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Research on the secondary structure of antibacterial silk fibroin scaffolds by FTIR and Raman spectroscopy

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The silk fibroin scaffold is a promising biomaterial for tissue engineering. Microbial infection may cause structural changes in the scaffold injected to the human body, affecting its function and repair efficiency. The development of scaffold with antibacterial properties can be the solution. Our work concerns studying structural changes of silk fibroin scaffold modified with polyethyleneimine (PEI) and phages which provide it antibacterial properties, with vibrational spectroscopy methods.

FTIR and Raman vibration spectroscopy allow the study of the structural and functional group properties of protein molecules, thereby observing the secondary structure changes of the modified scaffold. The SF scaffolds, PEI-polymerized SF scaffolds, and SF scaffolds incubated in Bs168 bacteria or AR9 phage were tested. By calculating the relative intensity parameters of each sample at characteristic peak positions, the functional group characteristics can be analysed to infer the secondary structure of protein scaffold samples.

In FTIR and Raman spectra it is shown that all of the SF based scaffold samples exhibited distinct peaks of Amide I, Amide II and Amide III. The Amide I band is mostly attributed to carbonyl group (C=O) stretching vibration. The Amide II band mostly combines peptide CN stretching and NH bending vibrations, indicating the β-sheets presence in SF. The Amide III band is mainly associated with CN stretching, NH bending, CC stretching and CH bending vibrations, while peaks at low frequencies are characteristic of vibrations of amide bonds in β structures.

By FTIR spectra the changes of vibrations of Amide II and Amide III were observed, indicating that the β-sheet structure in the scaffold increased under the action of bacteria; however, the scaffold with phage did not show significant changes. Furthermore, comparing with the control group, the samples with AR9 phage and Bs168 bacteria have a higher intensity in the line, corresponding to asymmetrical vibrations of COO- groups of amino acids, which may be due to partial hydrolysis of the protein.

By Raman spectra, the proportion of β structures in the scaffolds cultivated in Bs168 bacteria was significantly higher than in other samples, which is consistent with the information obtained by the FTIR method. Moreover, the increase in the vibration of the C-S bond of the scaffold with bacteria or phage that can show the presence of bacterial or phage residues on the surface of scaffolds.

The conducted research has shown that the structural changes of silk fibroin-based scaffolds can be analysed by Raman and FTIR spectroscopy. The changes in secondary structure, signs of partially degradation of fibroin in this samples and the presence of bacterial or phage residues were detected.