Electronic Supplementary Material (ESI) for RSC Advances.
This journal is © The Royal Society of Chemistry 2015

Supplementary Information

Bi-functional Roles of Ca-Y Zeolite in Treatment of Ethanol-HCl Induced Gastric Ulcer in a Mice Model

Xiaoqiang Shang,[a] Hao Chen,[a] Yingliang Qu[b,c] and Jie Fan.*[a]

[a] *Key Lab of Applied Chemistry of Zhejiang Province, Zhejiang University, Hangzhou 310027, China.*
[b] *Zhejiang University Institute of Technology Innovation, Hangzhou 310030, China*
[c] *Zeo-Innov Medical Technology Inc., Hangzhou 310027, China*
[*] *E-mail: jfan@zju.edu.cn*

1. Meterials

The following chemicals and drugs are used: ethanol, hydrogen chloride, formaldehyde aqueous solution (37% w.t), pepsin (Aladdin, JINCHUN biochemical tech. Co. Ltd, Shanghai, China), Omeprazole Capsule (CONBA Bio-pharm. Co. Ltd., Jinhua, China), and Bovine thrombin (Sigma-Aldrich Trading Co Ltd, Shanghai, China), thrombin Chromogenic substrate (BIOPHEN CS-01(38), HYPHEN BioMed), porcine plasma (YuHang District Slaughter House, Hang Zhou, China).

2. Ca-Y preparation by ion exchange[1]

The ion-exchange process was carried by mixing synthesized sodium Y zeolite with metal chloride solution. In a typical ion-exchange process, 1.0 g of zeolite was added into 10 mL of 5 M $CaCl_2$ at 25 ± 0.1 ℃. After 3 h stirring, the zeolite powder was filtered and dried. The process was repeated by twice.

3. Elementary analysis

Elementary analysis were conducted by X-Ray Fluorescence spectrometer. (ARL ADVANT'X IntelliPowerTM 4200, ThermoFisher, U.S.A) Those elements concentration under 0.5% w.t were ignored.

Table. S1 Molecular formula and Si/Al ratio of Na-Y and Ca-Y zeolite.

Zeolite	Molecular formula	Si/Al
Na-Y	$Na_{0.55}(SiO_2)_{1.03}(AlO_2)_{0.40}\bullet\chi H_2O$	2.56
Ca-Y	$Ca_{0.17}Na_{0.12}(SiO_2)_{1.08}(AlO_2)_{0.42}\bullet\chi H_2O$	2.57

After calcium(II) ion exchange process, the ion exchange degree (IED) was calculated as:

$$\text{IED} = \frac{n_{Ca}*Z_{Ca}}{n_{Ca}*Z_{Ca}+ n_{Na}*Z_{Na}} = \frac{0.17*2}{0.17*2+0.12*1} = 74\%$$

4. Ca-Y/HPC composite preparation

In a typical assay, 100 mg Ca-Y was mixed with 100 μL deionized water plus 100 μL porcine plasma for 10 minutes. Such made fresh Ca-Y/HPC composite can be used immediately or further dried under vacuum at 28 ^oC, and stored in a vacuum drier at room temperature.

5. X-ray powder diffraction (XRD)

X-ray powder diffraction patterns were collected on Ultima IV (Rigaku, JP) instrument using

monochromatic Cu K_α radiation. Patterns from 10 - 80 degree were collected with a scanning speed at 10 degree per minute. Data were analyzed using Jade 6.5 software.

