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Calcium Phosphate Scaffold Loaded with Platinum Nanoparticles for Bone Allograft

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Abstract

Inflammations are a predicament issue that leads to orthopaedic hard implants failure. Thereby, calcium phosphate platinum scaffolds possess anti-inflammatory properties, as powerful tools for successful bone regeneration. The effects of the platinum nanoparticles (PtNPs) on the scaffold's degradation and proliferation and attachment were evaluated. The scaffolds degradation rates ranged between 50-75 % and 80-95% for Scaffold with and without PtNPs respectively. Moreover, the cells proliferation and attachment on CPs-PtNPs scaffold were superior to CPs scaffold. The results warranted that, the synthesized scaffolds exhibit good biocompatibility and *in vitro* biodegradation, and also, it could be a used as a substrate for PtNPs delivery.

Keywords: Platinum Nanoparticles, Tissue Engineering, Bone Grafting, Calcium Phosphate.

1. Introduction

Tissue engineering is a fast growing field that provides unique alternative solutions for organ transplants, and thus offers dramatic potential improvement in human health(*1*). Autologous and allograft techniques for bone grafting are the most common choice for filling large bone defects (*2-3*). However, these approaches have their drawbacks, such as the need for second surgery at the donor site, limited

quantity and shape of available bone, and resorption of the bone graft(*4-5*). A range of porous materials, such as ceramics, polymers and metals have been developed in the field of bone grafts and scaffolds for tissue engineering(*2-10*). Some of the most favourable bioactive ceramic materials that were used in bone grafting and tissue engineering are calcium phosphate due to their exceptional physical and chemical properties (*2, 4-5*). One of the most unique properties for such materials are the mechanical, biological and protein absorption of composite used for scaffold synthesis(*2, 4, 6*). Consequently, they have been extensively used in biomedical implants for bone regeneration, such as coating for metallic implants, bone grafts and composites for middle bone implants (*4,7*). In addition to their biocompatibility and biodegradability, the osteoconductive properties of these composites make them ideal materials for bone regeneration(*7-8*). However, their poor mechanical properties, in particular their toughness, is a significant problem Successful incorporation of calcium phosphate with polymers has been achieved for the fabrication of scaffolds that have good porosity, degradability and protein absorption, while accomplishing acceptable mechanical properties in term of toughness (*7-8*). These scaffolds were subsequently used as an alternative autologous bone graft for tissue engineering(*8-9*). Generally, tissue engineering scaffolds should have suitable pore area micro-pore size, degradability and good mechanical properties (*10*). Thereby, designing a scaffold material from ceramics with other materials such as metals or polymers may lead to good simulation of the structure and properties of tissues to be replaced, and offers a tremendous potential for solving these problems (*9-10*).

Noble metal nanostructures are of considerable interest because of their unique properties and have been successfully introduced in many applications such as, antimicrobial applications, tissue engineering, and diagnostics (*11-16*). Therefore, noble metal nanostructures have been synthesized in many forms, such as wires, and flowers and have been successfully used in many applications such as medical (*11-16*). Platinum nanostructures are of particular interest for many applications, including sensing, and medical applications such as potential medicinal substance for oxidative stress diseases with suppressed mitochondrial complex (18-21) . Pt NPs show antioxidant properties that herald a promising future for the treatment of oxidative-stress-related conditions, such as neurodegenerative disorders, and including Alzheimer's diseases (18-21). Additionally, PtNPs have been shown to protect cells from oxidation-induced inflammation which inhibits pulmonary inflammation and induced bone loss by decreasing osteoclastogenesis(*18-22*). There are many versatile methods for biomaterials scaffolds fabrication such as freeze dryer, solvent casting, laser, and gas foaming.(*23-27* This method yield wide range of scaffolds varies in both chemical and physical properties. This methods result scaffolds with highly load bearing properties but with lack of degradations. Herein, we have focused on the synthesis of biodegradable and safe calcium phosphate scaffold loaded with Pt NPs.To the best of our knowledge this is the first report on the combinations between PtNPs and *CPs* as scaffolds for bone allograft. Also, this is the first report on the effect of Pt NPs on the scaffolds degradation rate.

