PREPARATION OF CALCIUM PHOSPHATE BIOCERAMIC POWDERS SYNTHESIZED IN SIMULATED BODY FLUID MEDIA

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ABSTRACT

In this study, calcium phosphate bioceramics powders were synthesized using $Ca(NO_3)_2.4H_2O$ and $(NH_4)_2HPO_4$ as the precursor by a biomimetic process. The condition of the synthesizing process is set mimicking the human physiological environment. The synthesized samples then were dried and calcined at several temperatures. The samples were studied after characterized by XRD, FT-IR, EDS and SEM. The process in this study was successful in synthesizing various types of calcium phosphate bioceramics.

Keywords: Calcium phosphate bioceramics, biomimetic process

PREPARASI POWDER BIOKERAMIK KALSIUM PHOSPHATE DISINTESA DI DALAM MEDIA *SIMULATED BODY FLUID*

ABSTRAK

Pada Penelitian ini powder biokeramik kalsium phospate disintesa mengunakan $Ca(NO_3)_2.4H_2O$ dan $(NH_4)_2HPO_4$ sebagai prekursor dengan methoda *biomimetic process*. Situasi proses sintesa di buat meniru lingkungan physiologi manusia. Sampel-sampel hasil sintesa kemudian dikeringkan dan dikalsinasi pada beberapa temperatur. Sampel-sampel hasil sintesa tadi dikarakterisasi mengunakan XRD, FT-IR, EDS dan SEM. Proses yang digunakan dalam penelitian ini telah berhasil disintesa beberapa jenis biokeramik kalsium phosphate.

Kata kunci : Biokeramik, kalsium phospate, proses biomimetik

INTRODUCTION

The need of better and faster healing method for patients has increased the demand of biomaterial in the biomedical field. One type of the biomaterial that interested scientist and clinician to be studied and developed is a synthetic "Calcium Phosphate" based bioceramics. As we know, calcium and phosphate are the main elements of the human hard tissue mineral.

The most widely used calcium phosphate based bioceramics are hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), β-tricalcium phosphate (β-TCP, $Ca_3(PO_4)_2$) and biphasic mixtures of these two. At present, synthetic calcium phosphate bioceramics are widely used in the area of orthopedic, dentistry and drug delivery. Further researches showed that calcium phosphate bioceramics are possible to be use as an ocular implant (*B. Kundu et al.* 2004) and also genetic therapy for certain types of tumors/cancers (*Zhi-Su Liu et al*. 2003) .

Considering the numerous applications of calcium phosphate bioceramics in biomedical fields, various synthesis techniques have been developed. Calcium phosphate bioceramics have been synthesized using several methods including the wet chemical precipitation , sol-gel, solid state reaction and hydrothermal method. Their presences are in the form of powders, granules, foams, dense and porous blocks, coating, cement, and also various composites.

Another possible technique to synthesize calcium phosphate bioceramics is by biomimetic (bio=life, mimetic=mimicking) method. In this method, calcium phosphate based bioceramics are precipitated in mimicking the condition of the physiological environment of the human body. The media used for the precipitation simulates body fluid (SBF), an inorganic solution with ion concentrations and a pH value similar to human blood plasma. Some studies of this method reported that this technique has been successful in synthesizing calcium phosphate bioceramics powders which are similar with those in the human bone (*T.V. Thamaraiselvi et al*. 2006; *A. Cüneyt Tas*. 2000; *Defne Bayraktar and A. Cüneyt Tas.* 2000).

METHODES AND MATERIALS

Processes of study and experiment conducted are given in the flowchart of the Figure1.

