

## IPD Project Details

**Project ID:** IPD7793

**Project Title:** Proteomic analysis of *Caenorhabditis elegans* wound model reveals novel molecular players involved in repair

**Description:** Now a days, wound healing is becoming a global threat that impact economy of the country severely. Though the steps involved in wound healing are well characterised, direct therapeutics for the accelerated wound healing is comparatively less. This is solely because of the incomplete mechanism of healing and the lack of knowledge on molecular players involved in wound healing. Hence, in the present study, we have investigated the molecular players involved in wound healing process using a versatile model organism *Caenorhabditis elegans* through proteomic analyses. Especially, we have employed the high through put proteomic analyses tools such as 2-D GE and LCMS/MS analysis to uncover the molecular players involved in wound healing. As a result of 2-D GE analysis, a total of 35 differentially regulated proteins were identified during injury in which 22 and 13 proteins were upregulated and downregulated, respectively. Bioinformatics analyses result suggested that the upregulated proteins have crucial roles in serotonin/ acetyl choline synthesis and calcium signalling, whereas downregulated proteins were majorly involving in ubiquitin mediated proteolysis. Additionally, the mRNA levels of up and downregulated proteins were validated using qPCR analysis. Overall, the study suggested that injury initiates multiple molecular mechanisms to repair the damage.

**Principal Investigator:** Professor Krishnaswamy Balamurugan

**PI Affiliation:** Professor

**Sample Preparation:** 1000 µg of proteins from both unwounded and wounded samples at 0 h were taken for 2-D GE analysis.

**Peptide Separation:** After 2-D separation, significantly differential regulated protein spots were excised from the gel and in-gel trypsin digestion was performed. Prior to in-gel trypsin digestion, the proteins samples were destained using 55 mM ammonium bicarbonate (AmBiC) prepared in 50 % ACN (Acetonitrile) followed by dehydrated with 100 % ACN and reduced using 20 µl of 10 mM DTT (Dithiothreitol) prepared in 50 mM AmBiC at 60 °C for 30 min. Subsequently, the protein samples were alkylated using 20 µl of 20 mM IAA (Iodoacetamide) at 37 °C for 30 min in dark and vacuum dried,

---

suspended in 5 µl of MS grade water. After that, the proteins were spotted along with 4-Cyano-4-hydroxycinnamic acid in 1:1 ratio and identified using MALDI-TOF-TOF analysis.

**Protein Characterization:** Spectrum obtained from MALDI-TOF analysis was subjected to peptide mass fingerprint (PMF) using MS Fit of ProteinProspector v 6.2.1 Tool and the proteins were identified using the following parameters. Taxonomy was set as *C. elegans*, Trypsin was selected as the primary enzyme with a specificity of at least one missed cleavage site. Mass tolerance of precursor ions was set to 1.2 Da for their respective fragment ions. Carbamidomethylation (cysteine residues) was selected as fixed modification and oxidation (methionine residues) was selected as variable modification.

**Experiment Type:** Gel-based experiment

**Species:** Data in species\_details No Data

**Tissue:** Data in tissue\_details No Data

**Cell Type:** Data in cell\_details No Data

**Disease:** Wounds and injuries No Data

**Instrument Details:** Data in instrument\_details Data in instrument\_details

**Protein Modifications:** iodoacetamide derivatized residue

**PubMed ID:** [33831597](#)