Ruprecht-Karls-Universität Heidelberg

Medizinische Fakultät Mannheim

Dissertations-Kurzfassung

MALDI TOF/TOF analysis of tissue surrounding a needle type sensor for continuous glucose monitoring

Autor: Asawari Kharkar
Institut / Klinik: Zentrum für Medizinische Forschung
Doktorvater: Prof. Dr. N. Gretz

Continuous glucose monitoring (CGM) is a core component of successful management of diabetes mellitus. The most common CGM devices commercially available are glucose-oxidase (GOD)-based biosensors, because of their enhanced sensitivity, better reproducibility, and easy maintenance as well as their low cost. However, implantable glucose sensors, which are known for excellent functionality in vitro, show a progressive loss of sensor function within a few days after implantation. Such sensor failure is commonly attributed to tissue reactions occurring around the implanted sensor, known as foreign body response (FBR).

The FBR is a term used to describe the non-specific immune response of biological tissues to any implanted foreign material. Characteristically, the inflammatory cells infiltrate the local site of implantation in order to remove the foreign body, followed by healing of the injured tissue. The inflammatory response can result in a formation of dense layers of fibrotic connective tissues, when the foreign body cannot be phagocytized and thus, isolating the foreign body from the surrounding tissues. These characteristic features of FBR significantly affect the performance and sensitivity of the implanted sensors. For instance, macrophages that are recruited to the implant site consume glucose present in the local microenvironment, and thus can gradually influence the inherent sensitivity of the glucose sensor. Although several studies have shown potential to address certain facets of FBR, only few approaches addressed challenges imposed by the entire cascade. FBR is a progressive phenomenon with a series of complex biological processes similar to those involved with wound healing. For comprehensive understanding of the cell-biomaterial interaction, it is necessary to combine FBR with more advanced characterization techniques. Mass spectrometry (MS) is a powerful proteomic tool, which recently utilized to study the cell-material interactions. This technique offers the opportunity to analyze differential protein expression in a single experiment without the need of specific immunohistochemical markers. In the present study, high-throughput proteomic analysis was carried out using a MS techniques, namely matrix-assisted laser desorption/ionization (MALDI) 'imaging' (MSI) and 'profiling' techniques to analyze series of events that drive FBR during early capsule development (day 10). This systematic approach allowed the study of the complex interaction between cells and their microenvironment at the molecular level. Although, MALDI has become a key technology in the emerging field of proteomics, its application in the field of FBR has been limited to biofouling and 'cutaneous' wound healing. Moreover, proteomic approaches have been rarely employed to study the complex nature of cell-biomaterial interactions at molecular level. To our knowledge, there is no single proteomic study available for in vivo foreign body encapsulation due to implanted biomaterials using MALDI-MSI and MALDI tissue profiling.
Additionally, we addressed several issues relevant to sample preparation for subcutaneous skin tissue samples for MALDI-MSI analysis. The limited adhesion of skin tissue sections to indium-tin oxide-coated glass slides was the major drawback in sample preparation of skin for MSI. In the present study, 3 different sample preparation protocols – Bruker, Single desiccation, and Warm slide were compared. The effects of these protocols on spectrum quality and reproducibility were quantified using Wilcoxcon Ranked test. Our results suggest that skin tissue samples prepared with Single desiccation protocol resulted in superior skin adhesion and improved spectrum quality in terms of peak intensity (c. 500 x 103 a.u.), number of peaks (2605 ± 667), resolution (6592 ± 37), and achievable reproducibility with a correlation coefficient of 0.99. These results provide unique guideline principles for designing proteomic studies with minimum sample handling and optimum MALDI-MSI analysis.

For targeted analysis of fibrous capsule, we combined laser capture microdissection (LCM) to obtain specific cell populations within the wound bed. In solution approach adopted in LCM was found useful for our MALDI MS profiling experiments. Using MALDI MS profiling, we identified characteristic proteins e.g., Tsp1, Tsp2, Opn, Mmp-9, Mmp-14 and caspase-9 associated with fibrotic process. Our bioinformatic perspective was useful to co-relate our experimental data with the published data and reduce the complexity of multiple datasets. A comparative peptide mass fingerprinting (PMF) studies was carried out using 3 different datasets resulting from a customized in-house Perl script, Mascot and MS-Fit. The total proteins resulting from PMF were categorized into different protein classes for NTS, HDPE, control and positive control. In addition, 24 cellular components, 29 molecular functions, 55 biological processes, 27 panther protein classes and 22 KEGG pathways were statistically significant and were commonly identified in all 4 groups. These functional patterns (encompassing cytoskeleton proteins, calcium ion binding, actin binding, intracellular signaling, cell proliferation, DNA replication, enzyme modulator, transferase, TGF-β signaling, Wnt signaling and MAPK signaling) are known to be involved in the pathogenesis of FBR. Our results provide a comprehensive outline of FBR using high-throughput MALDI profiling technology and offer important biological information on the key regulatory pathways, and characteristic proteins associated with fibrotic process. These candidates could be targeted using a promising strategy in the reduction of foreign body encapsulation, thereby extending the useful life of subcutaneous implants.