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Reproducing ancient Chinese ink depending on gelatin/chitosan and modern experimental methodology

Zhen Liu[1,2*] and Kun Liu[3]

Abstract

Chinese ink has very special significance for presentation of artistic effects and preservation of works of art; however, wasted nonrenewable resources, potential toxicity, and complex and inefficient production technologies have limited the development of inks for traditional Chinese culture. Herein, environmentally friendly, practical and antibacterial Chinese ink was prepared by facile heating-stirring of gelatin and the natural polysaccharide chitosan. The internal composition, viscosity, morphology, particle size and antibacterial properties of the ink were characterized by Fourier transform-infrared spectroscopy, X-ray photoelectron spectroscopy, scanning electron microscopy and bacteriostatic zone tests. The results showed that commercial ink (CM) spread easily on Xuan paper and led to imperfect artwork, while the chitosan and gelatin ink (CG) showed good adhesion and stability on Xuan paper. Based on this study, we believe that the good performance of CG ink should be attributed to restrictions arising from its internal chitosan and gelatin network, which restrict diffusion. Finally, the author used CG ink to display traditional Chinese calligraphy and landscape painting and believes that it has significant application prospects and will be used in large-scale production.

Keywords: Chinese culture, Chinese ink, Chitosan, Gelatin, Calligraphy

Introduction

The history of ink can be traced back for 3000 years to ancient China. The earliest written record was prepared in the Western Zhou Dynasty, and the ink-making process had reached maturity by the Eastern Han Dynasty [1]. During the Wei and Jin Dynasties, the “Mix-ink method” mentioned in *Qi Min Yao Shu* was written by Jia Sixie in the Northern Wei Dynasty and revealed the technology and formula of ink [2]. Compared with brush and paper, ink has very special significance for presentation of artistic effects and preservation of works of art. It is not only the result of brush, technique and artistic style but also an important medium allowing calligraphy and paint artists to express their feelings. Therefore, we hoped to avoid harmful substances and use modern technology to prepare a practical ink for calligraphy.

The person who specialized in making ink in ancient China was called the ink official, which was a low-level official position. This was mainly due to the contempt held for workers in ancient times; the status of handicraftsmen in society was relatively humble, which also limited the development of ink fabrication processes and materials. The complex and inefficient technology used for production of ancient ink was one of the disadvantages of ancient ink. In addition, the popularity of modern industrial ink has made studies of ancient ink relatively rare. However, modern industrial ink often contains potentially toxic substances, such as isopropanol, ethyl acetate, butyl acetate and phenol, which are corrosive to the human body [3].

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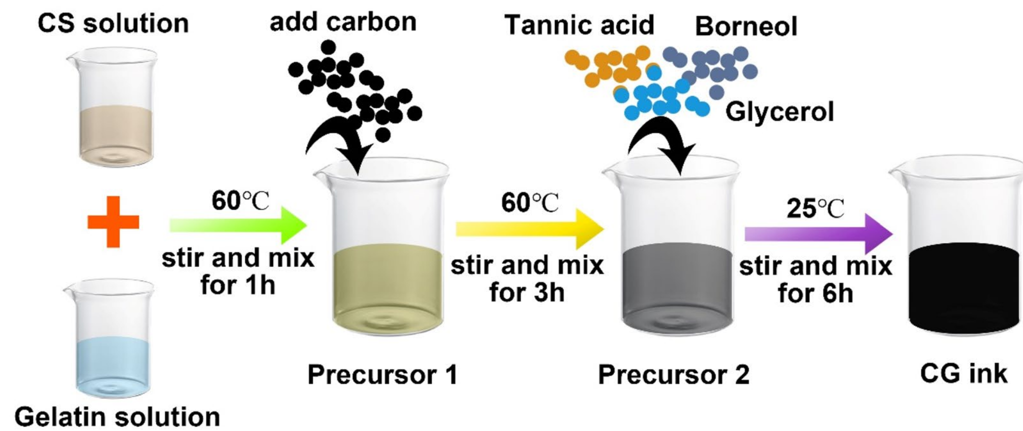

Fig. 1 Schematic diagram of preparation of CG ink

In ancient times, incomplete combustion was used to extract smoke and dust from pine branches or grease to prepare pine smoke and oil fume, respectively. During combustion, the fire temperature, tuyere and smoke collection time were strictly controlled to ensure soot blackness, fineness, and oil and ash contents [4]. First, the glue was enclosed by a gentle fire, pigment raw materials and raw materials were added, the mixture was fully stirred, and a pestle was used to pound the mixture evenly [5–7]. It was said that the mixture should be hammered repeatedly with "100,000 pestles" so that the smoke and binder reached a delicate and uniform degree and could be made into blanks [1]. In production of ancient ink, animal tissue was needed to make collagen [4, 8]. The addition of animal collagen improved the viscosity of the ink and enhanced the experience of writing with the ink, but sources of animal collagen were very limited, especially for some precious wild animals. Gelatin is a form of hydrolyzed collagen and is essentially a product of collagen decomposition; it is similar to collagen in composition and structure and is expected to replace animal collagen when added to the ink to improve the writing experience [9, 10]. Chitosan (CS) is a partially deacetylated product of the natural polysaccharide chitin, which has many functions, such as biodegradability, biocompatibility, bacteriostasis, and enhancement of immunity [11–13]. It is widely used in food additives, textiles, agriculture, environmental protection, beauty and health care, cosmetics, antimicrobial agents, biomedical fields and drug development and in many other fields and other chemical industries.

Hence, CS and gelatin were used in this study to develop proper formulas for producing Chinese ink (named CG ink). Combined application of CS and gelatin in inks was expected to enhance adhesion to Xuan paper and provide suitable inks for calligraphy and painting by adjusting the ratio of CS and gelatin. In addition, some natural products were also introduced into our ink, such as tannic acid extracted from plants and borneol extracted from borneol resin, which may have affected corrosion resistance and incense enhancement. Moreover, commercial (CM) ink was used as the control group for comparison with the properties of our CG ink. In particular, rare ink from the Qing Dynasty was also used to investigate the differences among inks. Based on these studies, we went further back into ancient Chinese culture to understand the special cultural roles of Chinese ink.

