The influence of demineralization process on physicochemical characteristics, BMP-2 quantification of human demineralized tooth matrix

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Abstract

Objectives: To examine the influence of demineralization process using 4 different concentrations and reaction times of HCl acid on physicochemical properties, BMP-2 releasing and degradation rate at 30 days of human tooth matrix.

Materials and methods: Tooth particles with size 500-1,000 µm were divided to 5 groups: 0M/0min, 0.5M/10min, 0.5M/20min, 1M/10min and 1M/20min. Chemical compositions were analyzed by XRD and XRF. Surface morphology was observed by SEM and BET analysis. Bradford protein assay was used to quantify total protein and human ELISA kit was used for BMP-2 quantification. Degradation rate was assessed by using 50 mM Tris-HCl solution for 30 days.

Results: Increasing reaction time led to more collagen exposed, larger dentinal tubules, less crystallinity, and less calcium-phosphate percentage but increased Ca/P ratio. The highest total protein and BMP-2 concentration were found in 1M/20min group compared to 0M/0min (p=0.000). Increasing HCl concentration led to more degradation rate.

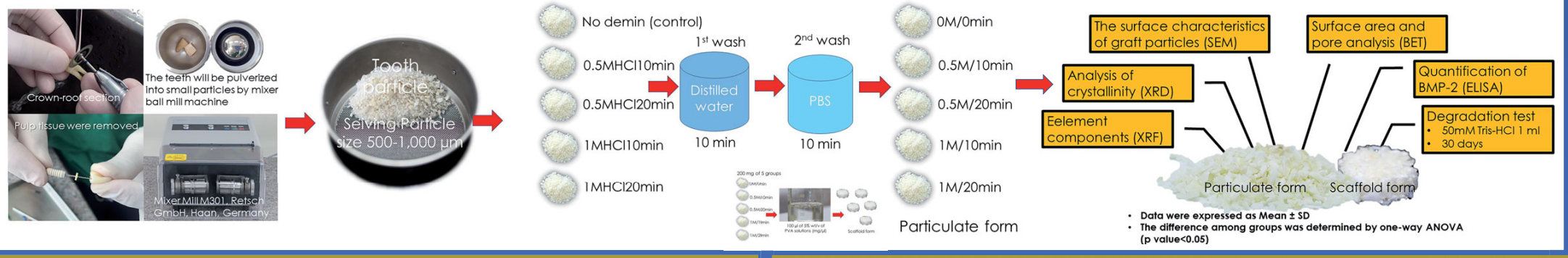
Conclusions: The reaction time of HCl had effect on tooth particles in aspect of surface morphology, crystallinity, element components, Ca/P ratio, quantity of BMP-2 releasing while increasing concentration led to more degradation.

Introduction and objectives

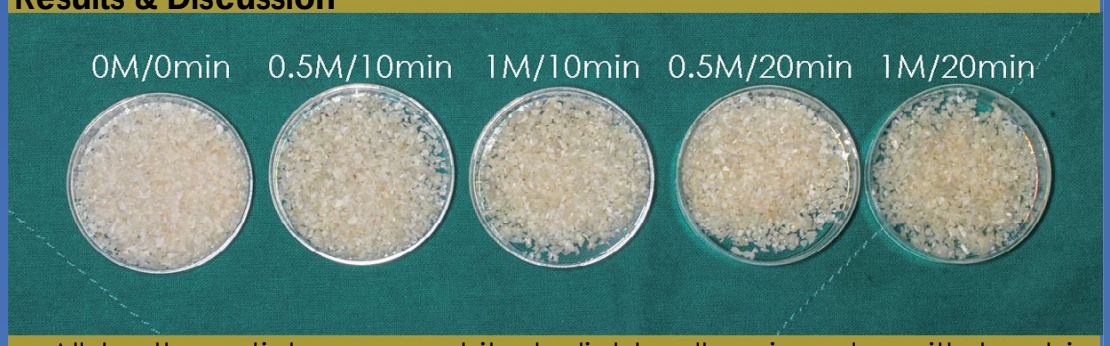
Demineralized tooth matrix has been considered a successful grafting material. However, there are various demineralization procedures that may result in products with different properties. The aim of this study was to examine the influence of the demineralization process of human tooth using 4 different concentrations and reaction times of hydrochloric acid (HCl) on the physicochemical characteristics, the amount of the bone morphogenetic protein-2 (BMP-2) protein and degradation rate of materials at 30 days.

