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Effects of OCP/PLGA Composites on MSC Differentiation and The Materials Hydrolyses

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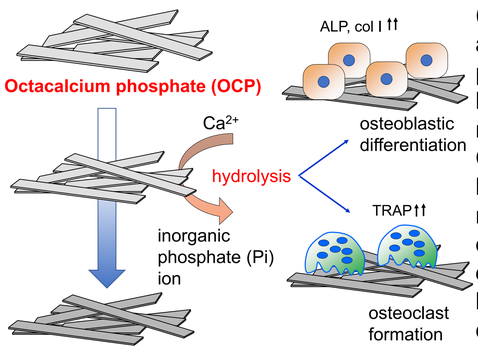
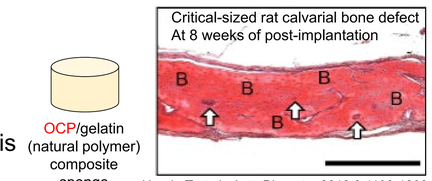
Introduction

Backgrounds

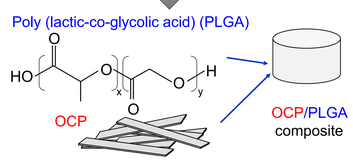


[1]Suzuki O. Jpn Dent Sci Rev 2013;49: 58-71.
[2]Brown WE et al. Prog Cryst Growth Charact 1981;4:59-87.

Octacalcium phosphate (OCP ($Ca_8H_2(PO_4)_6 \cdot 5H_2O$)), an acidic calcium phosphate, exhibits prominent osteoconductivity and biodegradability during the hydrolysis reaction into hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$) phase in various bone defect models [1]. The hydrolysis reaction controls chemical environments through the exchange of Ca^{2+} and inorganic phosphate ions between OCP and medium [2]. This chemical environment affects osteoblastic differentiation and osteoclast formation in vitro [1].

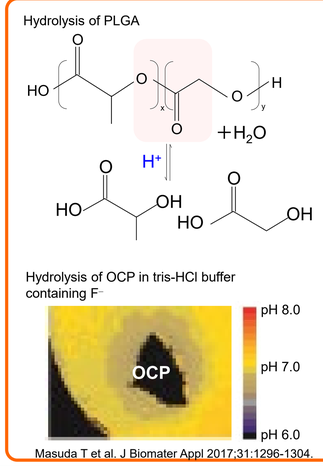


Handa T et al,. Acta Biomater 2012;8:1190-1200.
B, new bone; Open arrows, remaining OCP; Arrowhead, margin of defect.



[3]Handa T et al,. Acta Biomater 2012;8:1190-1200.
[4]Park TG. Biomaterials 1995;16:1123-1130..

However, improving the moldability of OCP is required to develop OCP-base bone substitute in clinical used. Thus, we have been investigating the fabrication of OCP/natural polymer composites and their bioactivities [1,3]. For tissue regeneration, synthetic polymers have also been used as biomaterials. The mechanical property and degradability of synthetic polymers are changed by their chemical structure. If the synthetic polymers are applied as the matrices of composites, controlling these properties of OCP-based bone substitute is expected. Poly (lactic-co-glycolic acid) (PLGA) is a synthetic polymer showing biodegradability regulated by its composition [4].



Masuda T et al. J Biomater Appl 2017;31:1296-1304.

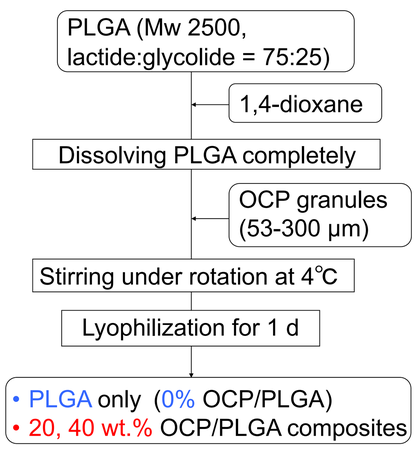
Aim of this study

The acid is known to be a catalysis of the hydrolysis of PLGA [4]. The hydrolysis reaction also induces decrease of local pH around OCP [5]. Therefore, it is hypothesized that OCP hydrolysis may affect PLGA degradation in those composite forms after the implantation into bone defects. In this study, **we examined how OCP in the presence of PLGA regulates their hydrolysis reactions and cellular activities relating to the bone regeneration in vitro.**

[5]Loo SCJ et al. Biomaterials 2005;26:2827-2833.
[6]Masuda T et al. J Biomater Appl 2017;31:1296-1304.

Materials and methods

Preparation of OCP/PLGA composites



Granules of OCP prepared through the wet synthesis method [7]. OCP was added in PLGA solution at 0, 20 and 40% (weight percent) to total weight of OCP and PLGA.

OCP/PLGA composites and PLGA with porous structure were prepared through the lyophilization process.