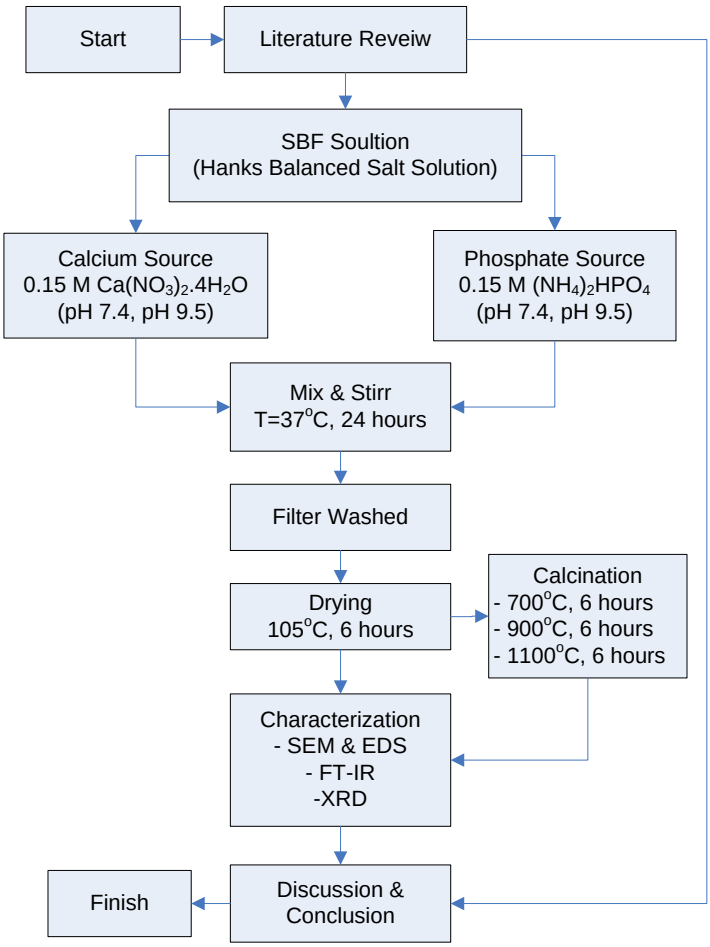


Figure 1 Process flowchart of the study

Simulated Body Fluid (SBF) used in this study was Hanks Balanced Salt Solution (HBSS). Pro-analyst grade of NaCl, $CaCl_2$, KCl, $MgCl_2.6H_2O$, $MgSO_4.7H_2O$, $NaHCO_3$, $Na_2H_2PO_4$, and $Na_2HPO_4.2H_2O$ were used in the preparation of SBF. Reagent were dissolved one by one in de-ionized water at appropriate quantities based on the

appendix of ASTM F 2129-04. The comparison of ion concentration between HBSS and human blood plasma can be seen in Table 1.

Table 1. Ion concentrations of human blood plasma and SBF (mM)

Ion	Blood Plasma	HBSS
Na^+	130-155	141.7
K^+	4.0-5.6	5.7
Mg^+	1.6-2.2	0.8
Ca^{2+}	4.0-5.5	1.7
Cl^-	100-110	145.6
HCO_3^-	24-30	4.2
HPO_4^{2-}	1.6-2.7	0.7
SO_4^{2-}	0.7-1.5	0.8

A series of experiment were performed over two different pH range. Pro-analyst grade of calcium nitrate tetrahydrate salt ($Ca(NO_3)_2.4H_2O$) were used in the precipitation as the calcium ion sources. Phosphate ion sources are obtain from pro-analyst grade of di-ammonium hydrogen phosphate salts ($(NH_4)_2HPO_4$). Each precursor was dissolved separately in SBF, depending on the set of experiment. The concentration of $Ca(NO_3)_2.4H_2O$ and $(NH_4)_2HPO_4$ were set as 0.15 M.

After the precursors were ready, each solution of precursor was adjusted at acidity of pH level 7.4 and 9.5 by adding some amount of 6 M NH_4OH solution. Then amount volume of 0.15 M $Ca(NO_3)_2.4H_2O$ and 0.15 M $(NH_4)_2HPO_4$ solution were added together with the molar ratio of Ca/P about 1.67. The solution then were stirred for 24 hours with reaction temperature maintained at 37^{o}C.

The resulting products were filtered, washed and dried in air oven at 105^{o}C. Then the products were calcined for 6 hours. The calcination temperatures were conducted at 700^{o}C, 900^{o}C and 1100^{o}C.

In this study the samples were characterized by Fourier-transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), Scanning electron microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDS). X-Powder software was used to help processing the XRD data.

RESULTS AND DISCUSSION

During the process, the acidity in the reaction increased. In the first sample, the precipitations occured mostly in the acid region. In the second sample, all of the precipitations occurred in the alkaline (base) region.