Materials and methods

Caries-free permanent teeth were processed for human tooth matrix. Periodontal ligament and pulp tissue were removed and the teeth were pulverized into particles range from 500-1,000 µm. The tooth particles were pooled and assigned to 5 groups with different protocols of HCl acid concentration and reaction time (0M/0min, 0.5M/10min, 0.5M/20min, 1M/10min and 1M/20min). The phase and chemical composition were analyzed by X-ray diffraction spectroscopy and X-ray fluorescence spectroscopy. Surface geometries were evaluated by scanning electron microscopy and Brunauer-Emmett-Teller analysis. Protein extraction was performed using a Guanidine-HCl method for 24 hrs. Bradford protein assay was used to quantify total protein and ELISA was used for BMP-2 quantification. A 200 mg of all groups were mixed with 5% wt/v of polyvinyl alcohol for scaffolds construction. Degradation rate of the scaffolds were assessed by using 50 mM Tris-HCl solution (pH 7.4) at 37°c for 30 days.



Results & Discussion

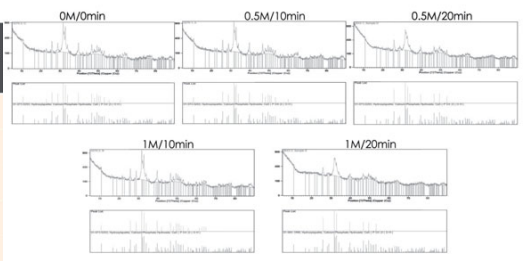


All tooth particles were white to light yellow in color with hard in consistency. Tooth matrix processed with longer reaction times revealed less opacity.

All demineralization processes decreased the Ca and P element content. The highest amount of Ca and P was in 0M/0min group while the lowest was in 1M/20min group. Contrast with Ca/P ratio, the highest was in 1M/20min group.

XRF element and XRD analysis

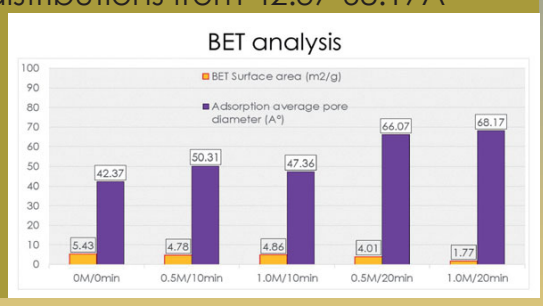
Groups	% element (% by wt)									Ca/P ratio	% Crystallinity	Inorganic Phase
	Ca	P	S	Mg	Na	Cl	K	Zn	Fe			
0M/0min	15.6	8.08	0.04	0.42	0.5	0.08	0.01	<0.01	0	1.492	63.09	HA
0.5M/10min	11	4.32	0.15	0.13	0.16	0.15	<0.01	<0.01	0	1.968	62.12	HA
1.0M/10min	8.25	2.71	0.19	0.01	0.15	0.17	<0.01	<0.01	0	2.352	61.91	HA
0.5M/20min	8.17	2.64	0.21	0.07	0.12	0.01	<0.01	<0.01	0	2.391	56.84	HA
1.0M/20min	3.43	1.07	0.25	0.05	0.08	0.2	<0.01	<0.01	<0.01	2.477	54.33	HA

