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Development of a 3D bioprinted patch incorporating probiotics for chronic wound treatment

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The rise of antimicrobial resistance renders traditional antibiotics ineffective for treating chronic wounds, such as diabetic foot ulcers, highlighting the urgency for alternative or complementary therapeutic approaches. Bacteriotherapy, which employs beneficial bacteria (i.e., probiotics) to compete with and displace pathogenic microbes, is an emerging solution; however, efficient biomaterial-based systems for probiotic delivery to the wound site need to be established.

This study focuses on fabricating a 3D patch by a novel hydrogel bioink incorporating probiotics to heal or prevent bacterial resistance in chronic wounds.

The custom-made bioink was formulated by dissolving 4 w/v% alginate (Mw = 427 kDa, Mannuronic/Guluronic ratio = 0.7) in a 25 v/v% glycerol aqueous solution. The 3D constructs were printed and crosslinked with 1 w/v% CaCl₂ in water. Commercial lyophilized probiotics, including *Lactobacillus plantarum*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus*, were incorporated into the bioink at a concentration of 165 mg/ml. Rheology, optimal printing conditions, and printability of both probiotic-free and probiotic-laden bioinks were evaluated. The biodegradability of the patches was tested in phosphate-buffered saline at 37°C to assess stability. Probiotic viability was determined by counting colony-forming units (CFUs) before and after bioprinting, while the antibacterial efficacy of the patches was tested against *Escherichia coli*, and wound-specific *Pseudomonas aeruginosa*, and *Staphylococcus aureus* by the halo assay.

The 3D-printed constructs, with different shapes and infill geometries, displayed high fidelity and good structural integrity for up to two weeks. After bioprinting, the CFU counts remained stable, and the inhibition zones against *E. coli* measured 30.5 mm, 42.5 mm and 56.0 mm after 24 h, 48 h, and 72 h of pre-incubation, respectively. Pre-incubation significantly enhanced inhibition zones against both *P. aeruginosa* and *S. aureus*. In a 3D infected wound model with a *P. aeruginosa* biofilm, the patch demonstrated substantial antibacterial activity, indicating its potential for treating infected wounds.

Keywords: 3D bioprinting, bacteriotherapy, probiotics