The EDS semi-quantitative analysis was conducted to calculate the approximate Ca/P mol ratio. This will help to identify and verify the compound result. The approximate Ca/P molar ratio for each sample was belowed the stoichiometric Ca/P of β-TCP (1.5) and HA (1.67). Only sample II with calcination was at 1100^{o}C.

The change of pH values and semi-quantitative EDS results for Ca/P molar ratios are presented in Table 2.

Table 2. Change of pH value and Ca/P molar ratio

No	pH		Calcination	Ca/P mol
	Initial	Final		
I	7.4	4.8	700°C	1.00
II	9.5	7.7	700°C	1.39
			1100°C	1.47

XRD and FT-IR analyses were conducted to identify the phase of compound synthesized. The main phase identified from the XRD analyses for each sample is given in Figure 2.

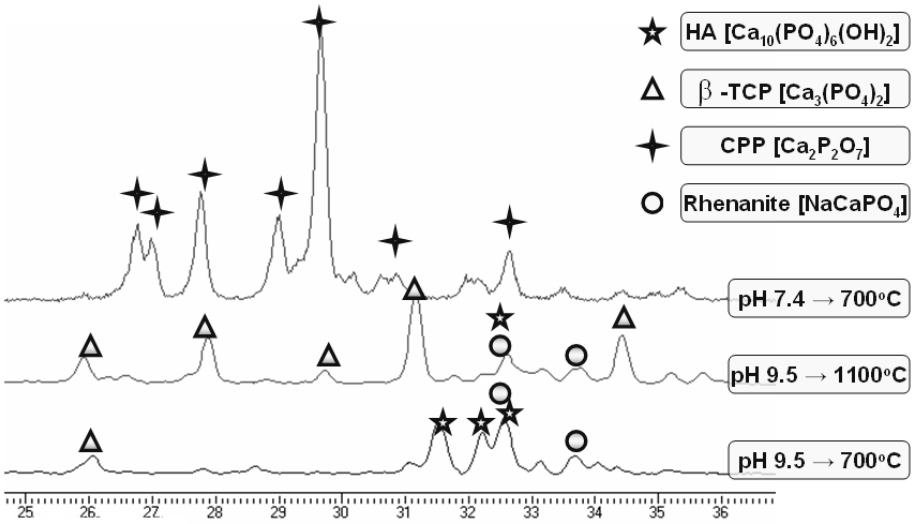


Figure 2 Peak search for the main phase

FT-IR analyses are needed to verify the XRD analyses. The spectra of FT-IR for each sample at various calcinations are presented at Figure 3 and Figure 4.