6. Scanning electron microscope (SEM) analysis conducted by SU 8010 (Hitachi, Japan)

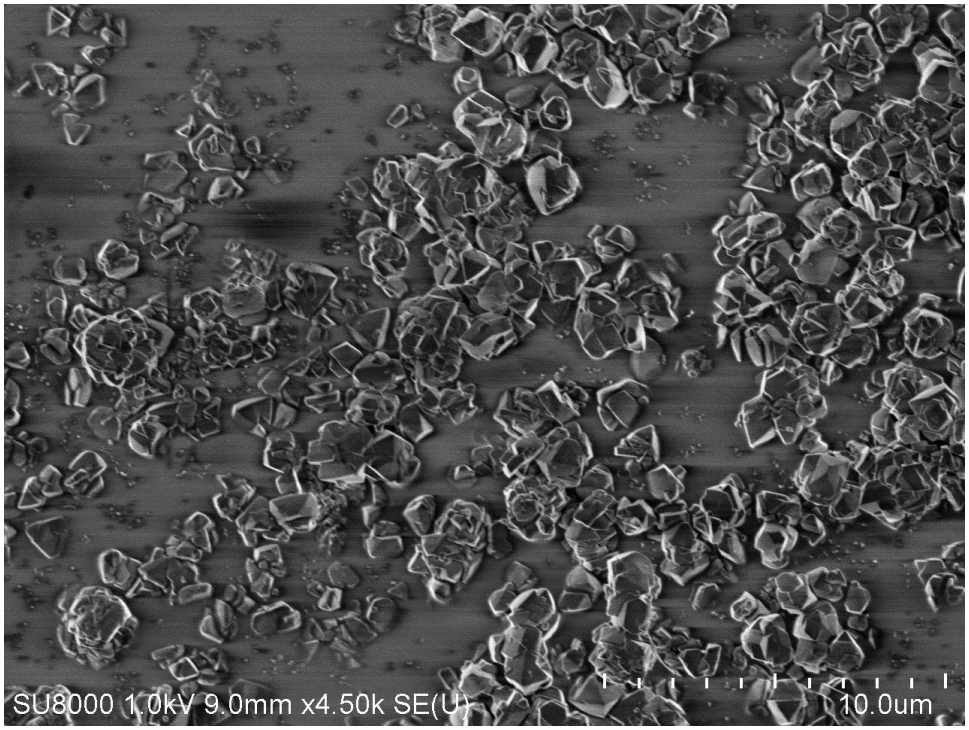

Fig. S1 SEM of Na-Y.

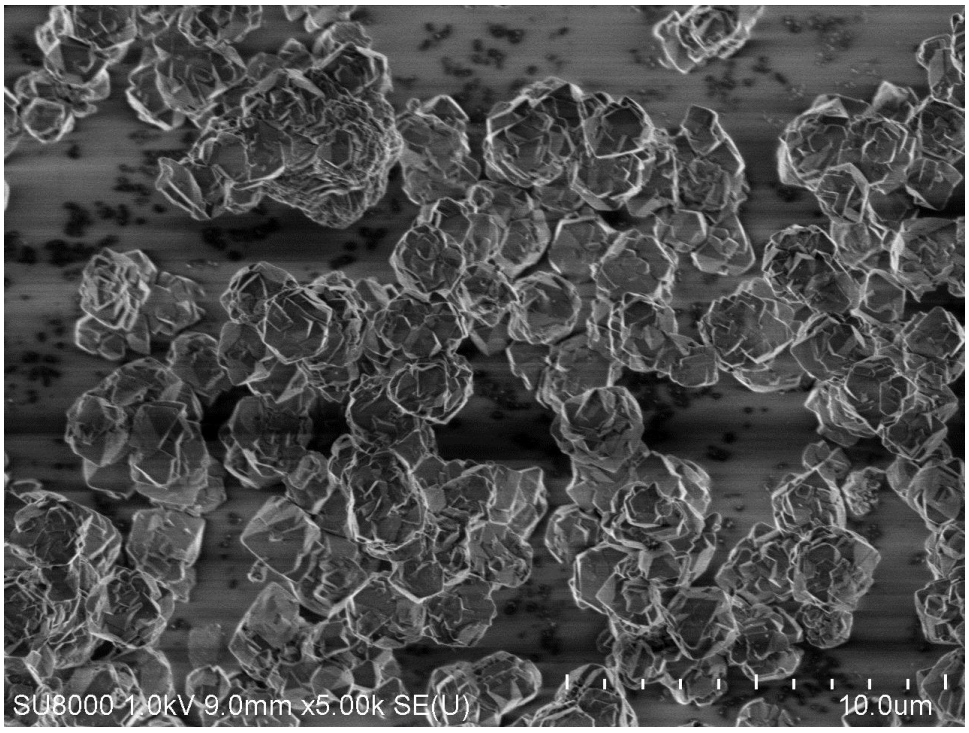

Fig. S2 SEM of Ca-Y.

The mean particle size of Ca-Y is 2.48 ± 0.93 μm, calculated by Digital Micrograph Software.

7. Procoagulant activity by *in vitro* clotting assay[1]

An *in vitro* clotting assay was used to measure the procoagulant activity of hemostatic agents after treatment with artificial gastric juice (1% w.t pepsin in pH 2.5 HCl solution, according to the Pharmacopoeia of the P.R.C) or deionized water separately. The assay measured the coagulant response in terms of a clotting time (CT), defined as the time required from activation of the intrinsic pathway of coagulation cascade to the appearance of a firm clot which stuck to the wall of a polystyrene tube. Hemostatic used are zeolite or zeolite with hard protein corona (zeolite/HPC) which was made by mixing zeolite with plasma, like mix 100 mg zeolite, 100 μL water and 100 μL normal plasma for 10 minutes. In a typical assay, 100 mg hemostatic agents were presented in a 5 mL polystyrene tube (52 mm × 12 mm), mixed with 0.5 mL artificial gastric juice or water for 5 mins, and then 2 mL citrated porcine plasma with 60 μL of 0.2 M $CaCl_2$ were injected meanwhile started timing. The capped tubes were rotated at 30 rpm on a mute mixer and the corresponding CT was recorded. The re-calcium ionization was always applied using 60 μL of 0.2 M $CaCl_2$, and 0.2 mL thrombin solution (100 NIH U/mL) were used to make a comparison. The clotting assays were carried at 25 °C.

