (E) Intactness % (F) Radiopacity optimization (G) Iodine Release kinetics from OC (H) ICP-MS analysis (I) Microstructure by SEM (J) Functional groups by FTIR spectroscopy (K) Crystallinity by XRD

## Results and Discussion

### *Assessment of biological efficacy*

#### Evaluation of Drug Release Profile




(b)

| Cumulative Release % | | |
|---|---|---|
| Antibiotic | OC | CS |
| Rifampicin | 57 | 77 |
| Vancomycin | 37 | 45 |
| Gentamicin | 38 | 51 |

| Groups | k | n |
|---|---|---|
| OC-R | 30.90 | 0.148 |
| CS-R | 38.02 | 0.173 |
| OC-V | 20.42 | 0.229 |
| CS-V | 19.05 | 0.158 |
| OC-G | 8.51 | 0.331 |
| CS-G | 13.49 | 0.382 |

Figure 5. a. Release kinetics of antibiotics RIF, VAN, GEN from CS and OC-based ceramic carrier b. Quantification of Drug Release

#### Long-term Antibacterial Activity

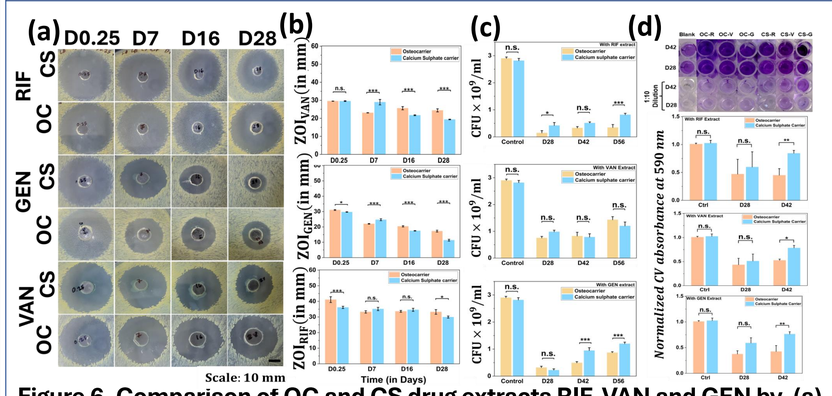



Figure 6. Comparison of OC and CS drug extracts RIF, VAN and GEN by (a) Disk diffusion assay (b)Quantified ZOIs (c) CFU count/ml by liquid inoculation (d) Quantified crystal violet absorbance

#### Scaffold Cytotoxicity Assessment

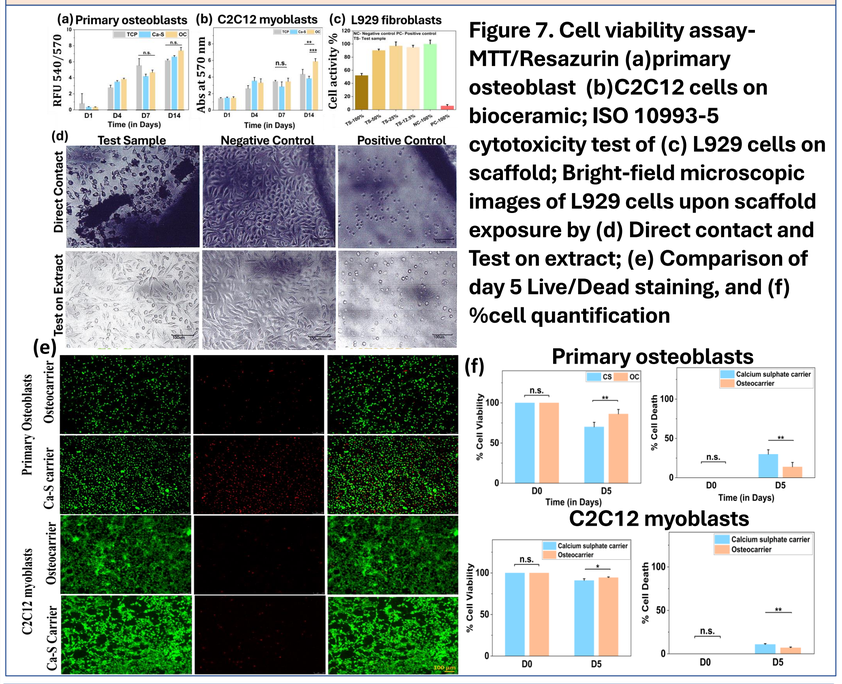



Figure 7. Cell viability assay-MTT/Resazurin (a)primary osteoblast (b)C2C12 cells on bioceramic; ISO 10993-5 cytotoxicity test of (c) L929 cells on scaffold; Bright-field microscopic images of L929 cells upon scaffold exposure by (d) Direct contact and Test on extract; (e) Comparison of day 5 Live/Dead staining, and (f) %cell quantification