2. Materials and methods

2.1 Materials

Hydrogen hexachloro platinate hexahydrate ($H_2PtCl_6 \cdot 6H_2O$; 99.99%), tri-sodium citrate ($Na_3C_6H_5O_7$, 98%), Dulbecco's Modification of Eagles Med (DMEM) ,glutamine, penicillin, streptomycin and (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, polyvinyl alcohol, Diammonium hydrogen phosphate $(NH_4)_2PO_4$, and Calcium nitrate $Ca(NO_3)_2$ were purchased from Sigma-Aldrich Chime GmbH (Munich, Germany). Ammonium Solution NH_4OH (35%), Poly Carbonate Filter paper, Hydrogen chloride HCL and Nitric acid HNO_3 were purchased from El-gomhouria Co, (Cairo, Egypt). Double deionized water (DDI) was prepared using a Milli-Q™ system (Direct-Q 3, Model ZRQS0P0WW, Millipore Corporation, Billerica, MA) with a resistivity of 18 MÙcm.

2.2 Synthesis of CPs-PtNPs Scaffold

The calcium phosphate and PtNPs were synthesized in our lap. In Concise, calcium phosphate platinum nanoparticles *CPs-PtNPs* scaffolds were fabricated by heat sintering. Briefly, calcium phosphate with different concentrations (namely: 20, 30, and 40 wt %) were dissolved in DDI. The powder was added to poly vinyl alcohol solution (2 wt %) to create ceramic slurries. Next, Pt NPs solutions of 10wt% were added to the slurries mixture with slow stirring and heating to complete mixing. As a cross-linker, a solution of 2 ml of 2.5 % glutaraldehyde was added drop wise. Finally the slurries were cast into a mould and dried at 100 ^{o}C for 24 hours and then sintered at 400 ^{o}C by incrementing 10 ^{o}C per minute for 8 hours. The moulds were allowed to cool at a rate of 10 ^{o}C per minute until they reached room temperature. The calcium phosphate scaffolds were synthesized as described above but free of PtNPs.

2.3 Cells proliferation and attachment

Scaffolds of size 14×10 mm were seeded with culture medium human osteoblast cells for a period of 21 days. The scaffolds were submerged in 70 % ethanol for sterility and autoclaved for two hours at 130^{o}C. Then, they were washed with phosphate buffered saline (PBS - pH: 7.4) and soaked in 5 ml of culture medium for one hour. Human osteoblast cells were grown in 24 well plates at a density of 5X10^{6} cells/ml in Medium (DMEM) containing fetal bovine serum (10%V/V), glutamine (2 mM), penicillin (100 U/mL) and streptomycin (1 µg/mL), and incubated in 5% CO_2 at 37 °C. The culture mediums were changed every 48 hours for a period of 14 and 21 days.