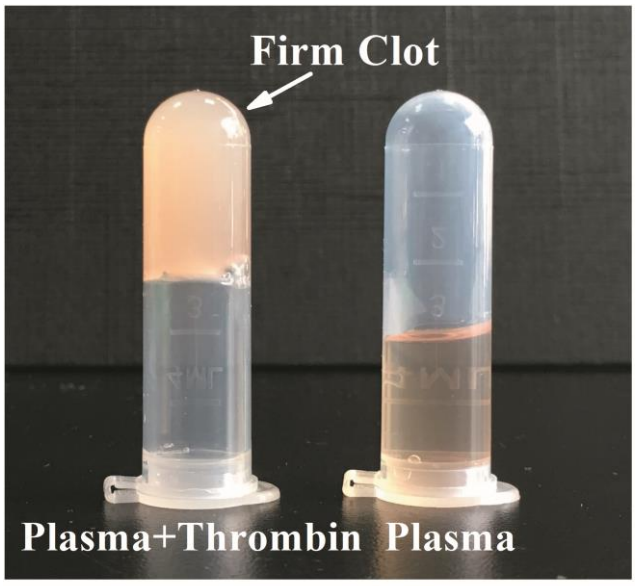

Fig. S3 Optical image of plasma with/without addition of thrombin solution (20 NIH U) under normal conditions. After addition of thrombin molecules, the plasma forms a firm clot sticks to tube within seconds.

8. Thrombin chromogenic substrate assays.

In a typical assay, 100 mg of Ca-Y/HPC or 0.2 mL thrombin (100 NIH U/mL) solution was mixed with 0.5 mL of artificial gastric juice or deionized water for 5 minutes. Then the suspension was added with 0.2 mL of 0.2 M Tris-HCl (pH 7.4) buffer and 0.1 mL of thrombin Chromogenic substrate solution (4 mg/mL, HYPHEN BioMed). After 2 min in water bath of 37 °C, the thrombin in the suspension was quickly deactivated by adding 100 uL of 1.0 M $Pb(NO_3)_2$ solution. The supernatant solution was removed by centrifugation (14000 rpm, 4 min) and the absorbance was determined at 405 nm by UV-Vis.

9. Experiment *in vivo*

Animals

Five-week-old male ICR mice weighing 20 ± 2g were used in this study, which are obtained from

Laboratory Animal Center of Zhejiang province, China. The mice are housed at 25 ^{o}C under a 12 hr light-dark cycle and have free access to food and water. All mice are randomly divided in six groups, each group contains 6-8 mice. All procedures relating to animal care and treatment conformed to the animal welfare guidelines of the laboratory animal center of Zhejiang University.

Ethanol-HCl induced gastric ulcer

Animals were fasted for 24 hours before the experiments, but allowed free access to water. Gastric ulcer lesions were induced by intragastric administration (0.1 mL/10g) of ethanol-HCl mixed solution (200 mM HCl in 50% ethanol).[2] Half hour before HCl/ethanol application, 0.2 mL solution of the zeolite (50 mg/mL, 250 mg/mL, 500 mg/mL), thrombin (250 NIH U/mL), and Omeprazole (450 μg/mL) were intragastric administrated. One hour after ethanol-HCl administration, the animals were killed by cervical dislocation, stomachs were quickly removed, measuring the acidity of remaining gastric juice, and then inflated with 2 mL of 4% formaldehyde for 1hour. After fixation, the stomachs were opened along the great curvature, irrigated with 4% formaldehyde, and then stretched out to capture images using D3100, Nikon.

Table S2. *In vivo* experiment of gastric ulcer model in mice

Group	**Ulcer area percentage (Mean ± SEM)%**	**Intragastric pH**
Control	35.1 ± 4.4	2.0 ± 0.5
Thrombin (2500 NIH U/kg)	32.9 ± 3.3	2.0 ± 0.5
Omeprazole (4.5 mg/kg)	19.8 ± 1.7	4.5 ± 0.5
Ca-Y (0.5 g/kg)	23.3 ± 2.2	2.2 ± 0.5
Ca-Y (2.5 g/kg)	17.8 ± 2.0	3.5 ± 0.5
Ca-Y (5.0 g/kg)	11.5 ± 1.9	4.5 ± 0.5

Statistical analysis

The images are analyzed by Image J software to measure the ulcer area. And the ulcer percentage (the ulcer area/entirety area) were used to indicate the degree of lesion. Data are presented as the means ± standard error of the mean (SEM). Statistical comparisons were made with Student's t-test or one-way analysis of variance (ANOVA), with $p < 0.05$ being considered to indicate a statistical significance.

References:

1. Y. Li, X. Liao, X. Zhang, G. Ma, S. Zuo, L. Xiao, G. D. Stucky, Z. Wang, X. Chen, X. Shang and J. Fan, *NANO RES*, 2014, **7**, 1457-1465.

2. A. Oyagi, K. Ogawa, M. Kakino and H. Hara, *BMC Complement Altern Med*, 2010, **10**, 45.