Methods

Materials and reagents

CS (M_w =100,000) and gelatin were obtained from Shanghai Macklin Biochemical Co., Ltd. Ethanol and other reagents were of analytical grade and purchased from Guangzhou Chemical Reagent Plant. The carbon used was water-soluble carbon black with high pigment (N330). Other ingredients (tannic acid, borneol and glycerol) were obtained from Aladdin Chemical Co., Ltd. (Shanghai, China).

Preparation of CS-gelatin ink

The formulas of the inks are listed in Table 1. The main components of the inks were CS, gelatin and carbon powders. In detail, as shown in Fig. 1 and 2% CS/acetic acid solution and gelatin aqueous solution were prepared first and stirred for 1 h at 60 °C to mix them well. Then, the carbon powders were added to the precursor and stirred for another 3 h. Subsequently, ingredients such as tannic acid, borneol and glycerol were introduced into the precursor. After 6 h, the CS-gelatin (CG) ink was obtained.

Table 1 The formula of inks

wt%	CS (2%)	Gelatin (20%)	Carbon powder	H_2O	ingredients
A	20	0	50	20	10
B	15	5	50	20	10
C	10	10	50	20	10
D	5	15	50	20	10
E	0	20	50	20	10

Characterization

The morphologies of the inks and calligraphic works were observed through a field emission scanning electron microscope (FESEM, ULTRA 55, Carl Zeiss, Germany). In detail, the samples were first dried and dehydrated and then glued to the aluminum table with conductive tape for observation.

The rheological properties of the inks were investigated at 25 ℃ with a rotary rheometer (TA Instruments, New Castle, DE, USA). The viscosities of the inks were tested with shear frequencies in the range 0.001–1000 s^{-1}. The viscoelasticities were tested at a strain of 0.5% and with frequencies ranging from 0.1 to 10 rad/s.

X-ray photoelectron spectroscopy (XPS) with an aluminum (mono) K_α source (1486 eV) (Thermo Scientific @ K-Alpha, USA) was employed to investigate the chemical compositions of membrane surfaces.

Fourier transform-infrared spectra of CS, gelatin and inks were acquired using a Bruker EQUINOX55 Fourier transform-infrared spectrophotometer (Germany) in the range 400–4000 cm^{-1}. The KBr pelleting method was used for sample preparation.

Dependent upon how the paper is produced, the "Xuan Paper" can be categorized into three types: raw Xuan, semi-processed paper (PiMade Xuan) and processed paper (Sized Xuan). In particular, PiMade Xuan owns the suitable water absorption and is commonly used in writing. All papers used during the writing tests were made of PiMade Xuan paper, and commercial (CM) ink and ancient ink refer to Guhuan ink and Qing Dynasty ink.

Bacteriostatic cycle experiments

Escherichia coli (*E. coli*, BNCC352086) on the slant of each test tube were picked up with the inoculation ring in aseptic water with glass beads, the spores were dispersed by hand for several minutes, and a mixed spore suspension was made after filtration. Then, 0.5 mL of a mixed spore suspension was injected into each Petri dish with a sterile straw. Molten agar medium, 15–20 mL, was injected into each Petri dish (approximately 45 ℃), and the bacterial liquid was mixed with the medium evenly and allowed to cool. Finally, tweezers were used to soak discs of filter paper in different concentrations of anti-mildewing agent, remove it, place it in the center of the medium plate with bacteria, and cover it with the lid. After culturing for 2–3 days at a suitable temperature, the sizes of the bacteriostatic circles around the ink filter paper disks were observed. The bacteriostatic effect was quantified by calculating the distance from the filter paper to the outermost periphery of the bacteriostatic zone, and the distance was calculated with ImageJ software.

Results and discussion

Analyses of the properties of gelatin, chitosan and inks

Chitosan and gelatin were the main components of the ink used in this study. As raw materials, their physical and chemical properties directly affected the experience of writing with the final ink, so we conducted detailed studies on the functional group compositions of raw materials and inks. As shown in Fig. 2A, the characteristic functional groups of chitosan were obtained by total reflection infrared spectrometry. Because the number of amino groups in the chitosan polysaccharide chains began to increase during chitin deacetylation [14, 15], more amino functional groups were detected on chitosan. The amine vibrations resulted in sharp characteristic peaks at 3200–3500 cm^{-1} [16, 17]. Due to partial deacetylation, amide groups were still retained on the chain segments and showed carbonyl vibrational peaks at 1720 cm^{-1} in the infrared spectrum. The above results showed that the chitosan had good purity and many amino functional groups. As shown in Fig. 2B, gelatin is composed of various protein

