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HYDROXYAPATITE DEPOSITION FROM AQUEOUS SOLUTION ONTO TIALV AND TIALV COVERED WITH COLLAGEN BY THE THERMAL SUBSTRATE METHOD

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INTRODUCTION

Hydroxyapatite (HA – $Ca_{10}(PO_4)_6(OH)_2$) is bioactive mineral, which is in focus of investigations deduced to developing of composite materials for bone implantation [1-2] and for producing bioactive covering of metal surgical implants [3-7]. The main task of implant covering study connected with possibility to develop such implants, which could have good osteointegration to reduce risk of their loosing or making intoxication of patient by metal ion diffusion into bone. From this point developing of implant covering, which could includes HA together with bioactive bone biomolecules looks very promising for achieving this aim. This requires developing of deposition technology under physiological conditions (temperature and pH), to avoid denaturation of biomoleculs. It is known numerous methods of metal surfaces covering by calcium phosphate compositions [3-7]. Among them can be useful for such approach recently developed by Kuroda et al [6-7] thermal deposition method permitted to produce HA coating from aqueous solution by heating surface of deposited plate up to 140° C with current 50 A. In our article we proposed developing of this method into direction of getting covering of HA at physiological temperature (37 ^{0}C) and with introducing collagen into the surface layer before making deposition.

EXPERIMENTAL METHOD

The basic scheme of experimental set up [Fig.1] is corresponding to Kuroda et al [6-7] experimental apparatus. In addition to it we introduced water-cooling system to keep stabile temperature gradient in solution between region of plate and bottom solution of deposition glass. During experiments the temperature of bottom solution was kept at the level of 19 ^{0}C and temperature on the plate surface and near by at 37 ^{0}C. Also additional equal referent glass with distillate water was used for precise measurement the temperature of plate surface during experiments by NiCrNi termocouple.

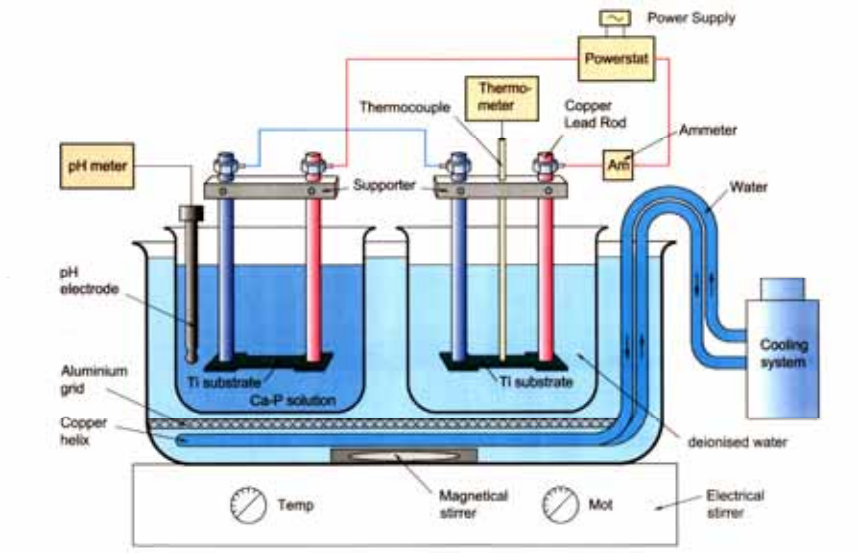
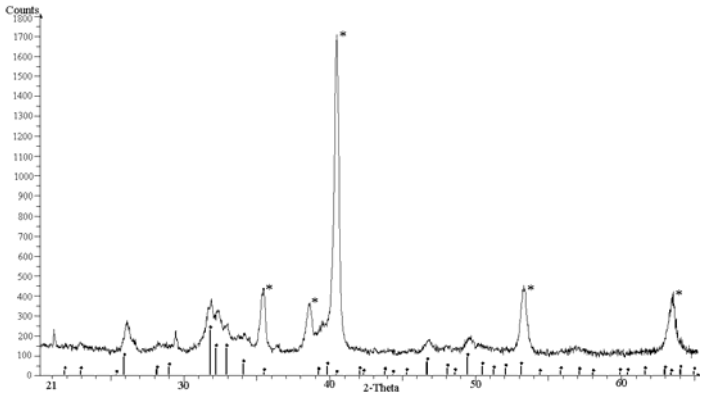


