Synthesis and Characterization of Carboxymethyl Cellulose-based Hydrogel Coating to Potentially Prevent Post-Surgical Adhesions

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Statement of Purpose: Post-surgical adhesions of polypropylene (PP) hernia mesh are one of the complications in hernia repair surgery. The purpose of this study is to synthesize and characterize the PP mesh with carboxymethyl cellulose (CMC)-based hydrogel incorporating the bepridil hydrochloride drug to potentially prevent post-surgical adhesions. By mixing CMC powder with bepridil hydrochloride (BHC), which is a calcium channel blocker, and adding citric acid (CA), a hydrogen bonded and crosslinked CMC will be formed. This may prevent the early formation of post-surgical adhesions which are triggered by calcium-dependent membrane bridges between mesothelial layers [4]. Different concentrations of CMC and the amount of drug will be evaluated. The properties of PP with CMC coated and drug loaded hydrogels will be observed by scanning electron microscopy (SEM). Fourier transform infrared (FTIR) spectroscopy will be used to define the chemical composition and identify the functional groups on the mesh structure. In addition, degree of swelling and weight loss during degradation will be measured. The bursting strength before and after coating the PP mesh will be performed to monitor the mechanical performance. *In vitro* experiments, including observing L929 cell viability using the MTT and live/dead cell assays will be undertaken to observe cell proliferation and the cytocompatibility of the coated PP mesh. Intracellular calcium levels will also be investigated *in vitro*.

Methods: Carboxymethyl cellulose (CMC) (Average Mn ~90,000), citric acid monohydrate (CA), and the bepridil hydrochloride (BHC) drug were supplied by Sigma-Aldrich. Different mole ratios of CMC/CA will be studied to determine the degree of swelling and crosslinking ratio of the hydrogel network. Different concentrations of drug loading with a fixed ratio of CMC/CA will also be included to observe the efficacy of preventing adhesions. L929 fibroblast cells (1x10^6 cells/well) will be seeded on the samples and incubated for 1 day, 3 days, and 7 days at 37ºC. Cells will be grown in culture flasks containing minimum essential medium (MEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, and 1% nonessential amino acids (NEAA). Cells will be maintained at 37ºC in a humidified 5% CO_2 atmosphere and monitored daily by using an inverted microscope. Subculture will be performed twice a week when the cells reach 80% confluency. The samples will be cut into 1cm^2 squares to cover the bottom of 6-well plates, sterilized with 70% ethanol overnight and rinsed three times with PBS. The culture medium will be changed every day and the cell viability will be determined by a live/dead cytotoxicity tool kit and imaged by fluorescent microscopy. Fura-2,AM indicator will measure the intracellular calcium level of human fibroblasts.

Results: Different mole ratios of CMC/CA will be carried out in 6-well dishes. Swelling behavior of CMC hydrogels, including the degree of swelling (%), the weight loss (%), the molecular weight between crosslinks, the mesh size and the crosslink density will be investigated. The degree of swelling and the weight loss of CMC hydrogel will be determined after immersion in PBS buffer solution at 37 ºC for 48 h [1,2]. The degree of swelling and weight loss decrease with increasing crosslinking ratio [1].

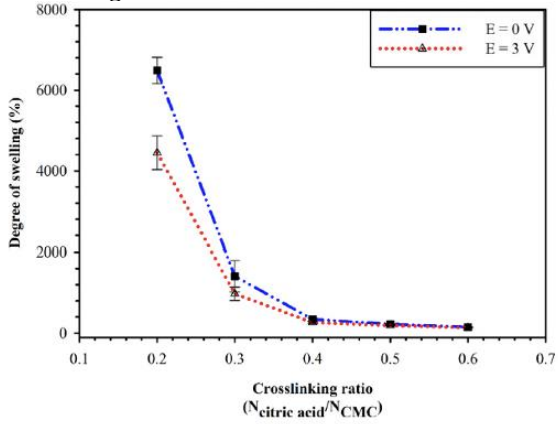

Figure 1The degree of swelling of carboxymethyl cellulose hydrogels under various citric acid crosslinker.[1]

The cytocompatibility of the CMC-based hydrogel coated PP mesh will be evaluated based on the cell viability of L929 cells using the MTT assay. The cell viability of the different mole ratio of CMC/CA samples shows significant differences ($p<0.05$) in cell proliferation on the samples [1,3]. The results also demonstrate the superior biocompatibility towards L929 fibroblast cells. The intracellular calcium level of the fibroblasts decreased as the concentration of drug increase indicating the hydrogel coating can potentially prevent post-surgical adhesions[4].

Conclusions: The aim of this study was to fabricate CMC-based hydrogels with drug loading for anti-adhesion applications and to measure the extent of hydrogel swelling and degradation. The findings demonstrate that CMC-based hydrogels are a potential method for creating an anti-adhesion layer on PP mesh. Future studies will include surface treatments to enhance cell-surface interactions and an *in-vivo* anti-adhesion trial.

References:

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