#### Evaluation of Osteogenic Potential

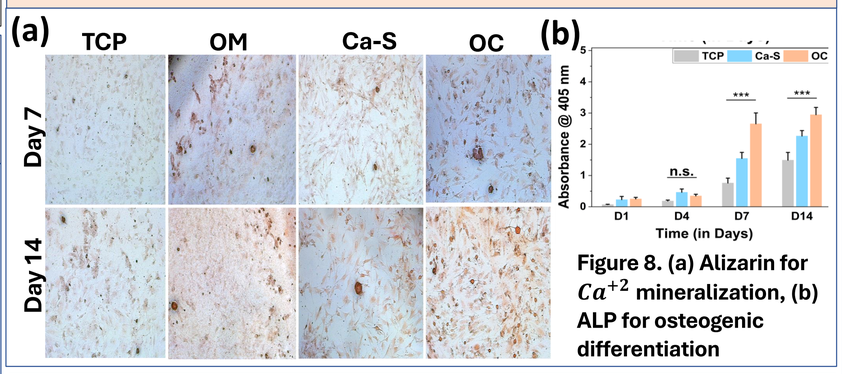



Figure 8. (a) Alizarin for $Ca^{+2}$ mineralization, (b) ALP for osteogenic differentiation

#### Scaffold Hemocompatibility

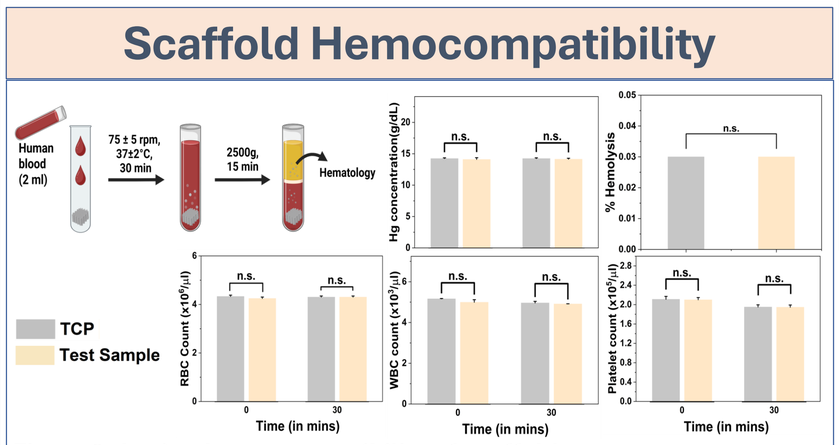



Figure 9. *In vitro* hemocompatibility of scaffold component as per ISO 10993-4

### *Safety and Toxicological evaluation*

#### Intracutaneous Irritation in Rabbits

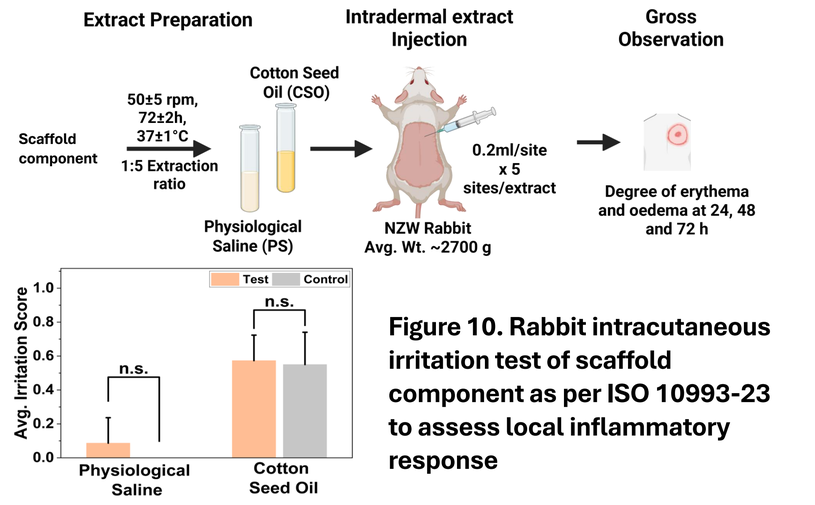
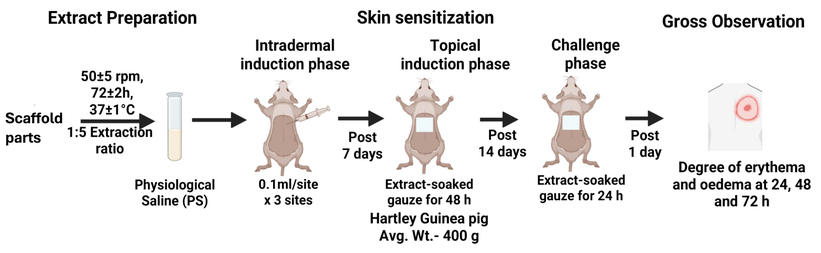



Figure 10. Rabbit intracutaneous irritation test of scaffold component as per ISO 10993-23 to assess local inflammatory response