Fig 1. The experimental set up for the hydrothermal substrate method with cooling system

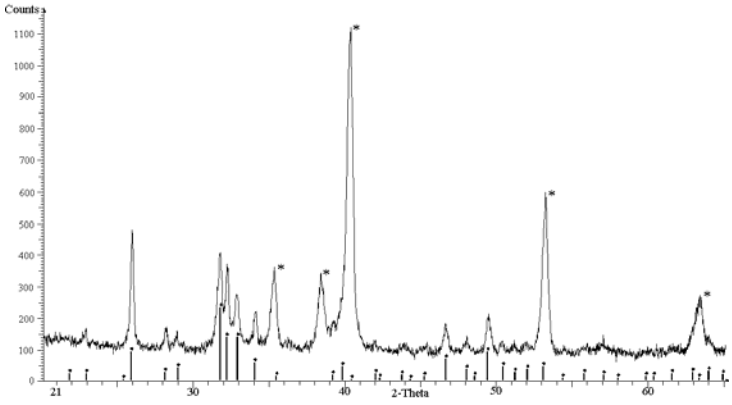
Both experimental pots were immersed into bigger common pot containing deionized water and were in direct contact with a cooling system, that is a copper grid with running tap water, cooled up to a needed temperature. In order to keep the same temperature in whole bulk an electrical mortal was used. For avoiding air bluebells, which appeared during thermal deposition on the plate surface, both solutions were degassed during 1 hour at temperature 55 ^{0}C by using water-cooling system. Working solution for HA deposition was 0,2 dm^3 aqueous solution of H_3PO_4 and $CaCl_2$ $6H_2O$ compounds (the molar Ca/P ratio was 1,67), starting pH 2.6. The pH of solution was adjusted up to 6.0÷6.3 by the addition of NaOH. An alternating current (13 A) was passed through the sample for it heating up to temperature (37 ^{0}C), time of deposition was 1 hour. Plates were cleaned with chloroform and distillate water before putting for deposition. The covering of plate surface with collagen was done by collagen type I from calfskin obtained from Fluka chemical company. Covering of sample plates by collagen was performed in water solution of collagen (5 mg/ml) and than obtained sample dried in warm cabinet at 37 ^{0}C. The weight of plates measured on electrical balance before and after deposition for obtaining information about mass distribution of HA on plate surface. The weight of collagen-covered plates was measured separately 3 times. The structure-morphological properties for samples were characterized using X-ray diffraction (XRD) and light microscopy. Different polycrystalline phases could be distinguished by comparison with reference data from the JCPDS database [8]. The data was collected with the diffraction angle 2Θ ranging from 5 to 65 °, a step size of 0.02 ° and a measurement time of 1 s per step. The X-ray source was Cu-K_α radiation from a Cu X-ray tube (run with 30 mA and 40 kV), with the disturbing Cu-K_β radiation suppressed by the insertion of a Ni filter.