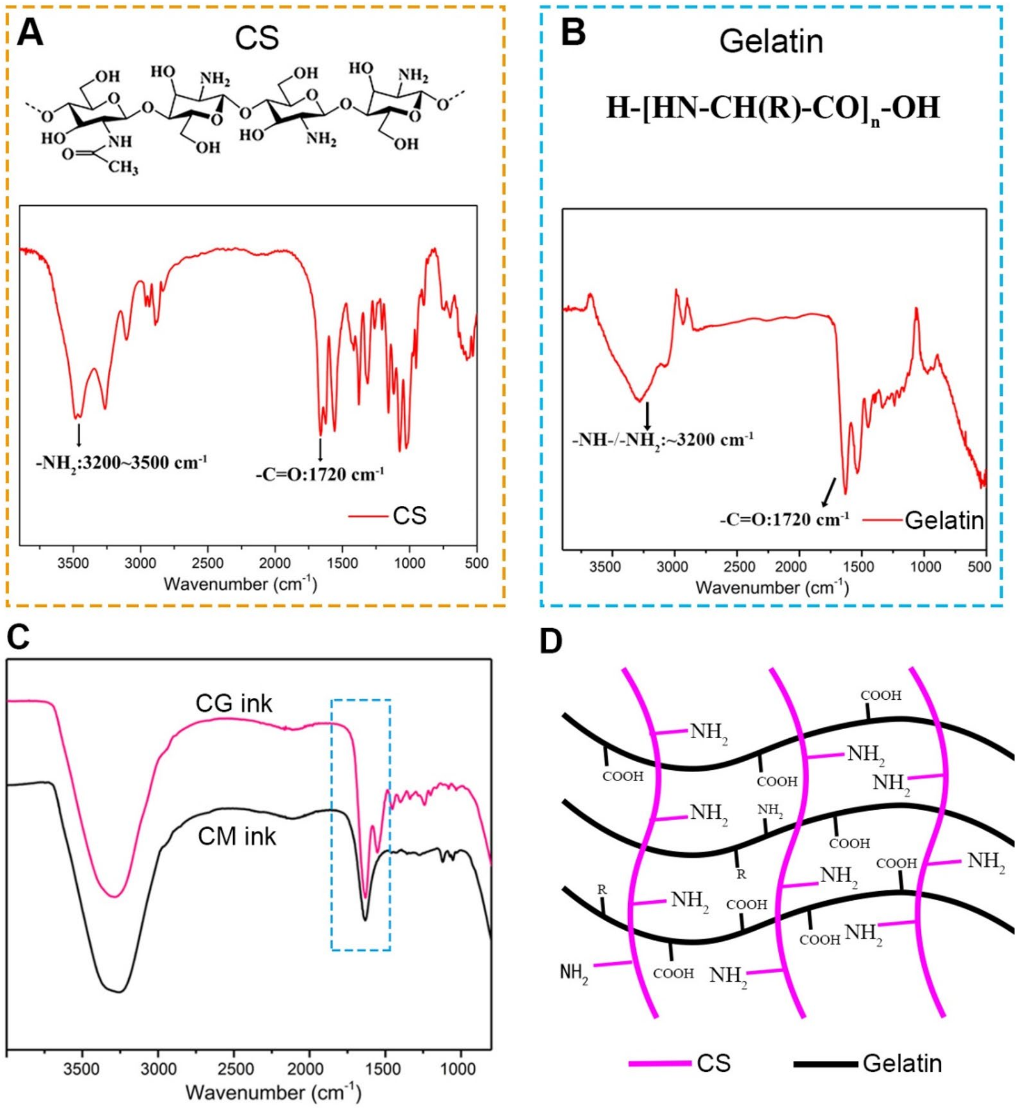

Fig. 2 Fourier transform-infrared spectra of CS, gelatin and inks (**A**–**C**). The schematic diagram of interaction of CS and gelatin in CG ink (**D**)

segments, so it contained many of the common functional groups of proteins, such as amino groups, carboxyl groups and even disulfide bonds [18, 19]. Similar to chitosan, there were typical amino peaks in the range 3200–3500 cm^{-1}, but there were large numbers of imine groups in gelatin, so there was no obvious peak similar to those of chitosan. In addition, there was a strong peak at 1720 cm^{-1}, which confirmed that there were more carbonyl groups in gelatin [19]. Then, we compared the main functional groups of modern commercial ink and antique ink (Fig. 2C). On the whole, the antique ink we prepared showed some similarities with commercial ink; for example, there was an obvious broad peak at approximately 3300 cm^{-1}, which was attributed to the hydroxyl and amino groups in ink [20, 21]. However, the difference was that the antique ink showed two peaks in the low wavenumber region, while the commercial ink had only one. This was attributed

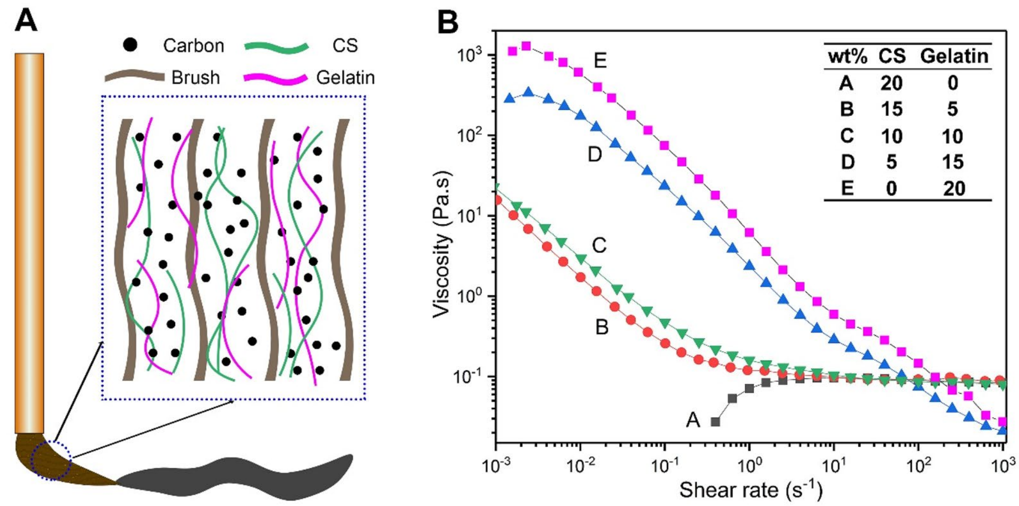

Fig. 3 The schematic diagram of interaction between the brush and CG ink (**A**). The viscosity analysis of inks with different formulas (**B**)

to addition of gelatin and chitosan to the antique ink, especially the presence of more carboxyl groups in the gelatin. As shown in Fig. 2D, we simulated the main functional groups of chitosan and gelatin molecules in antique ink. A large number of amino groups in chitosan and carboxyl groups in gelatin produced strong electrostatic interactions, which directly affected the viscosity of the antique ink. Additionally, the brush, which was made from animal hair, was also a protein and had a composition similar to that of the gelatin [22, 23], which made it easy to produce physical bonds involving electrostatic interactions and hydrogen bonding between the ink and the brush upon addition of chitosan and collagen; these were firmly absorbed by the brush and enabled the brush to retain the ink.

