# Preparation, Characterization, and *in vitro* Evaluation of Mineral/Osteogenic Growth Peptide Composite Grown on Calcium Phosphate Coated CP Titanium

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**Statement of Purpose:** An ideal dental implant would possess osteoinductivity and osteoconductivity as well as biocompatibility, matched mechanical properties, and corrosion resistance. Coprecipitation of osteoinductive biomolecules and osteoconductive calcium phosphate (Ca-P) mineral layer on titanium alloy implants is one approach for improving implant biocompatibility. An important advantage of this approach is the ability to prepare Ca-P mineral layers under a mild condition, therefore leading to the retention biological activity of biomolecules. In our previous studies, fibronectin, basic fibroblast growth factor were successfully incorporated with mineral layer on titanium and resulted in improving osseointegration of titanium implants *in vitro* and *in vivo*. Osteogenic growth peptide (OGP) is a short, endogenous 14-mer growth factor peptide present in the serum at μmol/l concentrations. As a soluble peptide, OGP regulates earlier proliferation, differentiation, and matrix mineralization of osteoblast lineage cells *in vitro*, and enhances fracture healing *in vivo* with administration systemically. In this study, we coprecipitated OGP and Ca-P mineral on titanium substrate.

**Methods:** Thin calcium phosphate (Ca-P) coating layer with an excellent bonding strength were deposited on titanium by ion-beam-assisted deposition (IBAD) method. The biomimetic coating process was carried out by immersing Ca-P coated Ti in Dulbecco's Phosphate buffer saline (DPBS) containing OGP (DPBSO) at 25 °C for determined intervals. Peptides presence in the mineral/OGP composite, the quantity of peptide incorporated, the morphology and structural features of the mineral with incorporated peptides, and the localization of the peptides were examined. To investigate the biocompatibility of coprecipitation layer, Sprague-Dawle (SD) rat mesenchymal stem cells (MSCs) were cultured on the coprecipitation surface, and the adhesion, proliferation, differentiation behavior of the MSCs were studied.

**Results:** The amount of incorporated OGP increased with prolonged immersion time from 0.43 μg for 15 min immersion to 2.36 μg for 24 h immersion. The absorption bands in the FTIR spectra could be assigned to be P-O of the $PO_4$ group at 1017, 963, 598 $cm^{-1}$, and to P-OH of the $HPO_4$ group at 870 $cm^{-1}$. An amide peak is shown for mineral/OGP coprecipitaion layer at 1644 $cm^{-1}$. An additional smaller amide peak was also detected at 1551 $cm^{-1}$. The presence of OGP in the coprecipitaion layer was also confirmed by the presence of a nitrogen peak in the XPS spectra. After only 15 min of immersion in DPBSO solution, some scattered deposits were visible on the substrate. After 30 min of immersion, larger part of the Ti substrate surface was covered with the deposits layer. With increasing immersion time, the deposits layer covered the whole part of Ti substrate. Then the mineral/OGP layer grew from a small, thin, curved, dense nucleating layer to flake-like, sharp edged, loose well-crystallized layer. The XRD pattern of mineral formed in DPBS solution could be indexed as a mixture of apatite and octacalcium phosphate (OCP). As addition OGP in the DPBS solution, the pattern of mineral/OGP coating layer was more specific to compose of apatite phase. The presence of OGP within the apatite was verified by the fluorescence, and the OGP-apatite composite was an even distribution of fluorescence. The MSC cells attached more to Ti immersed in DPBSO solution than in DPBS solution. On the mineral/OGP coated Ti, the actin-tracker green stained MSC cells revealed extensive cell spreading. A significant increase in MSC cells proliferation was found at day 1, compared to on control group, while an opposite trend was observed on day 3 but was not significant. However, by day 5 a significant increase in the proliferation of MSC cells cultured on OGP group was found compared to the control. At day 7, the proliferation of MSC cells cultured on both groups began to decrease. At day 3, ALP activity was significant higher on OGP group compared to negative control group and positive control group. As time progressed in culturing, ALP activity increased in all cases. However, at day 7, ALP activity of OGP group kept the similar trend as day 3 but was not significant compared to positive control group. Furthermore, at day 11, the ALP activity of positive control group was the highest, and OGP group possessed a significant higher ALP activity than negative control group.

**Conclusions:** OGP was successfully incorporated with mineral layer on Ca-P coated Ti substrate, and evenly distributed in the mineral layer. The *in vitro* results indicated that the incorporated OGP retained its bioactivity as regulating earlier proliferation, and differentiation of osteoblast lineage cells.

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