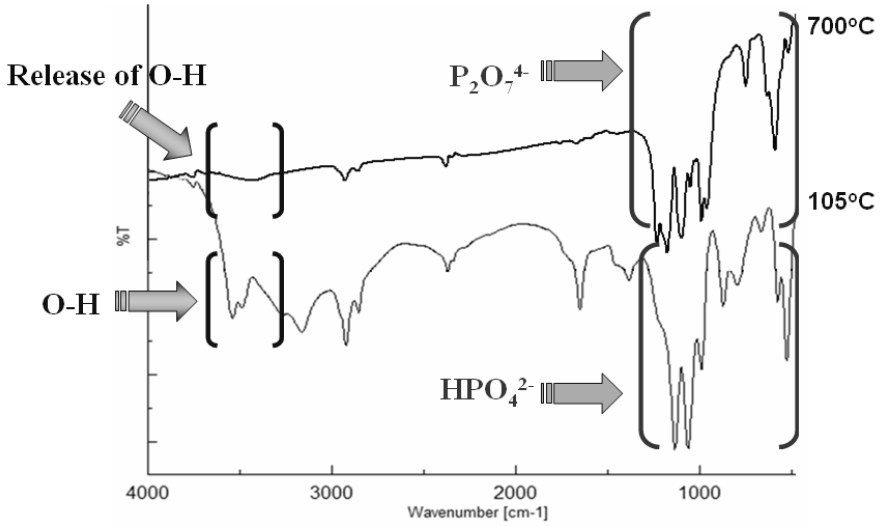


Figure 3 FT-IR spectra of sample I

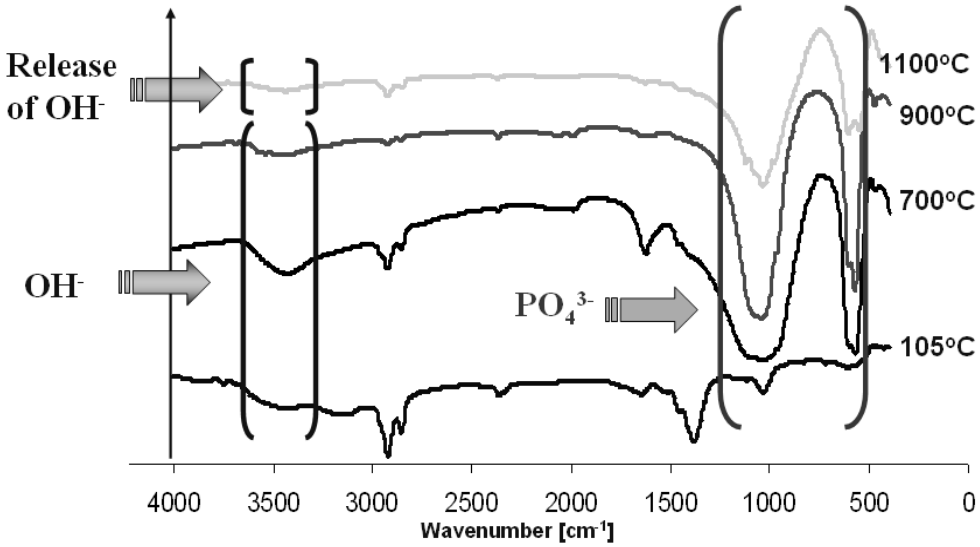


Figure 4. FT-IR spectra of sample II

All of the FT-IR spectra from the dried sample showed content of carbonate (CO_3^-) ions from the wavenumber of ± 2368 cm^{-1}, ± 1460 cm^{-1}, and ± 875 cm^{-1}. The carbonate ions probably occured from the impurities. Beside carbonate, possible bondings found in the spectra are from NH_4^- and NO_3^-. Another possibility

is the presence of hydroxyl (OH^-) ion from the moist of sample. Upon calcination, those bonding are slightly disappeared; this occurred due to the evaporation of the ions.

The main phase of the sample after calcinations at 700°C is Calcium Pyrophosphate (CPP, $Ca_2P_2O_7$). This is identified by matching the main peaks of XRD (Figure 2) and analyzing the fingerprint of FT-IR spectra (Figure 3). The XRD main peaks are matched with the PDF card number 09-0346 from JCPDS. The fingerprint of FT-IR from the range of 500 cm^{-1} - 1250cm^{-1} are matched with the fingerprint of pyrophosphate ($P_2O_7^{4-}$) studied by Tas and Badhuri (*A Cuneyt Tas and Sarit B Bhaduri*. 2004) and Parekh and Joshi (*Bharat B. Parekh and Mihir J. Joshi*. 2007).

The main phase synthesized in sample I confirms the study done Tas and Badhuri (*A Cuneyt Tas and Sarit B Bhaduri*. 2004). In their study, CPP was synthesized by the condensation of Calcium Hydrogen Phosphate salts (CHP, $CaHPO_4$). The CHP can be prepared using soluble calcium (e.g., $Ca(NO_3)_2.4.H_2O$, $CaCl_2.2H_2O$ or $Ca(CH_3COO)_2.H_2O$) and phosphate (e.g., $(NH_4)_2HPO_4$, $NH_4H_2PO_4$, Na_2HPO_4, NaH_2PO_4, KH_2PO_4 or K_2HPO_4) as long as the solution pH is kept in the acid range. This confirms that the FT-IR spectra fingerprint of the dried sample I from the range of 500 cm^{-1} - 1250cm^{-1} are similar with CHP salts studied by Tas and Badhuri (*A Cuneyt Tas and Sarit B Bhaduri*. 2004). When heating upon 700°C, CHP thermally decompose by a condensation reaction.

