Detection of α-defensins using surface-enhanced fluorescence methods on noble metal nanoparticles in the diagnosis of periprosthetic joint infections.

Autor: mgr Grzegorz Wołąkiewicz
Promotor: dr hab. Mariusz Szabelski, prof. UWM
Katedra Fizyki i Biofizyki, Wydział Nauki o Żywności
Uniwersytet Warmińsko-Mazurski w Olsztynie
Promotor pomocniczy: dr n. med. Marek Gojło
Oddział Kliniczny Chirurgii Urazowo-Ortopedycznej i Chirurgii Kręgosłupa Wojewódzki Szpital Specjalistyczny w Olsztynie

Summary

Periprosthetic joint infections (PJI) is one of the most serious complications after toral joint arthroplasty. Although the percentage of septic cases relative to the overall number of these procedures is relatively low, the issue affects an increasing number of elderly individuals suffering from joint dysfunction, which hinders normal functioning. Consequently, the number of arthroplasty procedures and related complications is on the rise. Despite established standards for the diagnosis of periprosthetic joint infections, laboratory test results are often inconclusive. Due to difficulties in isolating pathogens, diagnosis relies on inflammatory markers. One of the newest biomarkers used in PJI diagnostics is alpha-defensin, whose presence in synovial fluid has been linked to the body's natural response to infection. However, the widespread use of alpha-defensin testing is limited by the availability of only two commercial tests. This study proposes a new method for detecting alpha-defensin in synovial fluid, based on an immunosensor that enables peptide detection using specific capture and confirmation antibodies, labeled with different fluorophores. The study involved selecting markers, which, after binding to proteins, were spectroscopically characterized with respect to their use in the phenomenon of Förster resonance energy transfer (FRET). By immobilizing the labeled antibodies on the surface of a sensor coated with silver island films, an efficient biosensor was created, allowing detection through enhanced fluorescence. The developed measurement system, based on a simple design, enables selective excitation and fluorescence emission detection to confirm antigen presence. The studies demonstrated the possibility of detecting alpha-defensin over a wide range of concentrations. Validation of the test on fluid samples from patients with suspected PJI showed equivalence of the developed test with the commercial immunochromatographic test. Clinical evaluation of infection occurrence in the studied patient group confirmed the high sensitivity of the proposed test in predicting PJI.

Keywords: fluorescence, biosensor, alpha-defensin, periprosthetic joint infections