

INTISARI

Karakterisasi Extracellular Vesicles Periodontal Ligament Stem Cells Kuda dan Profil Proteinnya dengan Proteomik serta Potensinya untuk Terapi Luka Diabetes Mellitus

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Terapi bebas sel menunjukkan potensi besar dalam mempercepat penyembuhan ulkus diabetikum terutama melalui *extracellular vesicles* (eV), salah satunya *equine periodontal ligament stem cells-extracellular vesicles* (ePDLSCs-eV). Penelitian ini bertujuan untuk isolasi dan karakterisasi ePDLSCs, isolasi dan karakterisasi ePDLSCs-eV, melihat profil protein dari ePDLSCs-eV, dan evaluasi potensi terapi ulkus diabetikum secara *in vivo*. Metode yang digunakan dalam penelitian ini diantaranya karakterisasi ePDLSCs (morfologi, *colony-forming assay*, *surface marker* dan *stemness marker* dengan *Reverse transcriptase-quantitative polymerase chain reaction* (RT-qPCR), dan *multilineage differentiation*, koleksi sekretom ePDLSCs, isolasi dan karakterisasi ePDLSCs-eV dengan *Nanoparticle Tracking Analysis* (NTA) dan RT-qPCR, analisis profil protein proteomik ePDLSCs-eV dengan *High-resolution mass spectrometry* (HRMS), serta evaluasi gel ePDLSCs-eV untuk potensi kesembuhan ulkus diabetikum secara *in vivo* menggunakan tikus strain wistar berjumlah 24 ekor dengan kelompok kontrol, komersil, ePDLSC-eV, DM *base gel*, DM komersil, dan DM ePDLSC-eV. Hasil penelitian menunjukkan ePDLSCs merupakan *Mesenchymal Stem Cells* (MSCs) dari morfologi, *surface marker* (CD29, CD44, C90, CD18), *stemness marker* (NANOG), *multilineage differentiation* dengan *staining* (osteogenik, kondrogenik, adipogenik) dan ekspresi gen (RUNX2, SOX9, PPARG). Hasil NTA ePDLSCs-eV menunjukkan rata-rata ukuran partikel 100 nm dan mengekspresikan *surface marker* eV (CD9), memiliki 35 profil protein yang berperan dalam kesembuhan luka dengan tiga protein terbesar yaitu *Annexin 2* (ANXA2), *Actin Beta* (ACTB), dan *Vimentin* (VIM). Hasil evaluasi potensi secara *in vivo* membuktikan tingkat kesembuhan luka dengan ePDLSCs-eV secara signifikan ($p < 0,05$) lebih cepat dibandingkan kelompok lainnya dengan persentase penutupan luka 98,81%. Penelitian ini menunjukkan potensi xenogenik ePDLSCs-eV sebagai terapi regeneratif pada terapi ulkus diabetikum.

Kata kunci: *equine periodontal ligament stem cells, extracellular vesicles, kesembuhan luka, proteomik, ulkus diabetikum*

ABSTRACT

Characterization of Extracellular Vesicle derived from Equine Periodontal Ligament Stem cells, Proteomic Protein Profiling, and its Application in Diabetes Mellitus Wound Healing Therapy

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Cell-free therapy shows great potential to accelerate the healing of diabetic ulcers, especially using equine periodontal ligament stem cells-extracellular vesicles (ePDLSCs-eV). This study aimed to isolate and characterize ePDLSCs and ePDLSCs-eV, observe the protein profile of ePDLSCs-eV, and evaluate their therapeutic potential for diabetic ulcer treatment in vivo. The methods used in this study included the characterization of ePDLSCs (morphology, colony-forming assay, surface marker, and stemness marker using Reverse Transcriptase-Quantitative Polymerase Chain Reaction (RT-qPCR) and multilineage differentiation), collection of ePDLSC secretome, isolation and characterization of ePDLSCs-eV using Nanoparticle Tracking Analysis (NTA) and RT-qPCR, proteomic analysis of ePDLSCs-eV protein profile using high-resolution mass spectrometry (HRMS), and evaluation of ePDLSCs-eV gel for diabetic ulcer healing potential in vivo using 24 Wistar rats divided into control, commercial, ePDLSC-eV, DM base gel, DM commercial, and DM ePDLSC-eV groups. The results showed that ePDLSCs are Mesenchymal Stem Cells (MSCs) based on morphology, surface markers (CD29, CD44, C90, CD18), stemness marker (NANOG), multilineage differentiation with staining (osteogenic, chondrogenic, adipogenic), and gene expression (RUNX2, SOX9, PPARG). NTA results for ePDLSCs-eV showed an average particle size of 100 nm and expressed the eV surface marker (CD9), with 35 protein profiles involved in wound healing; the three most prominent were Annexin 2 (ANXA2), Actin Beta (ACTB), and Vimentin (VIM). The in vivo evaluation showed that wound healing with ePDLSCs-eV was significantly ($p < 0.05$) faster than in other groups, with 98.81% wound closure. This study demonstrates the xenogenic potential of ePDLSC-eVs as a regenerative therapy for diabetic ulcers.

Keyword: *equine periodontal ligament stem cells, extracellular vesicles, proteomics, ulcer diabetic, wound healing*