In sample II, the main phase of the sample after calcinations at 700°C were HA and Rhenanite ($NaCaPO_4$). Upon calcination at 1100°C, the main phase of of sample II change into β-TCP. This is identified by matching the main peaks of XRD (Figure 2) and the fingerprint of FT-IR spectra (Figure 4). The XRD main peaks are matched with the PDF card number 25-0166 for HA, 29-1193 for Rhenanite and 09-0169 for β-TCP. The fingerprint of FT-IR are matched with the studied by Tas (*A Cuneyt Tas.* 2000), Bayraktar and Tas (*Defne Bayraktar and A. Cüneyt Tas*. 2000), Markovic et. al. (*Milenko Markovic et al*, 2004), and Jalota et. al. (*Sahil Jalota et al.* 2006). The spectra of orthophosphate detected in the FT-IR is represent from the main phase (HA, Rhenite and β-TCP), which those three phases have orthophosphate ion in their compound. The hydroxyl ion detected in the range of 3400 cm^{-1} – 3600 cm^{-1} of the FT-IR spectra confirms the presence of HA compound. The decreasing spectra of hydroxyl ion at higher calcination temperature in the range of 3400 cm^{-1} – 3600 cm^{-1} of the FT-IR spectra confirms the thermal decomposition of HA into β-TCP.

The main phase synthesized in sample II calcined at 700°C shows different results with the biomimetic process done by Tas (*A Cuneyt Tas*. 2000) and Thamaraselvi et. al. (*T.V. Thamaraiselvi et al*. 2006). Although the reaction done by Tas (*A Cuneyt Tas*. 2000), Thamaraselvi et. al. (*T.V. Thamaraiselvi et al*. 2006), and the author occured in the base region but the results are different. In their studies, the powder synthesized by the biomimetic process result in HA powders with approximate purity over 90%. In this study, the powder synthesized results in

a biphasic mixture of HA and Rhenanite. This phenomena happened occured the different adjustment of pH level. Tas (*A Cuneyt Tas*. 2000) and Thamaraselvi et. al. (*Thamaraiselvi et al*. 2006) adjusted the acidity at a constant level of pH 7.4 by the continuous addition of NH_4OH. While the author used initial addition of NH_4OH, set up at pH level of 9.5 and finished at the level of 7.7. It seems that different kinds of pH treatment could significantly change the result.

The biphasic result of the second sample calcined at 700^oC is quite similar with the study of Jalota et. al. (*Sahil Jalota et al*. 2006). In their study, the synthesized powders calcined at 300^oC to 600^oC resulted in a biphasic mixture of approximate composition of 60% HA and 40% Rhenanite using different precursor ($Ca(NO_3)_2.4H_2O$, Na_2HPO_4 and $NaHCO_3$). They proposed their biphasic mixture as a new (potential) biomaterial for skeletal repair. While in this study, the main biphasic mixture of the sample calcined at 700^oC are approximately 41% HA and 34.3% Rhenanite. If this sample is studied at lower calcinations temperature, there is a great possibility that this sample could result in similar composition with the one studied by Jalota et. al. (*Sahil Jalota et al*. 2006).

The result of material characterization from XRD, EDS and FT-IR verifies the phase and compound identification, especially for the main phases and compound. The FT-IR fingerprint spectra show similarity of the main compound presence with the result of XRD analysis. The XRD analysis and the EDS analysis show similar trend of Ca/P molar ratio (Figure 5).