Analyses of rheological properties for inks with different formulas

The viscosity of an ink is directly related to the writing experience. As shown in Fig. 3A, we hoped that ink would be retained after the brush was dipped and then would flow out smoothly during writing, which was determined by interactions between the ink and brush. For example, when the interaction between ink and brush is strong, the brush is able to retain ink very strongly, which is reflected macroscopically by a sense of stickiness felt during brush writing; when the interaction is reduced, the brush enables smooth writing, but when the interaction is further reduced, ink in the brush will not be retained effectively. Therefore, we needed to prepare ink with a viscosity that facilitated direct writing. The viscosity changes resulting from different proportions of inks were determined with rheological tests. As shown in Fig. 3B, it was obvious that the gelatin content was directly related to the ink viscosity, and the viscosity of Group A was the lowest; this viscosity was similar to that of water and was not conducive to enveloping and retaining ink. On the other hand, Groups B and C had similar viscosities and maintained approximately 10 Pa.s. The viscosities of Groups D and E were higher; these inks had considerable viscosity after contact with the Xuan paper, which was not conducive to writing. In conclusion, Groups B and C were considered to have the proper viscosity, and Group C was selected for further testing.

Morphological and elemental analyses

The particle sizes and dispersion of materials in ink are also very important properties. As shown in Fig. 4A and B, we determined the particle sizes and dispersion levels of CM and CG inks. The sizes of the particles in the CM and CG inks were relatively similar, approximately 5–10 μm, while the difference was that the CG ink showed better dispersion and the particle dispersions were not concentrated. This meant that the CG ink in this experiment was comparable to the CM ink in terms of particle sizes and slightly better in dispersibility. This

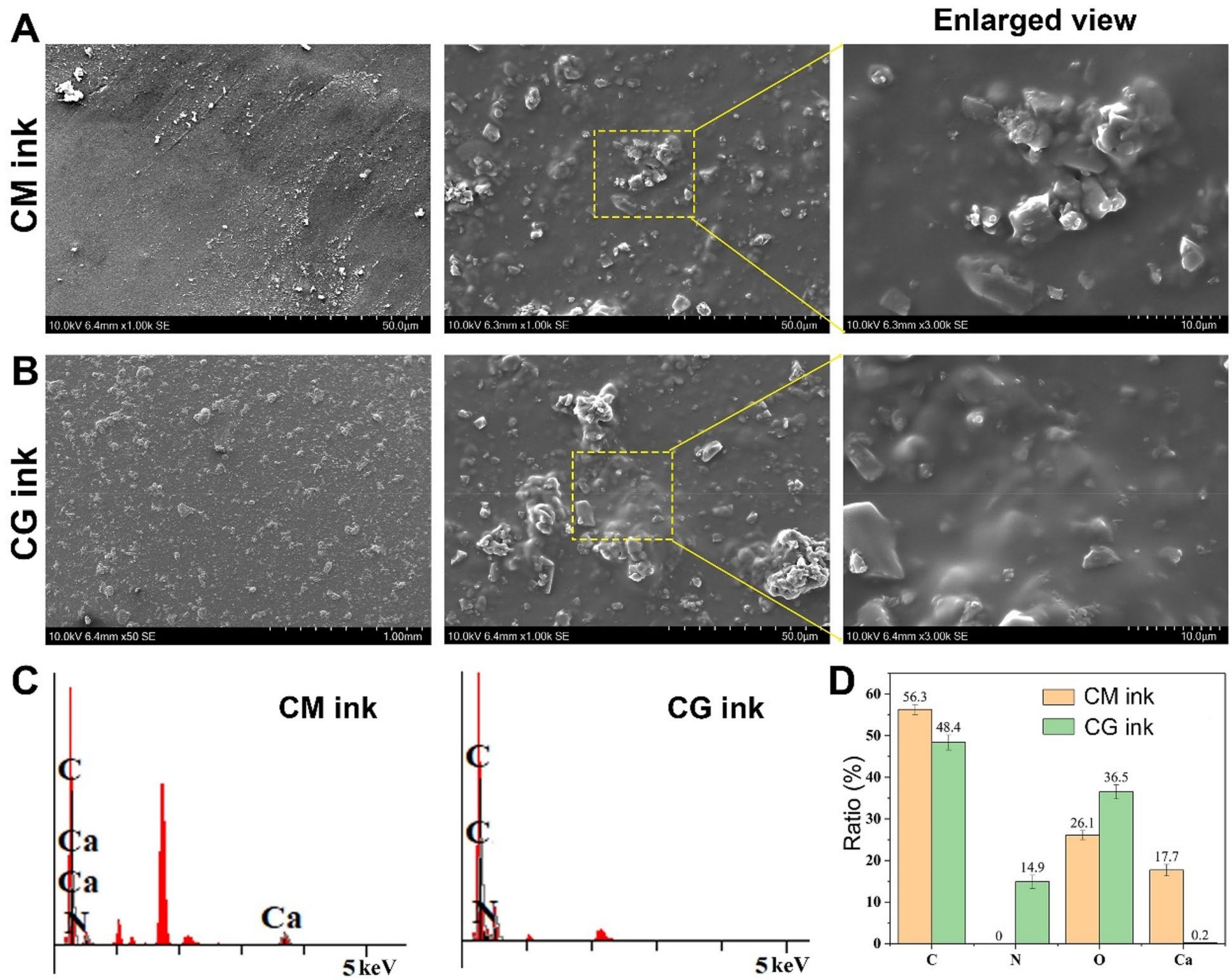

Fig. 4 The FESEM images (**A**, **B**) and element analysis (**C**, **D**) of CM and CG inks

should be attributed to the highly beneficial properties of CS in ink, which promoted dispersion of toner particles. On the other hand, we also carried out elemental analyses, and these results showed that there were differences in the compositions of the two inks (Fig. 4C and D). There was no elemental N in the CM ink, which meant that collagen was not present in the formula and it differed greatly from that of the CG ink. Furthermore, large amounts of calcium salts were present in the CM inks, while only 0.2% calcium salts were detected in CG ink due to the limited use of calcium salts.