The microstructure of composites was observed using scanning electron microscope (SEM).

[7]Suzuki O et al. Tohoku J Exp Med 1991;164:37-50.

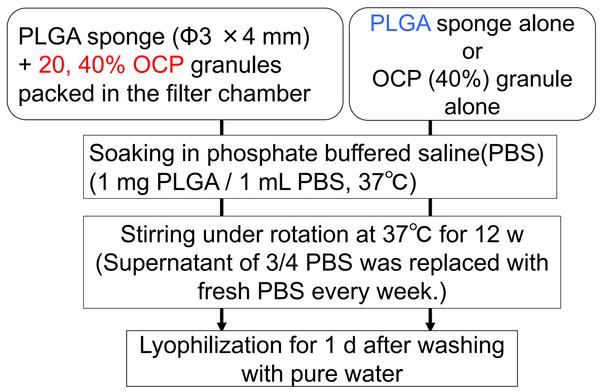
Cell culture experiments

Mouse bone marrow derived mesenchymal stem cell (MSC), D1 cell line, were seeded at 1.0×10^4 cell/well on 24 well plate.

Trans well inserts with 8.0 μm pores were set into each well. PLGA, 20 and 40% OCP/PLGA composites were placed on the bottom of the inserts. The cells with PLGA and OCP/PLGA composites were incubated in osteogenic medium at 37℃ in a humidified incubator with an atmosphere of 5% CO_2 and 95% air. The cells in the absence of materials were also incubated as control.

Alkaline phosphatase (ALP) activity normalized by DNA concentration in the cells was measured to evaluate the osteoblastic differentiation of cells at day 21.

Degradation test *in vitro*



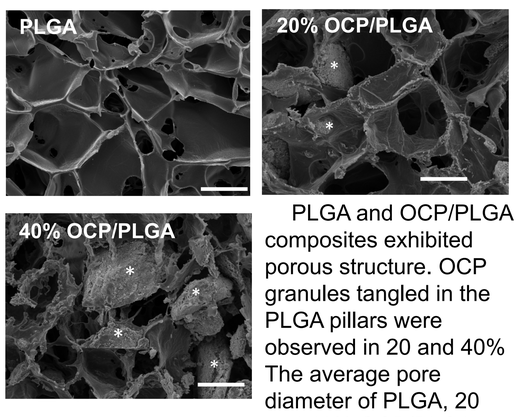
The degradation behavior of porous PLGA depending on the OCP content was examined in PBS for 12 weeks. pH and Ca^{2+} concentration in the supernatants collected every week was determined.

The weight loss rate of PLGA at 4, 8, and 12 weeks was measured.
The average molecular weight of PLGA was determined using size exclusion chromatography on HPLC system before and after the incubations.

The OCP after the incubations with and without PLGA was also analyzed using X-ray diffraction (XRD).

Results and discussions

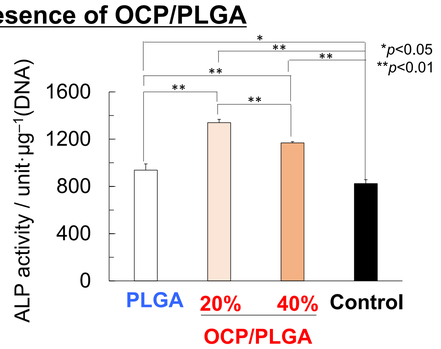
Microstructure of OCP/PLGA composites



*, OCP granule
Bars = 100 μm

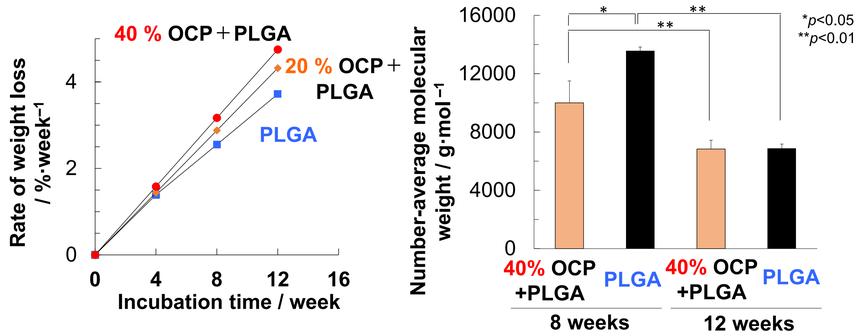
PLGA and OCP/PLGA composites exhibited porous structure. OCP granules tangled in the PLGA pillars were observed in 20 and 40% The average pore diameter of PLGA, 20 and 40% OCP/PLGA was 61, 57, and 52 μm, respectively.