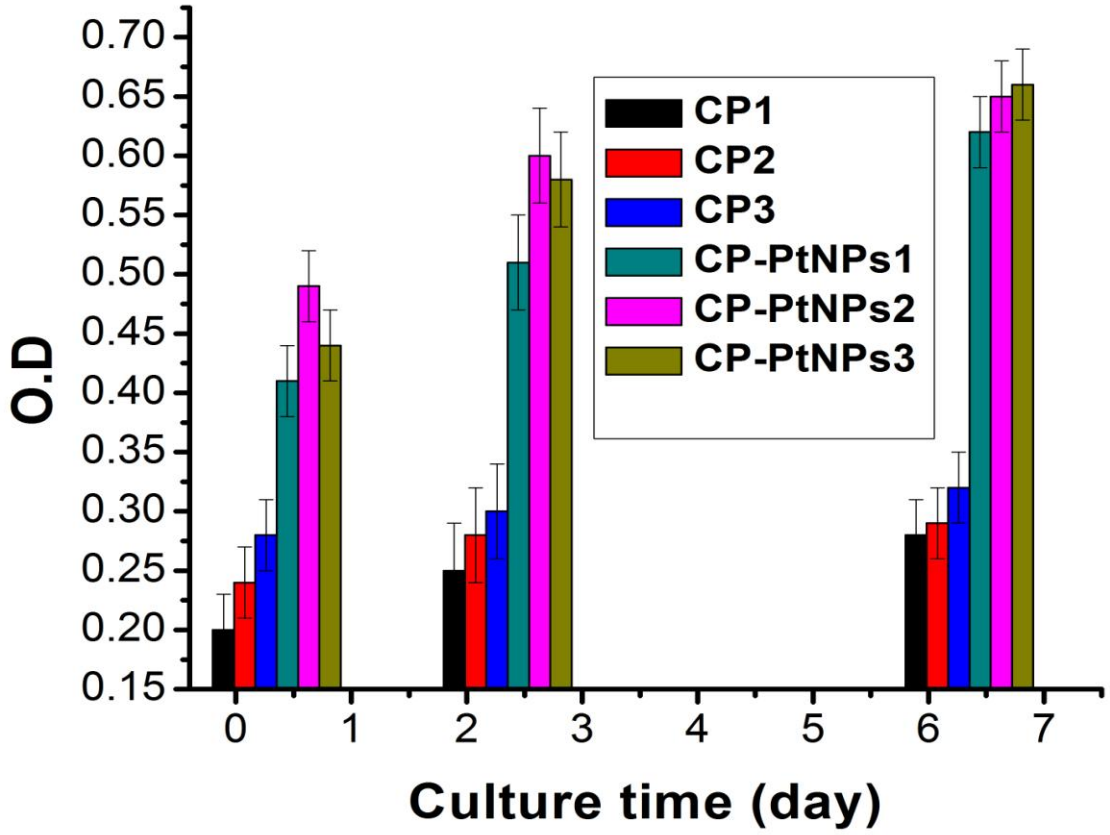

Figure 4. Cells proliferation and attachment on CP 1-3 that have 20, 30, and 40wt% of CPs powder while, CPs-PtNPs scaffold 1-3 have 20, 30, and 40wt% of CPs powder with 10%wt of Pt.

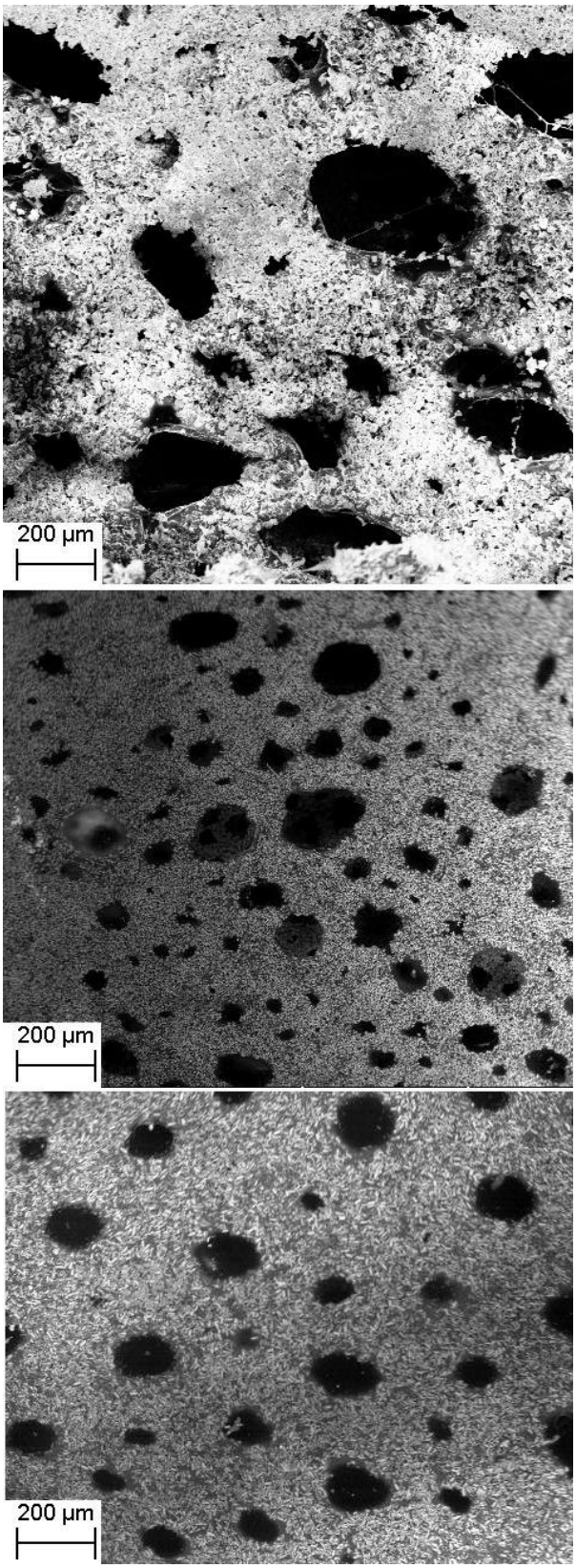

Figure 1. Ceramic scaffold that were synthesized upon using of 20, 30 and 40wt% of CPs nanoparticles without PtNPs [A-C] respectively.