Elemental analyses of CM, CG and Qing inks on paper

XPS was employed to determine the surface elemental compositions from survey spectra, C 1s, O 1s and N 1s core-level scans of CM, CG and Qing inks on paper, as shown in Fig. 5. The XPS survey spectrum showed that there was no N in the CM ink, which was consistent with the EDS results. With the CG and Qing ancient ink, the peaks for N were very obvious. These results indicated that animal collagen may have been employed in Qing ancient ink. Furthermore, the composition of CG ink was also proven to be similar to that of ancient ink

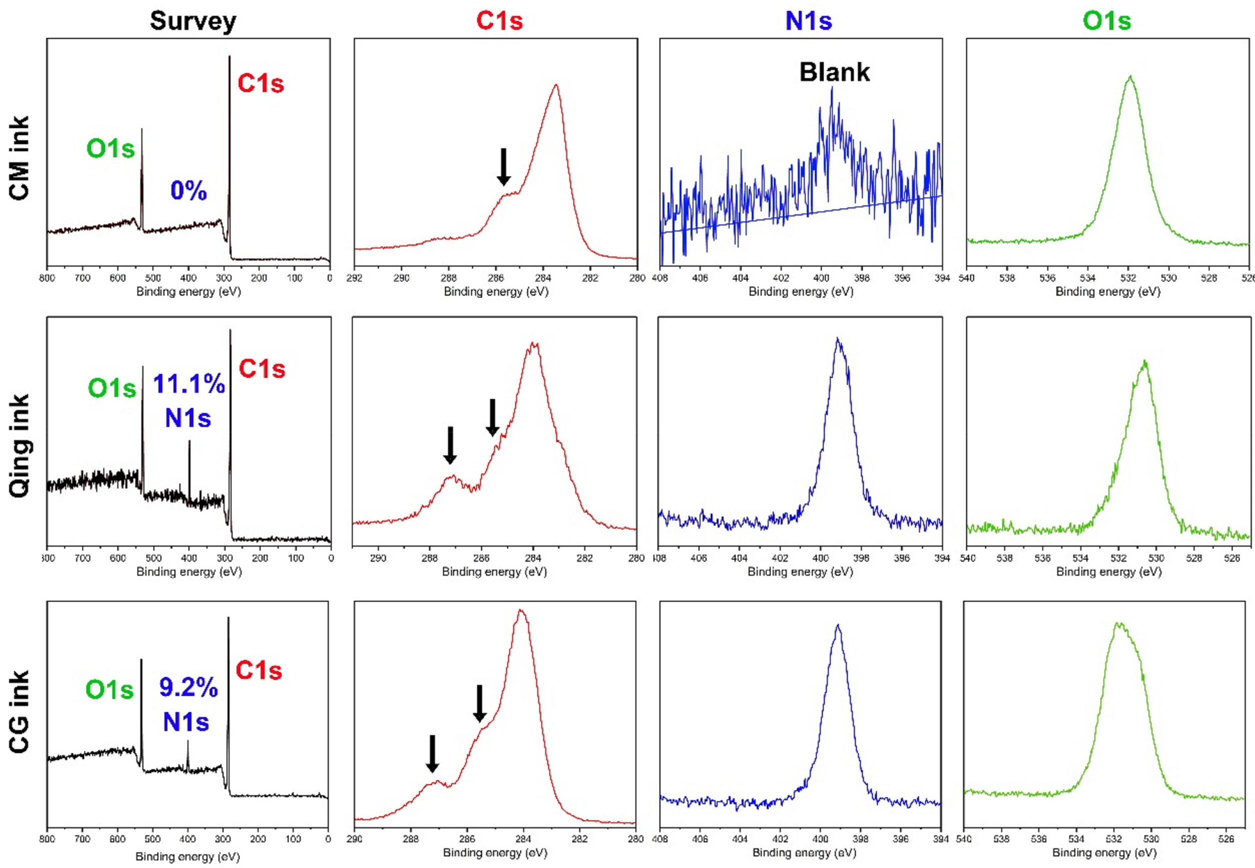

Fig. 5 XPS survey spectra, C1s, O1s and N1s core-level scan of CM, CG and Qing inks on paper

(11.1%), and the proportion of N was approximately 9.2%. Moreover, the C 1s spectra indicated that the peak shapes were very similar, and there were peaks at 287.2 eV and 285.3 eV. The above results revealed that the CG ink was similar to Qing ancient ink in terms of elemental content.

Stabilities of inks on Xuan paper

Inks with different compositions will show different spreading behaviors on paper; hence, the diffusivities of the as-prepared inks required further investigation. One of the important factors affecting writing is diffusion of the carbon powder in ink, which makes it difficult to show the edges and details of calligraphy. Therefore, to compare the differences in ink diffusivity for CG and CM, we used the same kind of paper to produce calligraphy. As shown in Fig. 6A and B, the head of the Chinese character "中 (zhong)" is the first position where the brush touched the paper; the brush contained more ink, and it was easily spread. As shown in Fig. 6C, the diffusion area seen on the paper for CM ink was filled with toner, which made it impossible to determine the original trajectory. For CG ink, although there was diffusion, the diffusion area did not contain toner, but diffusion of water would not lead to unclear ink. Subsequently, we used the dripping method to explore the stabilities of inks on paper. After a drop of water was dropped at the top of the Chinese character, the CM ink was impregnated, and a large amount of toner was spread and finally formed a stained area. In contrast, the CG ink only produced water stains and did not exhibit ink diffusion. The above results showed that there was good interfacial adhesion between the CG ink and the Xuan paper used in this study.

