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Biocompatibility and biological function of mesenchymal stem cells and MC3T3 cells on poly (ethylene glycol)-nanogold nanocomposites

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Backgrounds:

Vascularization and osteogenesis play a critical role for bone tissue regeneration. The main goal of this proposal was intended to design a kind of ideal biomaterial for bone tissue engineering application. A simple surface modification method, comprising of a thin coating with gold nanoparticles (AuNPs) and poly (ethylene glycol) (PEG) was developed to improve the biocompatibility required for bone tissue regeneration.

Materials and Methods:

The surface morphology of PEG-AuNPs was characterized by the UV-Vis spectrophotometry (UV-Vis), and Fourier Transform Infrared spectroscopy (FTIR). The biocompatibility effect and biological performance of the PEG-AuNPs was evaluated by in vitro study.

Results:

The behavior of human umbilical cord-derived mesenchymal stem cells (MSCs) and osteoblast (MC3T3) on PEG-AuNPs was further investigated. Cells on PEG-AuNPs particularly that containing 43.5 ppm of AuNPs showed had better cell proliferation, low ROS generation, less monocyte activation, as well as increases in the protein expression levels of matrix metalloproteinase-9 (MMP-9), which may account for the enhanced cell migration on the PEG-AuNPs.

Conclusion:

These results suggest that the PEG-AuNPs nanocomposite thin film coating may serve as a potential and simple solution for the surface modification of bone-contacting devices.

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Activated microglia promotes glioma growth in mice with deficiency of tumor necrosis factor receptor type II

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Microglia play a critical role in the immune response of the central nervous system (CNS). Similar to other neurodegenerative disorders, microglia accumulation is observed in glioma. The recent findings have indicated that activated microglia can enhance glioma progression. It has been known that systemic inflammation increase the production of proinflammatory cytokines, including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). These cytokines not only control local inflammation in peripheral tissues, but also trigger neuroinflammation in the CNS. The multiple actions of TNFα are resulted from the complexity of TNF receptor type I (TNFRI) and type II (TNFRII)-triggered signaling pathways. Our recent findings showed that intraperitoneal (ip) injection with lipopolysaccharide (LPS) injections into TNFRII$^{-/-}$ mice (0.5 mg/kg/day) significantly increased CD11b^{+} microglia/macrophages in the cortex. In addition, progressive C6-glioma cell formed tumor was observed in the implanted site of TNFRII$^{-/-}$ mice brain when compared to that observed in TNFRII$^{+/-}$ mice. We found that no significant survival rate of TNFRII$^{-/-}$ mice treated with peripheral LPS injection before C6 glioma cell implantation was observed when compared to that of WT and TNFRII$^{+/-}$ mice. However, pre-treatment with LPS increased tumor volume in TNFRII$^{-/-}$ mice bearing C6 glioma implantation. Alternatively, the lower survival of TNFRII$^{-/-}$ mice receiving peripheral LPS injection after the implantation of C6 glioma cells was observed when compared to that detected in wild type (WT) and TNFRII$^{+/-}$ mice. Yet, there was no difference in the tumor size between surviving WT, TNFRII$^{+/-}$, and TNFRII-/- mice with formed glioma. The low surviving rate of TNFRII$^{-/-}$ mice receiving post LPS injection might be due to systemic immune response. Together, the results suggest that TNFRII deficiency and microglia activation could play the role in enhancing glioma growth.

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Developments of chemical probes that label protein tyrosine phosphatases *in vivo*

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Backgrounds:

The goal of the research is to establish a method for the analysis of protein tyrosine phosphatase activities *in vivo*. Here we report the design, synthesize, and characterization of a series of new chemical probes that can be used for *in vivo* labeling of cellular PTPs.

Materials and Methods:

Chemical probes **LCL08037, LCL09012, LCL08021, LCL09011**, and **LCL10005** were designed and synthesized. These chemical probes were tested for their labeling specificity and efficacy toward PTPs *in vitro* and *in vivo*. Purified recombinant PTPs were used to evaluate the labeling properties of these probes *in vitro*. Cell based system was use to evaluate the labeling efficiency of these probes *in vivo*.

Results:

Chemical probes **LCL08037** was first designed and characterized that had a 1,4-fluorine reactive group to be recognized and labeled classical PTPs. However, this probe cannot enter cells to label phosphatases directly. We then applied multiple approaches including two-step labeling system, addition of acyloyxmethyl to neutralize the charge of the phosphotriesters, and adopted a non-polar BODIPY group as the reporter to facilitate the entering of these probes into cells. These approaches proved to be useful that effectively labeled PTPs when incubated with cells *in vivo*.

Conclusion:

Our newly designed probes could be used as a tool in detecting and monitoring the cellular activities of PTPs *in vivo*. These newly designed PTP probes can analyze PTPs active in diseases or identify unknown PTPs in cells.

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Identification and Characterization of *Bacillus* strains Isolated from honey

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A total of 86 bacterial strains were isolated from 29 honey samples collected from different countries. According to VITEK AutoMicrobic system tests and partial 16S rDNA sequences analysis, the isolates were belonging to several common species of the genus *Bacillus*. The *Bacillus* species identified included *B. subtilis, B. pumilus, B. megaterium, B. gibsonii, B. indicus,* and species of the *B. cereus* group, whereas a number of our isolates could not be classified. Among these strains, five strains were selected and subjected to analysis by API-ZYM commercial kit system based on presence of 19 different enzymes. The results showed that esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, β-galactosidase, α-glucosidase, and β-glucosidase were produced by all tested strains. However, the properties of their enzymes varied from strain to strain. In addition, all isolated 86 strains harboring riboflavin (*rib*) genes were detected by PCR amplification of the *rib* gene. As observed, 31 out of 86 strains yielded positive PCR result bearing *rib* gene. These results are encouraging as *Bacillus* strains bearing some useful enzymes are found in different country sources of honey. The present study is an approach initiated from diversity screening of *Bacillus* species to further potential enzyme production and application from the specified *Bacillus* strains.