Results and disscusion

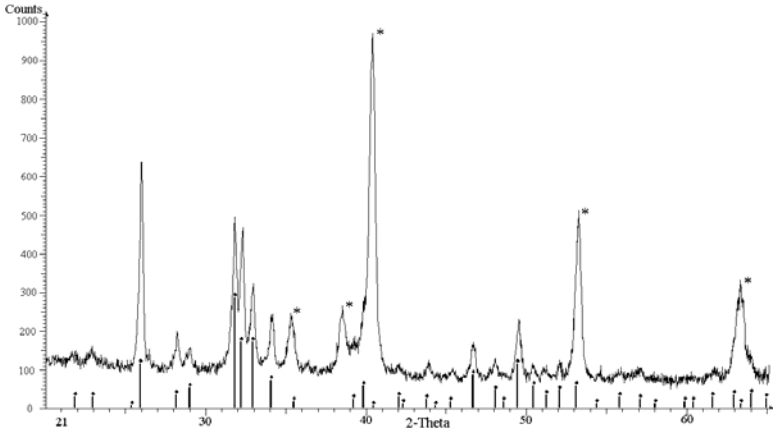
First the level of pH of experimental solution for deposition was investigated to get deposition of the pure HA phase at 37 ^{0}C. It was found that the most suitable start pH of solution for depositing HA at this temperature is between 6.0 and 6.3. pH below 6.0 still permits to get HA phase, but dramatically decreases speed of deposition, which already decreased by lowering temperature on the surface during deposition. Deposition at pH 6.3 gave pure HA coating with small crystal size and crystallinity and with presence some small rest of brushite ($CaHPO_4$ $2H_2O$) phase. pH value more than 6.3 changed phase composition of deposited calcium phosphates by adding more brushite phase. Next increasing of pH up to 6.5 with temperature 37 ^{0}C permits to get pure brushite phase on plate, with very high crystallinity. Figure 2 shows XRD patterns after 1hour deposition on pure plate at ph 6,3 (Fig. 2a), at pH 6,15 (Fig. 2b) and at pH 6,15 with addition of collagen (Fig. 2c).



(a)



(b)



(c)

Fig 2. The XRD patterns of hydroxyapatite coatings at 37 ^{0}C and pH 6.3 (a), pH 6.15 (b) and pH 6.15 with collagen (c). Titanium phase is shown by stars

All depositions were obtained with temperature of substrate 37 ^{0}C. One can see that resolution of the HA obtained at pH 6,15 (Fig. 2b) is much higher in comparison with pH 6,3, which reflected bigger crystal sizes of HA and higher level of crystallinity of those HA. The deference of the plate weight before and after deposition at pH 6, 15 without collagen is 0.0084, which gives a mass distribution . There are some peaks, which correspond to HA phase and peaks that correspond to Ti. It was observed some interesting points connected with texture of HA deposited on plate by thermal substrate method in comparison with powder HA. Their reflection peak at 2θ 26-degree shows bigger relative intensity than those for normal powder of HA. In order to control the stability of HA covering with time, we kept this sample during four months in glass box and than re-measured it again by XRD. We found that the phase composition of sample did not change, it still corresponded to HA and Ti. Also the texture future, which was determined initially, did not change. So, the sample was remained the same, only the intensity of peaks of HA became more pronounced.

The introducing of collagen into Ti surface before deposition of HA was connected with two main goals: 1) to develop possibility to make deposition of HA on protein layers by thermal substrate method, which in future could be extended to make co-deposition of HA together with other biomolecules; 2) to study influence of collagen onto HA formation during deposition. In fact bone growth is a complex biological process involving many biomolecules (collagen, proteins, growth factors etc.) and cells (e.g. osteoblasts and osteoclasts). At the same time collagen is one of the main protein involved in the process of mineralisation of bone, which is in a very simplified model occurs as the

precipitation of calcium phosphate from an oversaturated solution of calcium and phosphate ions on collagen matrix with following partial transformation of the amorphous phase to hydroxyapatite.

The amount of collagen on the plate within mass recording limit corresponded to 0.0004. According to light microscope observation collagen did not make full film on the surface, but surface still have free spaces.

The XRD measurements of 1 hour HA deposition on collagen at pH 6.15 gave from the first view practically the same results: high crystallinity HA and Ti peaks (see Figure 2c). Direct comparison of diffractogram shows that HA crystal size and thickness of covering for these two samples are different. The mass of HA deposition on collagen layer is 0.0065, which gave the mass distribution .

