Adsorption and Desorption of FGF-2 from Carbonate Apatite Granular

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Introduction: Combination of artificial bone substitute and growth factor is expected to mimic graft bone since bone contains not only carbonate apatite (CO_3Ap) but also growth factors such as bone morphogenetic protein(BMP), insulin-like growth factors (IGF), transforming growth factor (TGF) and fibroblast growth factor (FGF). In this study, adsorption and desorption of FGF-2 were studied to shed some light to understand the usefulness of the combination product of artificial bone substitute and growth factor.

Methods: CO_3Ap granular with 600-1000μm in diameter was made based on dissolution-precipitation reaction using $CaCO_3$ granular as a precursor. 0.18g CO_3Ap granular was immersed in 0.25ml FGF-2 solution under vacuum or under atmospheric condition at 0^oC. Concentration of the FGF-2 was measured by BCA method. In some experiments, FGF-2 was fluorescence stained using HiLyte Fluoro 488 acid, and its adsorption behavior to CO_3Ap granular was observed by fluorescence microscope. Desorption of FGF-2 from FGF-2 absorbed CO_3Ap granular was measured by ELISA method.

Results and discussion: No significant difference was observed between the vacuum immersion and simple immersion with respect to the adsorption of FGF-2 on CO_3Ap granular. In other words, FGF-2 was adsorbed on the surface of CO_3Ap granular but no penetration into the center of the CO_3Ap granular was observed even under vacuum immersion.

Fig. 1 shows the concentration change of FGF-2 solution when CO_3Ap granular was immersed in the 100μg/ml FGF-2 solution. FGF-2 concentration decreased quickly indicating quick adsorption of FGF-2 on the surface of CO_3Ap granular. It should be noted that complete resorption was not achieved at least within 12 h.

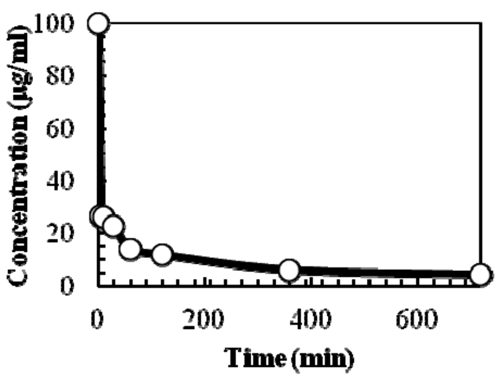

Fig. 1 Concentration of the FGF-2 when 0.18g CO_3Ap granular was immersed in 0.25ml 100μg/ml FGF-2 solution at 0^oC.

The adsorption behavior was the same even when the FGF-2 concentration was decreased to 10μg/ml, and approximately 4 μg/ml FGF-2 remain in the solution These results indicates that there is an equilibrium between adsorption and desorption of FGF-2 on CO_3Ap.

Fig. 2 and 3 show the concentration of FGF-2 released from the FGF-2 adsorbed CO_3Ap granular into water and saline, respectively. The released FGF-2 was again adsorbed by CO_3Ap granular in both case.

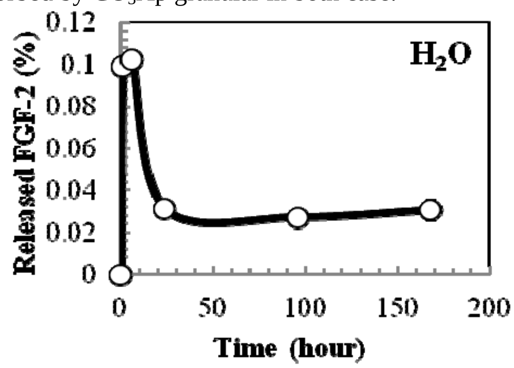

Fig. 2 Concentration of the FGF-2 when 0.18mg FGF-2 adsorbed CO_3Ap granular was immersed in 0.25ml water at 0^oC.

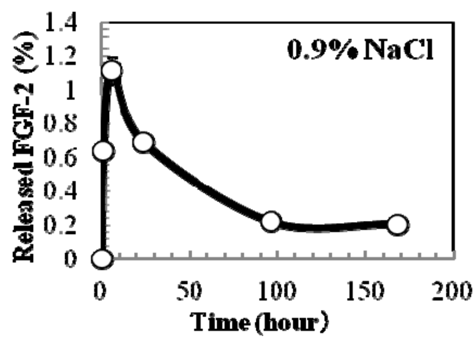

Fig. 3 Concentration of the FGF-2 when 0.18g FGF-2 adsorbed CO_3Ap granular was immersed in 0.25ml 0.9% NaCl at 0^oC.

However, the amount of released FGF-2 was different up to the solution used for the immersion. 0.03% of the adsorbed FGF-2 was released into water from FGF-2 adsorbed CO_3Ap granular whereas 0.3% of the adsorbed FGF-2 was released into 0.9% NaCl solution. In other words, the amount of released FGF-2 was ten times larger when immersed in electrolyte. These result indicate that the ionic adsorption of the FGF-2 to CO_3Ap granular is present at least in part. Also, it should be noted that the amount of released FGF-2 is limited even in the 0.9% NaCl solution. Most of the FGF-2 was kept absorbed on the surface of CO_3Ap granular.

Conclusion: FGF-2 was adsorbed on the surface of CO_3Ap efficiently. At least, ionic bonding plays some role for the resorption. Further studies are awaited based on this initial results obtained in the present study.