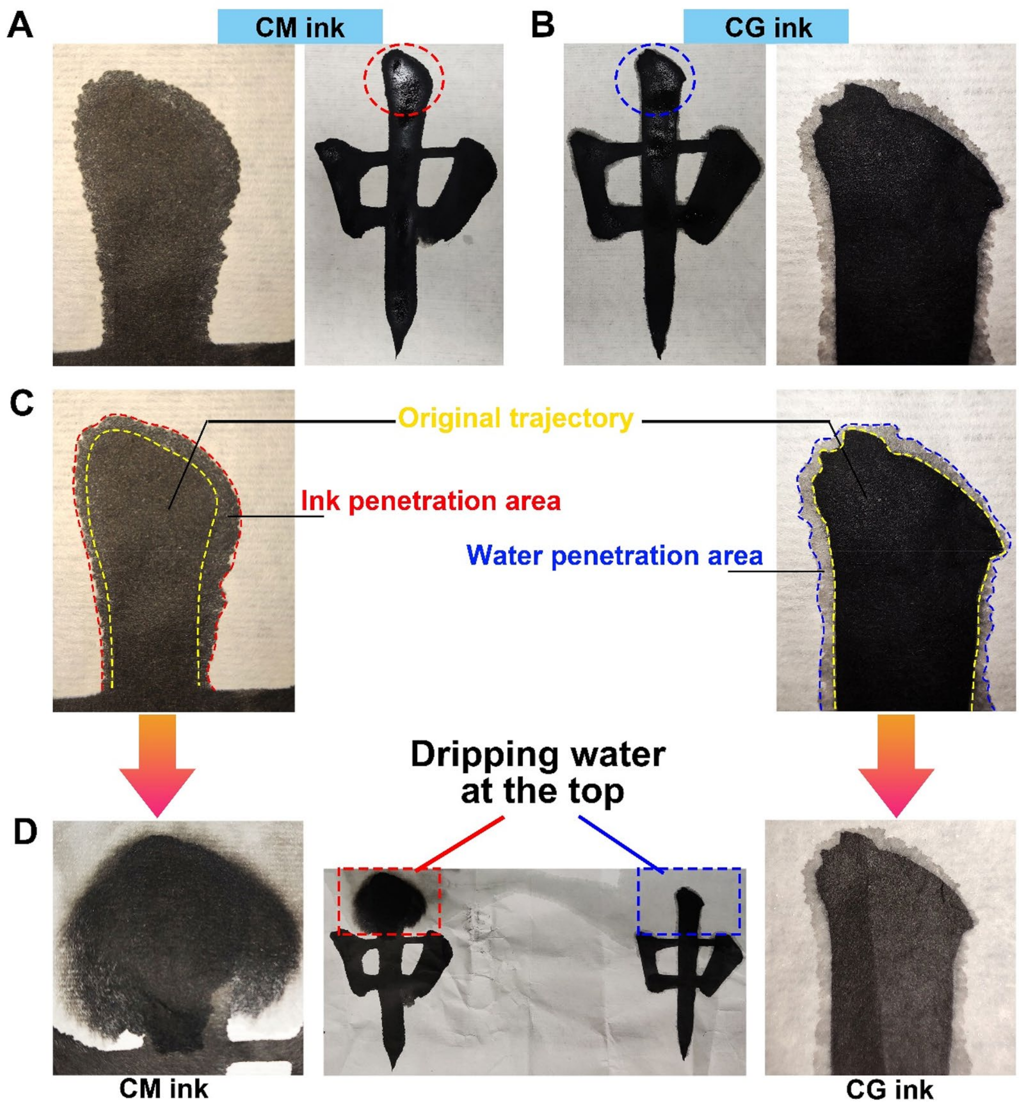

Fig. 6 The ink written on Xuan paper shows different traces and diffusion states before (**A**–**C**) and after (**D**) dropping water

We also verified the combination of ink and materials microscopically through FESEM. As shown in Fig. 7, ink samples were taken from the edge and center of the paper. In Fig. 7A, there is no obvious boundary at the junction of the CM ink and the Xuan paper, which may be because the edge was the weakest part of the ink mark due to diffusion of the gradient. CG ink in the Xuan paper appeared with a very clear dividing line (yellow dotted line); at the same time, even after larger magnification, the boundary was still clear. This result verified that CG ink showed excellent interfacial bonding without any diffusion. Subsequently, we observed the central positions of the inks. It was clearly seen that the molecular networks of chitosan and gelatin and the fibers of the Xuan paper cross-fused with each other and formed a solid adhesion layer. However, these phenomena were

