

Supplementary Materials

Long-term Effect of Biomaterialized Insulin Nanoparticles on Type 2

Diabetes Treatment

Yun Xiao^{1,3}, Xiaoyu Wang², Ben Wang³, Xueyao Liu¹, Xurong Xu² and Ruikang Tang^{1,2}*

1. Center for Biomaterials and Biopathways, Department of Chemistry, Zhejiang University, Hangzhou, Zhejiang 310027, China
2. Qiushi Academy for Advanced Studies, Zhejiang University, Zhejiang University, Hangzhou, Zhejiang 310027, China
3. Institute of Translational Medicine, Zhejiang University, Zhejiang University, Hangzhou, Zhejiang 310027, China

Corresponding author: Professor Ruikang Tang, Department of Chemistry, Zhejiang University, Hangzhou, Zhejiang 310027, China; Tel/Fax: +86-571-87953736; E-mail: rtang@zju.edu.cn

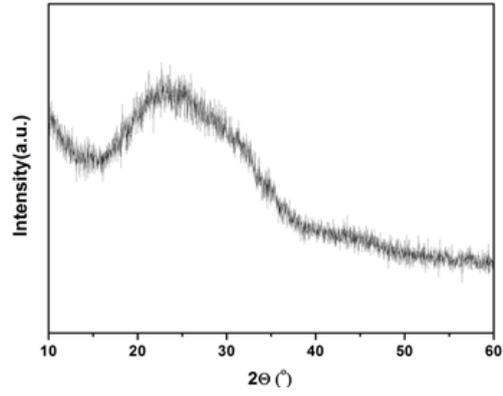


Fig S1. XRD pattern of BINP showed that the mineral phase in BINP was similar to amorphous calcium phosphate, indicating a poor crystallinity.

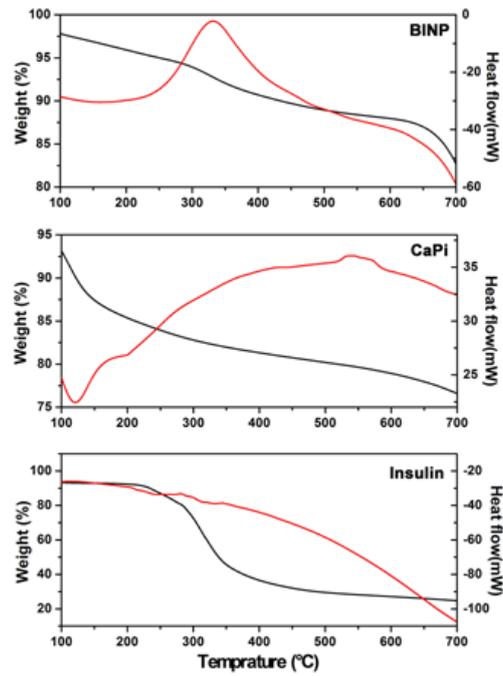


Fig S2. TGA (black) and DSC (red) curves of insulin, CaPi and BINP.

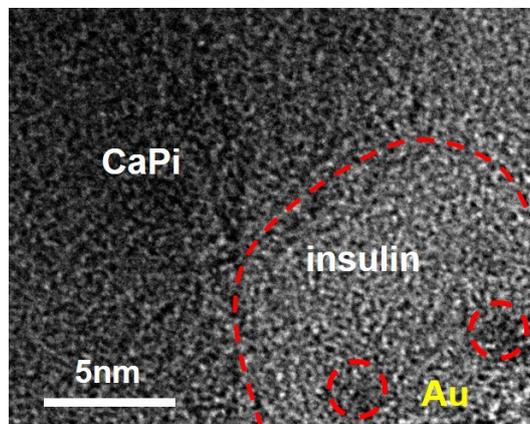


Fig S3. HRTEM image of Au-labelled BINP.

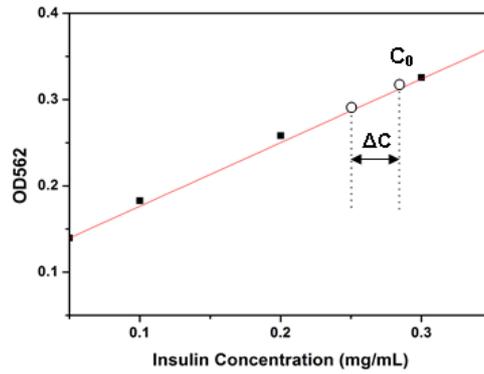


Fig S4. Standard curve of insulin concentration in DMEM using BCA Protein Assay Kit. C_0 was the original insulin concentration prior to the biomineralization. ΔC was the concentration decreasing after the biomineralization. The result showed that the percentage of biomineralized insulin was about 11% in our cases.