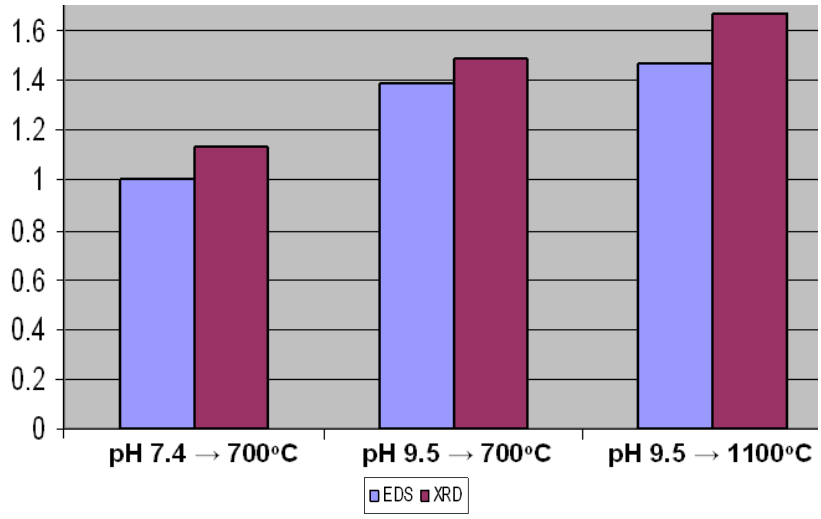
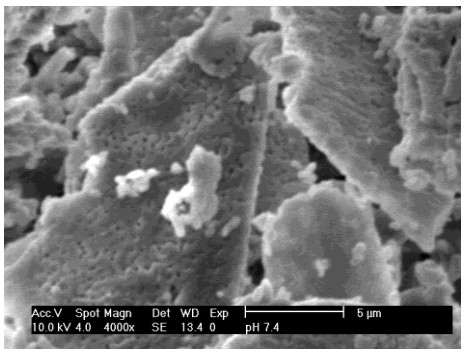
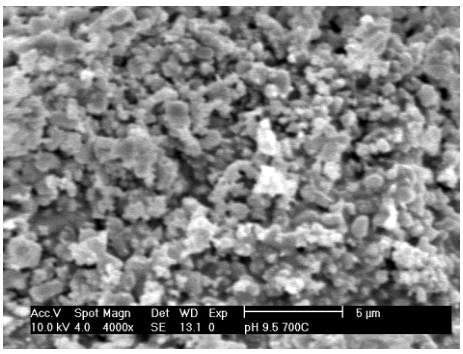


Figure 5. Comparison Ca/P ratios in the study

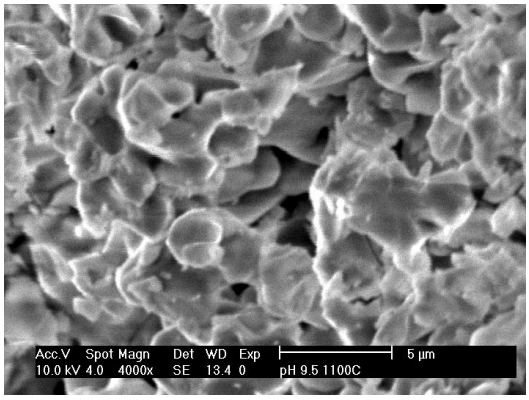
The SEM images of the samples (Figure 6), show that the particle synthesized are in the range of sub-micron to micron. Sample I result in a flake-like particles while sample II results in spherical particles.



(a)



(b)



(c)

Figure 6. SEM images of (a) sample I calcined at 700°C; (b) sample II calcined at 700°C and (c) sample II calcined at 1100°C

The summary of the main phase synthesized in this study is given in Table 3.

Table 3. Summary of the main phase

No	Calcination	Main Phase
I	700°C	CPP
II	700°C	HA & Rhenanite
	1100°C	β -TCP

The authors realize that the human mimicking condition in the experiments of this study is still far away from the real human condition. It is amazing to see how nature could work efficiently in a very complicated mechanism. Developing the biomimetic method with reproducing all the sequence of biomineralization steps and condition will lead to unbearable cost.

The biomimetic process has been applied also for coating of medical implant. Based on ASTM 2024-00, the requirement for the coating must have a minimum HA content of 50%. While in this study, the HA content of biomimetic process (sample II calcined at 700^{o}C) results in 41%. For applying in biomimetic coating, the authors recommends some modification of the process. Those are by adjusting the acidity of the process at certain constant level of pH at the base range and also increasing the concentration of precursor.

CONCLUSION

Calcium phosphate bioceramics has been successfully synthesized in this study. The body mimicking condition of synthesized process did not result in calcium phosphate bioceramics that have significant similarity with the one produced in the real body system. Finding of biphasic mixture of HA and rhenanite ($NaCaPO_4$), similar to research done by Jalota et. al., (*Sahil Jalota et al*. 2005); while main phases, Ca/P trend confirmation by means of XRD and FTIR shape.

ACKNOWLEDGEMENT

The authors would like to acknowledge the support from Materials Science and Engineering Study Program - ITB for the SEM and EDS characterization. School of Pharmacy - ITB for the laboratory facilities. Colleagues from Materials Science and Engineering Study Program - ITB for the discussion. Colleagues from School of Life Science and Technology - ITB for the physiology tutorial and information.

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