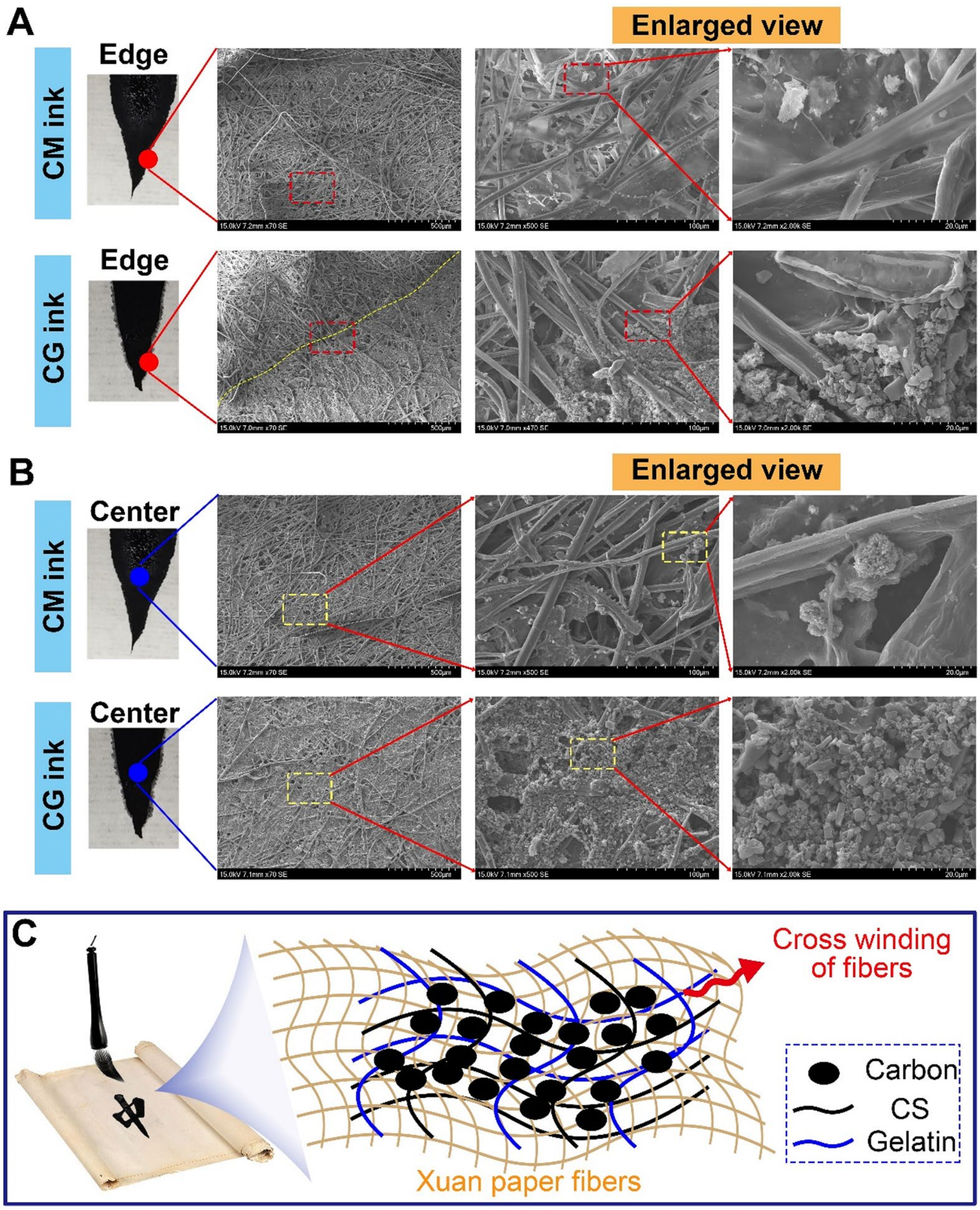

Fig. 7 The FESEM images of Xuan paper with ink (**A**, **B**). The schematic diagram of the interaction among the CS, gelatin and Xuan paper fibers (**C**)

not observed for the components of the CM ink. The above results showed that the network of chitosan and gelatin restricted the flow of toner effectively, and after contact with Xuan paper, chitosan and gelatin crossed deeply into the fibers [24] so they were not diffused by water. This is of great practical significance for preservation and dissemination of calligraphic works.

Bacteriostatic properties of inks

During long-term preservation, ink is easily attacked by bacteria, fungi and other microorganisms, which can result in ink failure. Therefore, we introduced the antibacterial components chitosan and tannic acid into the ink. Chitosan is positively charged, while tannic acid has many phenolic hydroxyl groups, which have a strong

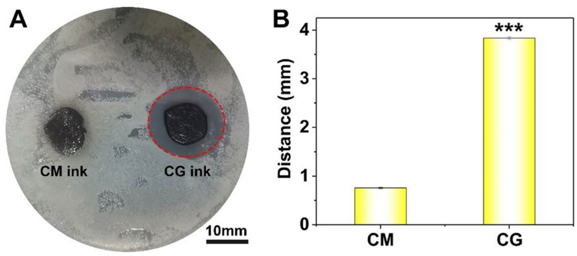

Fig. 8 The bacteriostatic property of CM and CG inks using the *E. coli*. The existence (**a**) and size (**b**) of the bacteriostatic circle around the inks filter paper disk (n = 5). The quantitative results were exhibited as mean ± standard deviation, and one-way analysis of variance was employed to make the statistical analysis. When $p < 0.05$, it means a significant difference (*); when $p < 0.01$, it represents a very significant difference (**); when $p < 0.001$, it indicates a very high significant difference (***)

antibacterial effect. In this study, the bacteriostatic properties of CM ink and CG ink were tested with preliminary bacteriostatic zone experiments. As shown in Fig. 8, growth of bacteria around the CG ink were inhibited and showed a clear culture medium, while there was some decomposition of the bacterial community around the industrial ink but no obvious bacteriostatic circle. These results showed that the antique ink we prepared had good antibacterial properties and is expected to be preserved for a long time.

Display of traditional chinese calligraphy and painting

The author employed the CG ink to copy the representative work *Zijin Research Post* in Fig. 9. Originating from the author's understanding of the copied object, the author mainly tried to realize a perfect combination of likeness and spiritual resemblance during the process of copying. According to this work (Fig. 9C), CG ink presented a good writing process, which showed the edge and turning well. Moreover, a strong ink color and rich sense of layers also appeared. Furthermore, we applied CG ink to create landscape paintings, as shown in Fig. 9D. Compared with calligraphy, painting has loftier requirements for the descriptions of objects and the presentation of ink. In the painting, the brush moved slowly when trees were created, which was intended to test the black and light effects of the CG ink. The stone was mostly done on the side in order to test the ink adsorption capacities in different parts of the brush tip, belly, root and so on. On the whole, the performance of the CG ink was similar to those of mainstream inks, but the effects of CG ink on different papers remain to be studied further.