Osteoblastic differentiation of MSC in the presence of OCP/PLGA



The ALP activity significantly increased in 20 and 40 wt.% OCP/PLGA groups compared to PLGA group, while it was higher in 20% OCP/PLGA group than in 40% OCP/PLGA group. The results of cell culture experiments suggest that **the composites consisting of OCP exhibit higher ability to induce osteoblastic differentiation compared to PLGA alone.**

Degradation behavior of PLGA regulated by the presence of OCP



The weight loss rate of PLGA increased with increasing the dose of OCP co-present at each incubation period. The molecular weight (M_n) of PLGA decreased after incubations in PBS at 8 and 12 weeks compared to original PLGA (93350 g·mol $^{-1}$). The molecular weigh of PLGA incubated with OCP was significantly lower that of PLGA incubated without OCP at 8 weeks. These results indicate that **the degradation of PLGA was accelerated by increasing OCP dose through the cleavage of the molecular chains of PLGA polymer in the PBS.**

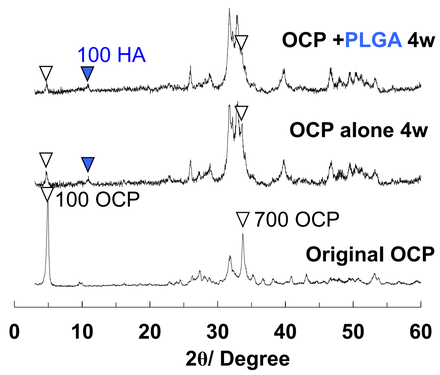
Changes in pH, Ca^{2+} concentrations in PBS during the incubations

Supernatants	Periods/ weeks	pH	Ca / mM
PBS	0	7.43	0
	1	7.44	–
PLGA	8	7.46	–
	12	7.20	–
	1	7.00	0.053
20% OCP +PLGA	8	7.33	0.018
	12	7.15	0.005
	1	7.02	0.064
40% OCP +PLGA	8	7.37	0.014
	12	7.12	0.019

The pH in PBS with PLGA only was unchanged over 1 to 8 weeks and then decreased at 12 weeks. The values of pH remarkably decreased after incubations with OCP+PLGA at 1 week. The pH in PBS with OCP+PLGA increased at 8 weeks and decreased at 12 weeks. **The PBS incubated with OCP+PLGA tended to maintain lower pH compared to PLGA only over 1 to 12 weeks.**

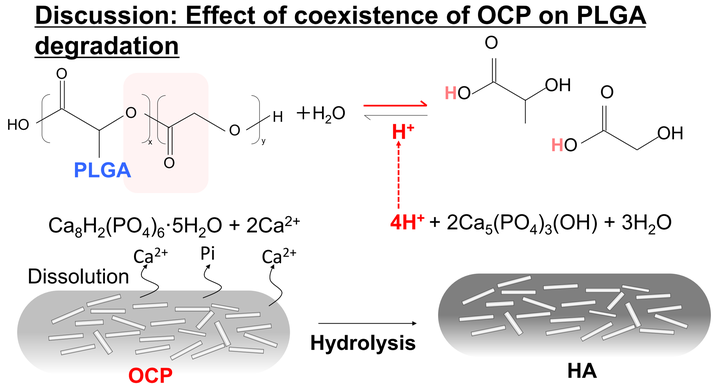
The concentration of Ca^{2+} in PBS with OCP+PLGA increased at 1 weeks and subsequently decreased over 8 to 12 weeks. The Ca^{2+} concentration in PBS with 40% OCP+PLGA tended to be higher than that in PBS with 20% OCP+PLGA at each week.

Hydrolysis of OCP regulated by the presence of PLGA



The diffraction peaks corresponding to 100 and 700 of OCP were observed at 4.7° and 33.6° in the XRD patterns of original OCP. At 4 weeks, the peak attributed to 100 of HA was detected at 10.9° in the patterns of OCP incubated in PBS with and without PLGA. The intensity of diffraction of 100 and 700 was decreased after the incubations in PBS. These intensities in OCP incubated with PLGA tended to be lower than those in OCP without PLGA. These patterns suggest that **the hydrolysis reaction of OCP with PLGA could progress compared to OCP without PLGA in physiological conditions.**

Discussion: Effect of coexistence of OCP on PLGA degradation



The degradation product of PLGA acts as an acid catalyst in the hydrolysis reaction of ester bond in PLGA in the absence of OCP. In the degradation tests, the dissolution and hydrolysis of OCP occurred in PBS. Thus, the OCP could provide the acid catalysis from early stage of incubations in PBS. The results in this study suggest that **OCP in the composites accelerate the degradation of PLGA through the progress of the hydrolysis reaction of OCP.**

Conclusion

- The results in this study suggest that coexistence of OCP and PLGA affected the progress of mutual hydrolysis reactions and OCP in the matrices of PLGA stimulated the osteoblastic differentiation of MSCs in vitro.
- The combination with OCP could regulate the biodegradability of composite bone substitute materials using biodegradable synthetic polymers as the organic matrix.