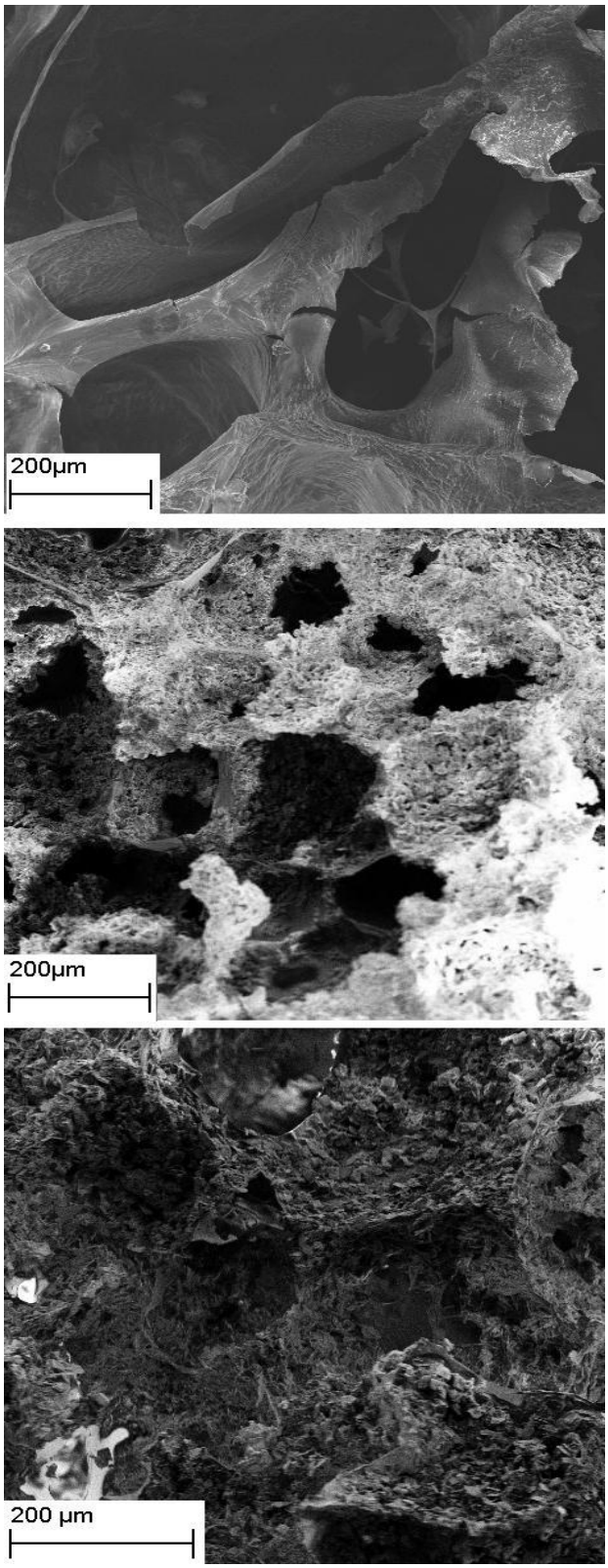

Figure 2. Ceramic scaffolds that were synthesized upon using of 20, 30 and 40wt% of CPs nanoparticles without PtNPs [A-C] respectively.

2.4 *Scaffold Morphology*

The Micro-structure of the synthesized scaffolds were studied using field emission scanning electron microscope (SEM, LEO SUPRA 55; Carl Zeiss AG, Oberkochen, Germany) at an accelerating voltage of 8-12 kV, with magnification in the range of 50-400 Kx. The scaffold samples were mounted on an SEM grid and imaged as it is without sputter coating. The micro-structures were selected randomly from different visions through imaging process.

2.5 In vitro Degradation Test of the scaffold

In vitro degradation of the scaffolds were investigated by cutting the scaffold samples to size 12×5 mm and soaking them in PBS 7.4 at 37ºC in shaking water bath for 6 hours, 1, 3, 7, 14, and 28 days. The solutions were refreshed every day and were measured using changes in dry weight after incubation for a specified time period. For such tests, the specimens were removed, rinsed in distilled water, and dried in a vacuum oven for 1 day at 120ºC. All the values presented are the average of three specimens. The percentage of weight loss was computed using the following equation:

$$\text{Weight loss} = \frac{W_t - W_0}{W_0} \text{X } 100$$

Where W_0 is the starting dry weight and W_t is the dry weight at time t.