Conclusions

Although the process for preparation of ancient ink has been handed down to this day, it is so hard to use ancient technology to make ink today due to the harsh preparation and preservation conditions required for ancient Chinese ink. Therefore, to promote the spread of this intangible cultural heritage of mankind based on ancient ink, such as in Chinese calligraphy, it is necessary to explore new technology and a material system for Chinese ink used in calligraphy and painting. In the laboratory, an environmentally friendly, practical and antibacterial Chinese ink was prepared with a facile heating-stirring process based on gelatin and the natural polysaccharide chitosan. We found that the ink had the proper viscosity when the ratio of chitosan to gelatin was 1:1. Then, we observed the micromorphologies and elemental compositions of the two inks with SEM and found that although there were great differences in the elemental compositions of the CG ink and CM ink, the particle sizes of the CG ink were comparable to those of modern industrial inks. We further analyzed this difference through XPS, and the results showed that the elements of CG ink were very similar to those of ancient ink. Then, the performance of each ink was evaluated by writing on Xuan paper and with SEM analysis. The results showed that CM ink spread easily on Xuan paper, while the CG ink showed good adhesion and stability on Xuan paper. Based on this study, we believe that the good performance of CG ink should be attributed to restriction of toner by its internal CS and gelatin network, which limit diffusion. Finally, the author used CG ink to display traditional Chinese calligraphy and landscape painting and believes that it has significant application prospects and will be used in large-scale production.

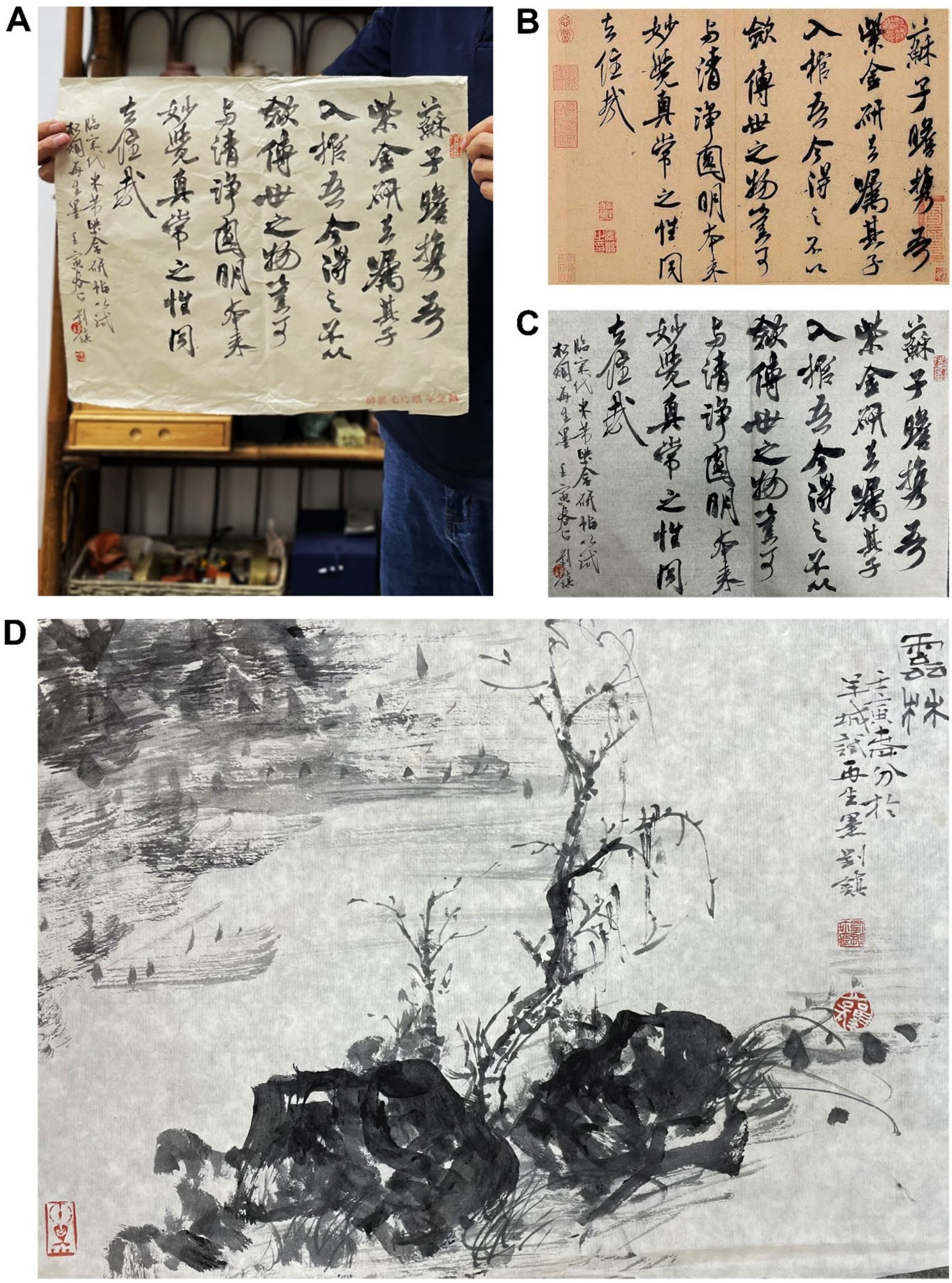

Fig. 9 Prof. Liu took a group photo with his calligraphy work (**A**). The image of *Zijin Research Post* of Mi Fu on Song Dynasty (**B**) [25], and the imitation of the *Paste* created by Prof. Liu with CG ink (**C**). Prof. Liu applied CG ink to create landscape paintings (**D**)

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Author contributions
ZL: conceptualization, methodology, software, validation, formal analysis, writing—original draft, supervision. KL: conceptualization, methodology, software, validation, formal analysis, writing—original draft, writing—review and editing. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
There are no competing interests to declare.

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