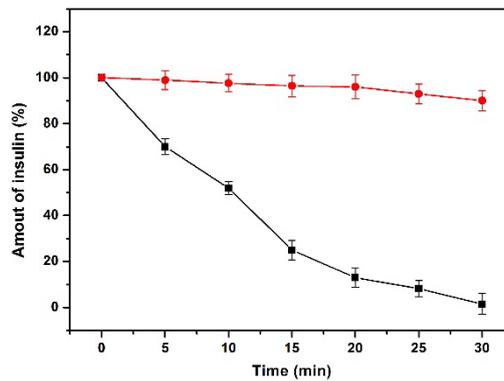


Fig S5. Insulin remaining in the presence of protease (α -chymotrypsin, 30 $\mu\text{g/mL}$) at 37 °C. The insulin in BINP (red curve) is much more stable than the native one (black curve) against the degradation.

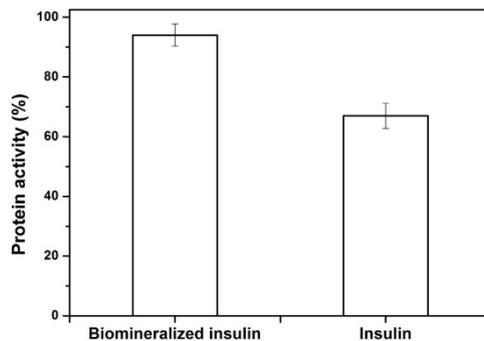


Fig S6. Stability of BINP and activities of enclosed insulin after 1 month's storage in DMEM (temperature of 37°C).

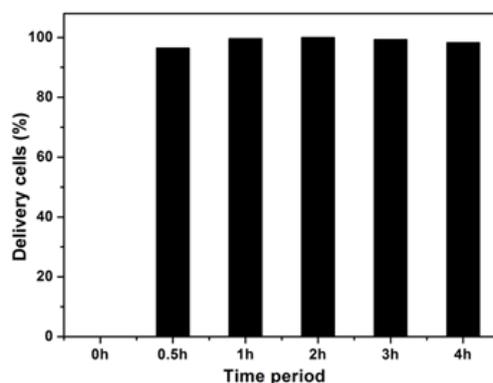


Fig S7. Flow cytometry about the BINP internalization by R-HepG2. Within 30 min, 96% cells contained the internalized BINP and the value could reach 100% at 2 h, implying a rapid internalization kinetic of BINP.

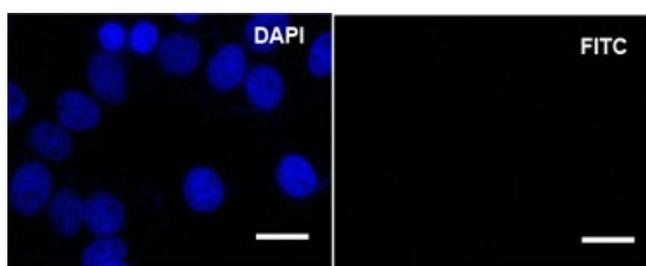


Fig S8. Confocal laser scanning microscopy image of R-HepG2 after the treatment of the insulin. No green fluorescent emission was detected in the cells. It demonstrated that Insulin was labelled by FITC (green) and cell nuclei were stained with DAPI (blue) (bar = 20 μ m).

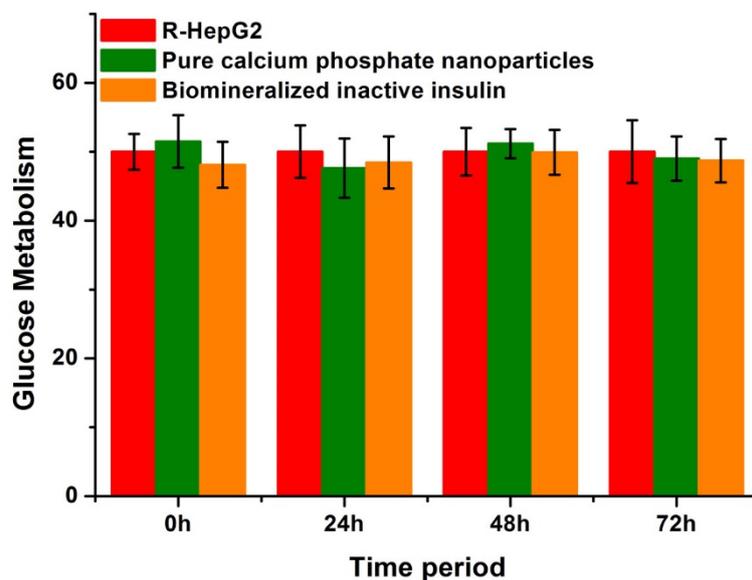


Fig S9. Glucose metabolism of cells after the treatments using the pure CaPi nanoparticles, the mixture of pre-synthesized CaPi and insulin or biom mineralized inactive insulin. No influence was observed and the results indicated their internees on glucose metabolisms.