3. Results and discussion

3.1 *Scaffolds morphology and micro-structures*

The size and morphology of the CPs-PtNPs were studied using SEM, and the particle size. The *CPs scaffolds* poses a moderate porosity but with lake of interconnectivity. Briefly the scaffold sample synthesized by using of 20wt% of CPs powder shows the best porosity ranged between 30-35% with pore size ranging between 100-400 µm. Also the pores have canal like. The porosity for scaffolds samples synthesized upon usage of 30, 40 wt% displayed porosity in the range of 20-10% with pore size ranged between 100-300 µm. These results demonstrated that, the porosity decreases with increasing of CPs powder concentration with lake of interconnected pores.

The fabricated *CPs-PtNPs* scaffold displayed a highly porous and interconnected open-pore structure with pore size distribution ranging between 100-800 µm, as shown in Fig.2 A-F. Briefly, Fig.2 A-C presents the scaffolds samples that were synthesized by using 20, 30 and 40wt% of CPs nanoparticles with 10% of Pt NPs. Other scaffolds were synthesized with 20, 30, and 40% Pt NPs with 40wt% of CPs nanoparticles to manipulate and study the effects of varying PtNPs on the scaffolds morphology as is demonstrated in Fig.2(D-I). The observed final porous structures of the scaffold are clearly a function of CPs-PtNPs ratios and PtNPs concentration. Thus, it may be deduced that increasing CPs-PtNPs and/or PtNPs concentrations increases both of the porosity and interconnectivity of the scaffolds. Therefore, the scaffolds open and form interconnected pores due to the presence of PtNPs with CPs powder that may act as non-solvent that lead to enhanced porosity and interconnectivity. The Interconnected pore structure is important to bone cell-growth, tissue regeneration, and interface support. Furthermore, the micro-porous structure is beneficial to capillary growth, nutrient transport, and biological properties of the implant (*1-7*). Several groups reported that, the porous surface improves the mechanical interlocking between the implant and the surrounding natural bone, providing greater mechanical stability at the critical interface(*7-10*, *22-25*).

3.2 In vitro Degradation Test

To support the differentiation and proliferation of cells, a scaffold should be biodegradable and bioactive. The degradability of the scaffold samples were monitored at 3, 5, 10, 15, and 25 days in culture, as shown in Fig. 4. Obviously, the degradation rate for calcium phosphate scaffolds sample 1-3 synthesized upon usage of 20,30,40wt% of CPs, increased regularly up to day 25 and ranged between 10-90% . The degradation rates increased hastily at the first 15 days and ranged between 8-78%.

Their small difference between degradation rates for samples. Also, both of samples 1 and 2 degrade correspondingly. Apparently, the degradation rates of CPs-PtNPs scaffolds sample 1and 2, increased gradually and ranged between 8-70%. Briefly, the degradation rates were faster through the first 15 days and whereas it decreased at day 15 ranging between 10-60%, followed by a steady increase in the rate degradation up till day 30, up till 70%. While, the decrease in degradation rate at day 20 and 25 for the scaffolds sample 3 and 5 may due to the depositing and partially saturating the SBF solution by CPs-PtNPs powder or may due to the unknown behaviours of nanoparticles(*11-16,18-25*). These results indicate that Pt NPs delayed the degradation rates of the scaffolds via decreasing the solubility of the scaffold. Rather than relying on the biodegradable scaffold alone, the enhanced mechanical and biological properties of Pt NPs loaded scaffold will definitely be useful when the portion of bone to regenerate is large.

Furthermore, the results clearly revealed that, the PtNPs could induce powder CPs particles to deposit in SBF, and thus has a better bioactivity *in vitro*, compared to the ceramic scaffolds.

