ABSTRACT

Most chronic wounds do not heal and are frequently the result of bacterial infection or underlying medical conditions like diabetes. Infections with bacteria on wounds might exacerbate the site of the wound state and slow down its healing process. Diabetic patients face additional challenges in healing from wounds due to various factors that impede wound healing. Conventional dressings like plasters, gauze, and bandages are reactive rather than proactive. Despite being preventive in nature and utilizing cytotoxic silver, current antibacterial therapies do not eliminate bacteria. The efficacy of FDA-approved diabetic wound therapies is further limited by their contraindications. *Pseudomonas aeruginosa*-induced injuries are on the rise, and the enormous financial costs associated with bacterial resistance pose a serious threat to health systems throughout the world. It is crucial to find alternatives to antibiotics that have greater antibacterial action while posing less risk.

In recent decades, there has been an outburst in scientific concern in polymer composites. These materials find application in tissue engineering, pharmaceutical delivery, and wound bandages and dressings. Many polymers have been explored in an attempt to find the exquisite therapy for treating wounds. It is essential to make these polymer composites biocompatible, porous, bioactive, and highly absorbent in order to direct the growth and differentiation of cells and minimize any negative effects they may have on integrated drugs. In current thesis work, a variety of materials such as copper-MOF, 2-deoxy-D-ribose, copper doped nickel nanoparticles, and carbon-wrapped copper nickel nanoparticle, have been exploited for their wound healing activities.

In the first research project, a facile lyophilization process was used to develop chitosan membranes (CS) loaded with two different amounts of copper-MOF: 10 mg/20 ml (Cu-MOF10/CS) & 20 mg/20 ml (Cu-MOF20/CS). The absence of any chemical changes following Cu-MOF loading was confirmed by FTIR spectra

of Cu-MOF10/CS and Cu-MOF20/CS dressings. Interconnected porous structures were visible in the SEM images of the synthesized materials (CS, Cu-MOF10/CS, and Cu-MOF20/CS). The materials' cytocompatibility was determined using fibroblast cell culture, and its hemocompatibility was demonstrated by a blood clotting index of less than 5%. Comparing Cu-MOF20/CS to Amikacin, CS, and Cu-MOF10/CS membranes, the former demonstrated significantly greater effective antibacterial activity against the tested strains of *E. Coli* (149.2%), *P. aeruginosa* (165.5%), *S. aureus* (117.8%), and *MRSA* (142%). Cu-MOF20/CS dressing also effectively removed the biofilms of *MRSA* (52 %) and *P. aeruginosa* (37 %). On day 23, Cu-MOF10/CS and Cu-MOF20/CS enhanced wound healing up to 86.7 percent and 82.2 percent, respectively, in the full thickness infected wound rat model. While trichrome staining revealed higher collagen deposition, H&E staining of injured tissues treated with Cu-MOF10/CS & Cu-MOF20/CS showed more neovascularization and reepithelization along with reduced inflammation.

In the second research project, X-ray diffraction analysis and Fourier Transform Infrared spectroscopy (FT-IR) were used in the characterization of prepared Cwrapped Copper-Nickel NPs. Hydrogels (composed of propylene glycol, sodium alginate, and 2-phenoxy ethanol), ALG-30 and ALG-50, loaded with two different amounts of C-wrapped Cu-Ni NPs (30mg and 50mg) were developed.

When fibroblasts were used in a cell culture studies, the synthesized gels were found to be cytocompatible. Significant antibacterial activity was demonstrated by Cwrapped Copper-Nickel nanoparticles, ALG-30 and ALG-50, against Gram-positive (*S. aureus* and *MRSA*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria. The amount of *Pseudomonas aeruginosa* and *MRSA* biofilms was considerably reduced after a 24-hour exposure to nanoparticles and hydrogels (ALG-30 and ALG-50). Using ALG-30, a Pseudomonas aeruginosa-infected wound in a rat full-thickness infected skin wound model healed approximately 87.5% of the wound in 16 days.

Masson trichrome stained tissues revealed higher collagen deposition in the ALG-30 treated group, whilst H&E stained tissues exhibited enhanced angiogenesis, reduced inflammation, and stimulated re-epithelialization.

Cu-NiO NPs, or copper doped nickel nanoparticles, were formed in the current study via a redox reaction and were examined using X-ray diffraction. Collagen, sodium carboxymethylcellulose (CMC), and 2-phenoxy ethanol were simply mixed in deionized water to create collagen-based hydrogels. These hydrogels were then combined with synthesized nanoparticles and 2-deoxy-D-ribose (2dDR) to create three different types of hydrogels: Coll (collagen hydrogel without Cu-NiO NPs & 2dDR), CDR (collagen hydrogel with 2dDR), and CDRNP (collagen hydrogel with 2dDR & Cu-NiO NPs). By using Dische's diphenylamine test in PBS at 37°C to estimate 2dDR release, it was discovered that 1.74 mg and 1.34 mg of 2dDR, respectively, were released over 7 days from CDR and CDRNP hydrogels. Out of all the manufactured gels, the CDRNP gel shown up to 67% and 72% antibacterial activity against *E. Coli, P. aeruginosa, S. aureus,* and *MRSA*. Cu-NiO NPs was examined against fibroblasts at a range of concentrations (60, 30, 15, 3.75, and 1.875 mg/ml) in *in vitro* cell metabolic tests, and no concentration was observed to alter cell viability. In a similar vein, on days 1 and 5, the CDRNP hydrogel had no cytotoxic effects and cell metabolic activity comparable to the control. In the *ex vivo* chorioallantoic membrane experiment, CDRNP also showed increased angiogenesis in comparison to Coll and CDR. Rats with full-thickness *P. aeruginosa* biofilminfected wounds underwent an *in vivo* trial in which CDRNP eliminated bacterial biofilms and produced up to 99% wound closure in 17 days.

Overall, among all the three projects, hydrogels [C-wrapped Copper-Nickel NP embedded hydrogel (ALG-30) and Cu-NiO NPs loaded collagen hydrogels] exhibited more promising results as compared to Cu-MOFs loaded chitosan membranes. These, due to having excellent antibacterial activity, are anticipated to be widely used in clinical practice. These may have significant potential to address the present antibiotic resistance crisis in chronically infected wounds.