Electrically stimulated polypyrrole-gelatin/silk fibroin hydrogels for skin regeneration

Chronic skin injuries accounts for about 1 to 2% incidents worldwide. The major challenge while addressing this issue is healing time as chronic wounds decelerates the rate of wound healing by interfering with the biochemical events. When skin is wounded, it disrupts the transepithelial potential that exists between dermal and epidermal layers. While electrical stimulation has therapeutic effects in skin regeneration, application of electrical stimulation directly to skin has several limitations for various reasons. Therefore, the delivery of stimulation through hydrogel has benefits in keeping wound site moist. The effect of electrically stimulated conductive hydrogel is being explored for faster wound healing. The present study aimed to develop an electrically stimulated polypyrrole-gelatin/silk fibroin matrix for skin tissue engineering. To mimic skin, dual component hydrogel composed of gelatin and silk fibroin was reinforced with polypyrrole. Gelatin hydrogels were crosslinked with 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide (EDC/NHS) or glutaraldehyde (GTA) to improve its mechanical properties. Raman spectroscopic approach was deployed to understand the interaction of fibroblast cells with crosslinked hydrogels. It was concluded that GTA crosslinked samples were cytotoxic. Thus, gelatin was crosslinked with EDC/NHS, which showed better cytocompatibility as per Rhodamine-DAPI staining results. Dual step activated/crosslinked matrices of gelatin-silk fibroin were prepared to further tailor the properties of gelatin. With increase in silk fibroin loading in gelatin, enhanced cell proliferation was observed. However, taking into account the solid weight of gelatin, the sample loaded with 25% of silk fibroin was found to exhibit superior mechanical characteristics. Although the fabricated gelatin/silk fibroin matrices are mechanically stable, the hydrogels lack conductivity. Thus, polypyrrole was reinforced by using interfacial polymerization. After the modification with 100 mM of polypyrrole, conductivity got increased from 10^{-7} to 10^{-3} S/cm. Also, the samples showed scavenging activity, blood compatibility and were cytocompatible. The sample formulation with 30 mM polypyrrole and containing 75% gelatin and 25% silk fibroin was investigated for wound healing by electrical stimulation. An indigenously designed device having graphite electrodes is proposed capable of delivering pulsed stimulation to electroconductive hydrogels for wound healing studies. Human dermal fibroblasts stimulated with 300 mV/mm of electric fields showed enhanced proliferation at 2 Hz frequency and 20% duty cycle. Upregulation in the expressions of TGFβ, αSMA, and Collagen I supported the hypothesis of accelerated wound healing upon electrical stimulation.

Keywords: Biomaterials, Hydrogels, Wound Healing, Conductive Polymers, Electrical Stimulation, Bioelectricity