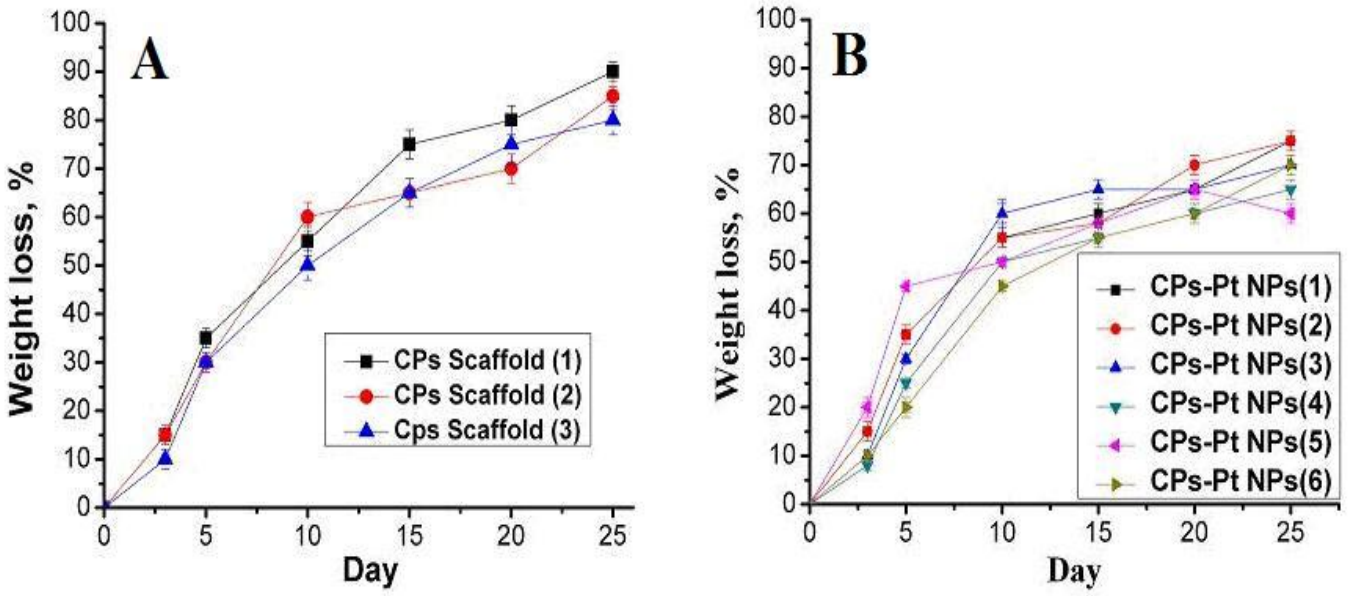

Figure 3. A The degradation rate for calcium phosphate scaffolds sample 1-3 using 20, 30,40wt% of CPs. While B is the Effect of platinum on degradation rate: Scaffolds using of 20,30,40wt % of CPs with 10% of PtNPs sample [1-3] and 20,30,40% of PtNPs with 40wt% CPs sample [4-6] on day 3-25.

3.3 Cell proliferation and attachment

Proliferation and attachment of Human osteoblast cells cultured on the CPs and CP-PtNPs scaffolds were assessed using the MTT assay. Optical density (O.D.) absorbance values were measured by micro-plat reader at day (1, 3, and 7) which it is accurate and fast methods for an indication of cell proliferation on various biomaterials. Figure 4 reveals that O.D. values for all scaffold samples increased gradually from day 1 to day 7. Also, CPs-PtNPs were significantly higher than those of CPs scaffolds at 7 days. There is a significant difference between CPs and CPs-PtNPs samples. Also, the cells proliferation on CPs-PtNPs 1-3 that have 10% of PtNPs were higher than CPs scaffold free of PtNPs. These results clearly indicated that, cell growth and proliferation on CPs-PtNPs was superior to CP, which suggesting that CP-PtNPs

scaffolds assist cell growth and could promote cell proliferation.

4. Conclusions

Calcium phosphate scaffold have successfully achieved bioactivity, degradability, and suitable mechanical properties compared to conventional polymeric scaffolds. Herein, we fabricated calcium phosphate platinum scaffolds with good degradability properties. The effects of varying Pt NPs on the scaffold morphology degradability and cells viability have been studied. The Pt NPs have unambiguous effects on the degradation rates. The *in vitro* degradation rates of calcium phosphate platinum scaffolds ranged between 8-70% after 25 days of immersing in SBF. The cells proliferation and attachment on CPs-PtNPs scaffold were superior to CPs scaffold also; the results suggested that the calcium phosphate platinum scaffold's exhibits good biocompatibility and *in vitro* biodegradability. To the best of our knowledge, platinum nanoparticles have not been incorporated previously with calcium phosphate for fabrication of scaffolds for bone allograft.

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