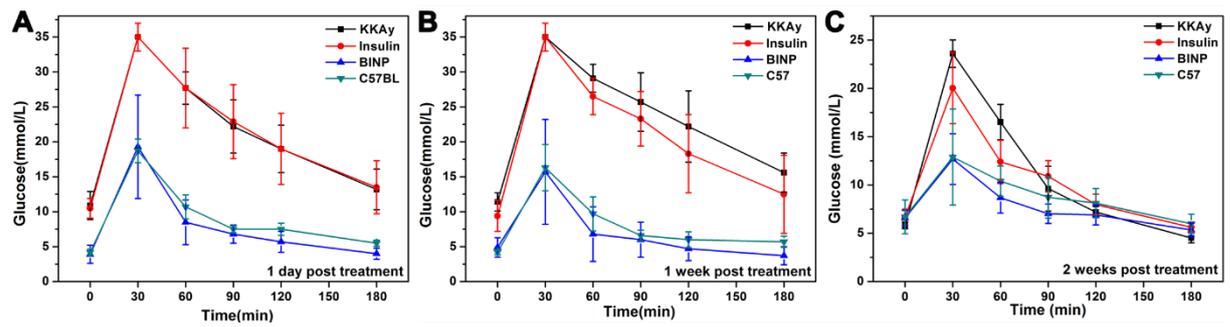


Fig S10. OGTT curves post the treatment. The results were consistent with the other independent experimental results, which were shown in the manuscript. It confirmed the conclusion about the long term treatment effect of biomaterialized insulin on diabetes.

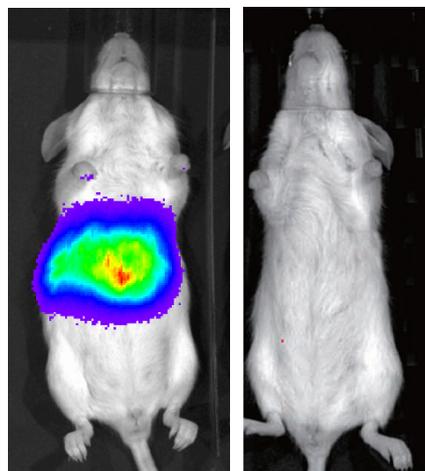


Fig S11. *In vivo* imaging of BINP treated mice 4 h (left) and 24 h (right) after the intraperitoneal injections. The BINP phase was labeled by luciferase prior to the administrations. It demonstrated that BINPs accumulated in liver organ of the mouse and they were degraded within 24 h under the *in vivo* condition.

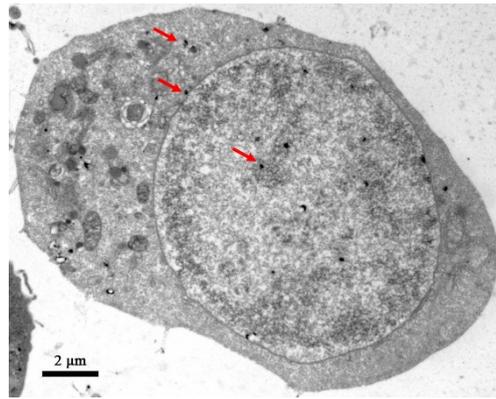


Fig S12. Biological TEM image of a sectioned R-HepG2 cell after BINP treatment. The existence of BINP could be observed within the cell.

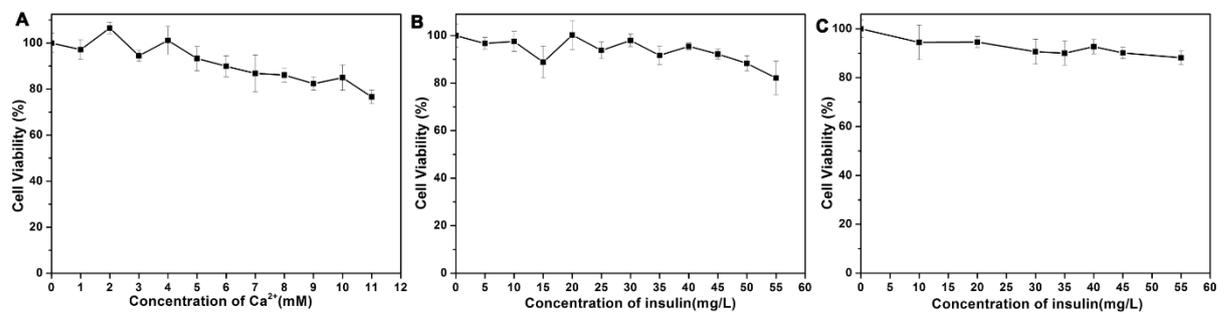


Fig S13. MTT cell viabilities under different biom mineralization conditions. (a) DMEM with different calcium concentration (Note: the *in situ* mineralization of insulin could be started at Ca concentration of 1 mM and the calcium concentration was proportional to the BI amounts in the solution). It showed that the amount of precipitated CaPi nano phase had no significant influence on cell viability (in our experiment, the mineralization condition was fixed at the Ca concentration of 5 mM). (b) DMEM with different insulin concentration of insulin and the result was that, insulin did not affect on cell viability. (c) In the presence of 5 mM calcium, the biom mineralized insulin-DMEM solution exhibited no effect on the cell viability (in this experiment, the insulin concentration was fixed at 40 mg/ml).