#### Maximization Test in Guinea Pig




| Group | No. of animals | Grading of Skin Reaction observed post-challenge phase | | | | | |
|---|---|---|---|---|---|---|---|
| | | Erythema | | | Oedema | | |
| | | 24h | 48h | 72h | 24h | 48h | 72h |
| Control | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Test | 10 | 0 | 0 | 0 | 0 | 0 | 0 |

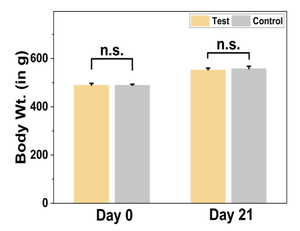
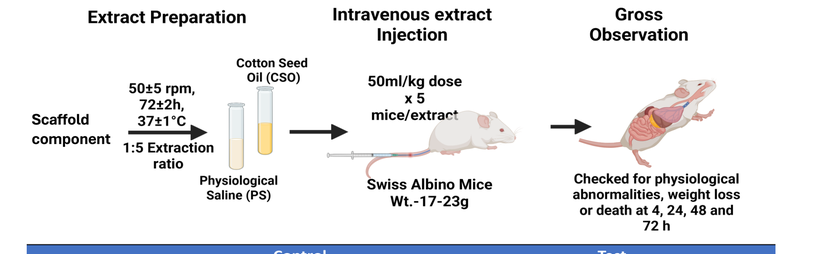



Figure 10. Guinea pig maximization test of scaffold component as per ISO 10993-10 to assess delayed hypersensitivity response

#### Acute Systemic Toxicology in Mice




| Clinical Observation | Control | | | | | | | | Test | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4h | | 24h | | 48h | | 72h | | 4h | | 24h | | 48h | | 72h | |
| | PS | CSO | PS | CSO | PS | CSO | PS | CSO | PS | CSO | PS | CSO | PS | CSO | PS | CSO |
| Respiratory | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Motor | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Convulsion | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Reflexes | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Ocular signs | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Cardiovascular | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Salivation | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Piloerection | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Analgesia | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Muscle tone | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Gastrointestinal | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Skin | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |

Figure 11. Mice acute systemic toxicity test of scaffold component as per ISO 10993-11 to analyze toxic effects to different organ system

#### Pyrogen Test in Rabbits

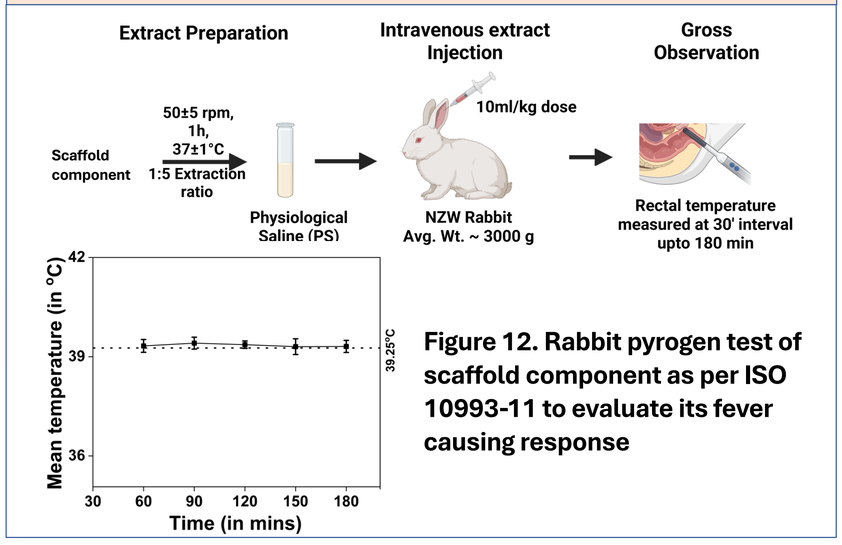



Figure 12. Rabbit pyrogen test of scaffold component as per ISO 10993-11 to evaluate its fever causing response

## Conclusions

Based on in vitro studies and ISO 10993 evaluation, the OC–3D scaffold system demonstrates strong translational potential for academic clinical trials in India. It exhibits osteoconductivity, enables sustained drug release, and provides sufficient mechanical stability for managing bone defects associated with trauma, non-union, and osteomyelitis.

## Contact


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## References


1. Kumar, I., et al. (2025). Limb Reconstruction System for Infected Nonunion: A Retrospective Study. Journal of orthopaedic case reports, 15(4), 313–319.
2. Xu, Y., et al. (2023). Treatment of tibial large bone defects: A comparative study of bone transport over an intramedullary nail in combination with antibiotic-impregnated calcium sulphate versus bone transport alone with antibiotic-impregnated calcium sulphate. Injury, 54 Suppl 2, S78–S85.
3. Wildemann, B., et al. (2021). Non-union bone fractures. Nature reviews. Disease primers, 7(1), 57.


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