Conclusions

In this work the method for getting pure HA covering at physiological temperature on TiAlV plates was developed on the basis of thermal substrate method. Method was extended to producing the collagen -HA protective covers on TiAlV plate. It was found that collagen improved crystallinity of protective covers. The further experiments should go along direction to introduce of the drugs or other bioactive substances toward bone tissue into protective HA alloys covers. This will permit to reduce the risk of inflammations and repulsions of alloys from bone tissue with time, which is very important for long-time staying implants in patient body.

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Hydroxyapatite deposition from aqueous solution onto TiAlV, and TiAlV covered with collagen by the thermal substrate method

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Thermal substrate method recently proposed for getting hydroxyapatite coating on titanium was used to obtain deposition of high crystallinity hydroxyapatite onto titanium-based plate (TiAlV) on physiological temperature. The coating was produced onto free plates and onto collagen covered surfaces. The collagen was introduced to surface of titanium-based plates in order to modulate possibility to get bioactive layers of hydroxyapatite by the thermal substrate method, what is important for improving osteointegration of titanium implants into bone. The influence of pH value of solution on the processes of deposition was investigated. The experimental conditions were: temperature = 37 ^{0}C, deposition time = 1 hour, pH = 6,00 - 6,3 and Ca/P = 1,67. The best pH value for depositing high crystallinity hydroxyapatite on target corresponding to 6,15, going down with pH reducing speed of deposition and increasing pH to 6,3 decrease crystallinity of hydroxyapatite. Collagen improved speed and crystallinity of layers.
Key words: thermal substrate method, hydroxyapatite, and collagen.

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Гідроксилапатитні покриття на TiAlV , осаджені з водного розчину в присутності колагену методом термічної депозиції.

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Нещодавно запропонований в літературі метод термічної депозиції гідроксилапатитних покриттів на титані був використаний для отримання висококристалічного гидроксилапатиту на пластині з титанового сплаву (TiAlV) при фізіологічній температурі. Покриття було нанесене на чисту пластину і на пластину, покриту плівкою колагену. Колагенова плівка була нанесена на поверхню титанової пластини з метою промоделювати можливість утворення біологічно-активного гідроксилапатитного покриття методом термічної депозиції, що є важливим чинником для поліпшення остеоінтеграції титанового імпланту в кісткову тканину. Вплив величини pH розчину на процес депозиції був досліджений. Умови експерименту були наступними: температура 37^{0}C, термін депозиції 1 година, Ca/P= 1,67, pH= 6,0-6,3. Значення pH=6,15 є найбільш оптимальним для утворення плівки висококристалічного гідроксилапатиту на титановій пластині. Зниження pH призводить до сповільнення депозиції, підвищення ж рівню pH до 6,3 зменшує кристалічність гідроксилапатиту. Колаген сприяє прискоренню депозиції та підвищує кристалічність осаду.
Ключові слова: метод термічної депозиції, гідроксил апатит,колаген.

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Гидроксиапатитные покрытия на TiAlV , нанесенные из водного раствора в присутствии коллагена методом термической депозиции.

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Недавно предложенный в литературе метод термической депозиции гидроксилапатитных покрытий на титане был использован для получения высококристаллического гидроксилапатита на пластине из титанового сплава (TiAlV) при физиологической температуре. Покрытие было осаждено на чистую пластину и на пластину, покрытую пленкой коллагена. Коллагеновая пленка была нанесена на поверхность титановой пластины с целью промоделировать возможность образования биологически активного гидроксилапатитного покрытия методом термической депозиции, которое является важным фактором улучшения остеоинтеграции титанового импланта в костную ткань. Влияние значения pH раствора на процесс депозиции было исследовано. Условия эксперимента были следующими: температура 37^{0}C, время осаждения 1 час, pH=6,0-6,3, Ca/P=1,67. Значение PH=6,15 наиболее оптимально для образования пленки высококристаллического гидроксилапатита на титановой пластине. Снижение pH приводит к замедлению осаждения, повышение pH до значения 6,3 уменьшает кристалличность осадка. Коллаген способствует ускорению депозиции и повышает кристалличность осадка.
Ключевые слова: метод термической депозиции, гидроксилапатит, коллаген.