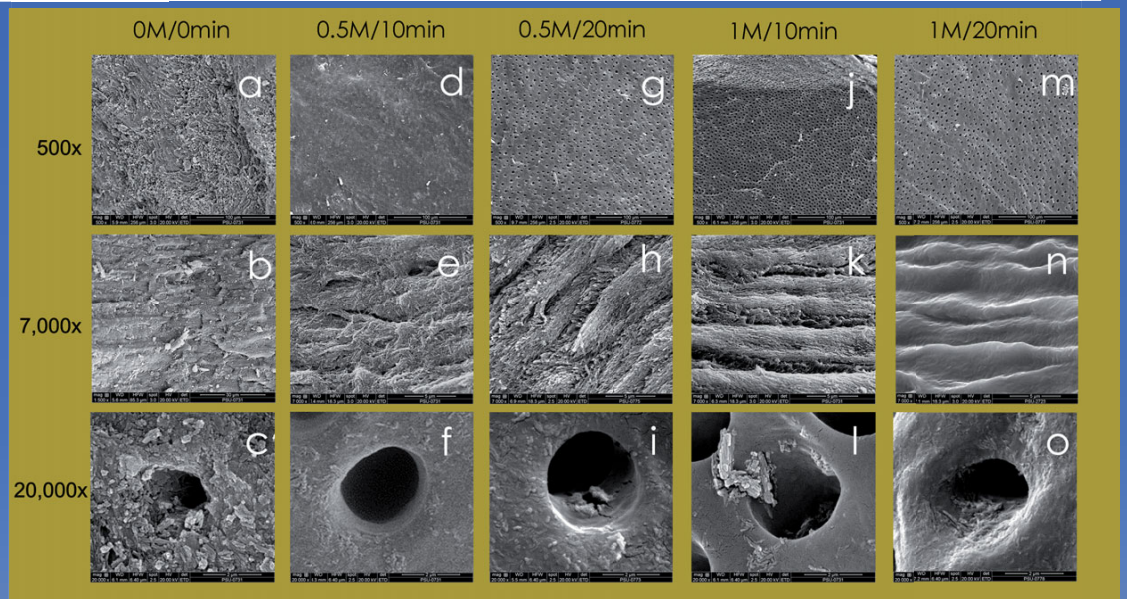


XRD confirmed that there was only hydroxyapatite phase. the highest percentage of crystallinity was in 0M/0min group and the lowest was in 1M/20min group.

BET analysis demonstrated pore distributions from 42.37-68.17A° with the highest in 1M/20min group. In contrast to the surface areas range from 1.77-5.43 m2/g with the lowest in 1M/20m group.

A reaction time may effect to Physicochemical properties of materials.

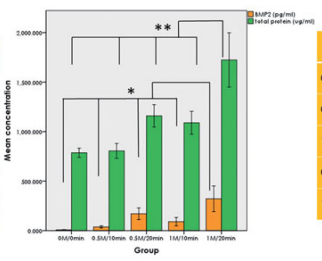




From SEM images, 0.5M/20min and 1M/20min DTM groups exhibited a smooth surface with larger dentinal tubules and abundant of exposed collagen fibrils when demineralization increased.

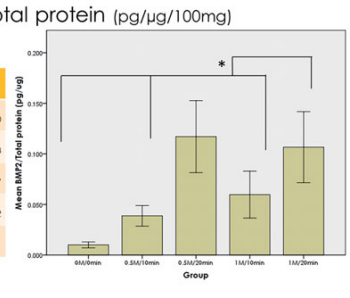
BMP-2 and total protein

		Means±SD	sig
Total protein (µg/ml)	0M/0min	975.000±80.854*	0.000
	0.5M/10min	1,166.875±307.661*	0.000
	1M/10min	1,843.125±105.541*	0.000
	0.5M/20min	1,876.875±105.876*	0.000
	1M/20min	3,526.250±238.410	
BMP2 (pg/ml)	0M/0min	2.668±5.336*	0.000
	0.5M/10min	111.538±6.679*	0.000
	1M/10min	309.718±237.095*	0.000
	0.5M/20min	528.364±154.775*	0.000
	1M/20min	1,181.529±193.766	

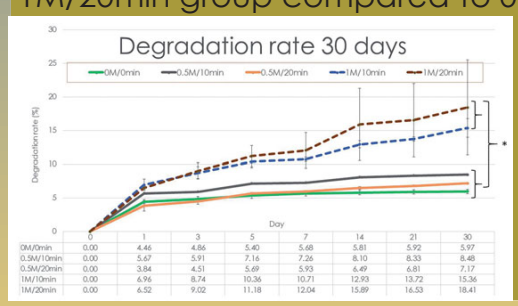


BMP-2/total protein (pg/µg/100mg)

	Means ±SD	sig
0M/0min	0.002±0.005*	0.000
0.5M/10min	0.101±0.029*	0.003
1M/10min	0.167±0.132*	0.037
0.5M/20min	0.278±0.070	0.802
1M/20min	0.336±0.060	



The highest total protein and BMP-2 concentration were found in 1M/20min group compared to 0M/0min(p=0.000).



The lowest degradation rate was 0M/0min group. The degradation of 1M/10min and1M/20min groups were significantly higher than other groups,(p<0.05).

So, concentration may effect to degradation rate of materials.

Conclusions

The reaction time of HCl has a greater effect on demineralization process of human tooth more than the concentration in aspect of surface morphology, crystallinity, element components, Ca/P ratio and the quantity BMP-2. While increasing the concentration leads to more degradation of the demineralized human tooth matrix. The demineralized process using 0.5M HCl acid for 10-20 minutes are suitable protocol for fabricating demineralized tooth matrix and will benefit for further clinical application.