Combined Polyhexamethylene Quanidine and Nanocellulose for the Conservation and Enhancement of Ancient Paper

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1 Combined polyhexamethylene guanidine and 2 nanocellulose for the conservation and enhancement 3 of ancient paper

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13 **Abstract** Here a new reinforcement and mold-resistant method for ancient books is proposed based on
14 the suspension of nanocellulose (CNCs) and polyhexamethylene guanidine (PHMG). The paper
15 documents were treated with the reinforcement suspension under the optimum ratio obtained by
16 response surface methodology (RSM), then tested for mildew resistance and accelerated aging
17 experiments to evaluate the anti-mildew effect, mechanical strengthening properties and durability of
18 CNCs/PHMG compound solution on ancient books. The results showed the mechanical properties of
19 the paper treated by CNCs/PHMG were significantly improved at the optimum ratio (0.3wt%:0.3wt%),
20 with the folding endurance increased by 1.78 times and the increase ratio of tensile strain was 46.62%.
21 Meanwhile, the treated paper maintained the same good zero-span tensile strength after aging 7 days as
22 the sample unaged untreated, indicating superior aging resistance of this treatment method. In addition,
23 excellent biocidal activity with the growth trace of mold less than 10% was observed on treated paper.
24 In conclusion, it is a method that simple to operate, which can be a promising treatment of
25 conservation and enhancement of ancient paper.

26 **Keywords** *ancient books ▪ mildew resistance ▪ reinforcement ▪ strength ▪aging*
27 *resistance*

28

29

30 **Introduction**

31 Ancient literatures are an important historical and cultural heritage with great
32 collection and academic value. The main form of them was made of paper, which as
33 the mainstream carrier of literatures is usually passed down to the present through
34 writing and printing. According to statistics, the literatures in collections of China
35 now reaches 30 million volumes (Wang 2006). However, due to the irregular
36 preservation conditions, such as changes of environment and inherent instability of
37 the paper itself, literatures are damaged to different degrees in the process of
38 preservation, which damage the valuable information (He et al. 2019). Therefore, it is
39 urgent for the enhancement of the paper documents.

40 Ancient books are damaged by various reasons, such as acidic substances absorbed
41 from air, additives added during paper-making and mold attacking. Actually, all those
42 factors may cause the fiber degradation, an important intrinsic factor during paper
43 aging. Acidic substances derived from the absorption of pollutants (NO_x) and
44 oxidation of additives (Isca et al. 2018) can result in the fracture of fiber-glucoside
45 bond, significantly weaken the mechanical strength of paper (Erhardt 1995; Simona
46 Margutti 2001). Mold is another threat for ancient books, which possess strong
47 adaptability and rapid reproduction, producing acidic byproducts on the surface of
48 paper (Sarantopoulou et al. 2006) that depolymerized cellulose fibers (Piantanida et
49 al. 2006; Ponce-Jimenez 2003). In addition, harmful mycotoxins produced by some
50 mold may harm people's health (Konkol et al. 2012).

51 To address the reinforcement and mildew resistance of ancient books, several
52 strategies have been developed in recent years. The enhancement techniques could be
53 summarized as composite reinforcement, synthetic reinforcement and natural
54 reinforcement, according to the type of enhancement materials (Zhang 2020).
55 Generally, the synthetic materials mainly include silane coupling agent and

polyurethane, etc. 3-aminopropyl-diethoxymethylsilane (AMDES) and aminopropyl triethoxy silane (APTES) cause a polymer network, which forms a cross-linking effect with the paper's fiber molecules to achieve reinforcement effect on newsprint (Piovesan et al. 2018). The pure cellulose paper was treated by the synthetic material AMDES with the tensile strength and folding resistance significantly improved (Dupont et al. 2010). Composite materials usually combined natural materials with synthetic materials to strengthen paper (Zhang 2020). TMSC-Mg(OH)₂-HMDSO reinforcement solution is formed by uniformly dispersing trimethylsilyl cellulose (TMSC) in hexamethyldisiloxane (Amornkitbamrung et al. 2015). However, there are many problems such as poor polymer stability and inferior compatibility with paper fibers in composite and synthetic materials. In contrast, natural materials (chitosan (Li et al. 2017), guar gum, cellulose (Kukharensko et al. 2014), etc.) play an important role in the protection of ancient books due to the extensive sources, friendliness to environment and good compatibility with paper. Among these, nanocellulose have emerged as a promising reinforcement material relying on its own unique superiority, such as optical transparency (Jia 2013), excellent biocompatibility, high specific surface area (Yuri Kobayashi 2015) and colloidal dispersion stability (Xiao et al. 2020). Ancient paper is treated by CNCs with a striking improvement in strength, which have confirmed by many reports in the past decades (Bergamonti et al. 2020).

Mildewproof of paper is mainly carried out by treating with Ag nanoparticles (Dai et al. 2017), TiO₂, chitosan (Salama et al. 2020), quaternary ammonium salt (He et al. 2019). Anyway, there are a lot of shortcomings such as high price, inferior antibacterial ability and poor chemical stability. Recently, guanidine cationic polymers attracting considerable attention due to their high biocidal activity (anti-fungal (Zhang 1999), antiviral (Pinto et al. 2010; Thakkar 2009), antibacterial (Manetti et al. 2009; Qian et al. 2008) and low toxicity (Zhang 1999) to the human body). In particularly, the antibacterial rate of polyhexamethylene guanidine

83 hydrochloride (PHMG) reached more than 90% while its concentration was only 1
84 ppm (Feiertag 2003). Due to its strongly positive charges, the cationic oligomers were
85 electrostatically bonded with the negatively charged bacterial envelope, changing the
86 phospholipid environment on the cell membrane (Feiertag 2003), resulting the
87 destroy of membrane, infiltration of bacterial cell components, and death of bacteria
88 (Brogden 2005; Oule et al. 2008; Qian et al. 2008).

89 The positive PHMG can form electrostatic attraction with the negative fibers,
90 which may contribute to the formation of the hydrogen bonds between chloride ions
91 of the PHMG and the hydroxyl of the nanocellulose (Kukharensko et al. 2014), thus
92 improve the mechanical properties of paper. In view of the preservation and
93 reinforcement of ancient books, we combined PHMG and CNCs to strengthen paper.
94 PHMG was dispersed in the suspension of CNCs to form a compound solution, and
95 then sprayed on the surface of the paper with the spray gun. The paper documents
96 were processed under the optimal conditions explored by response surface
97 methodology (RSM) to obtain the strengthening and anti-mildew properties.

98 **Experimental Methods**

99 **Materials**

100 Polyhexamethylene guanidine hydrochloride (PHMG) was purchased from
101 Macklin Biochemical Technology Co., LTD. Sulfuric acid was purchased from
102 Guangzhou chemical reagent factory. Hardwood dissolving pulp was supplied by
103 Huatai paper Co., LTD (Shandong China). Paper samples were taken from the
104 Whatman paper and an old book that published in 1954, respectively.

105 **Preparation of CNCs and PHMG suspensions**

106 CNCs was prepared from hardwood dissolving pulp hydrolyzed by 55wt% sulfuric
107 acid applied at 45 °C for 40 min according to the reported work of our group (Peng et
108 al. 2018). The length of the CNCs was between 40 and 800 nm, while the diameter
109 was 10~20 nm. CNCs suspensions (1% w/v) were diluted to different concentrations
110 (0.1~0.5wt%) and mixed with PHMG to obtain CNCs/PHMG compound solution.

111 **Experimental design and optimization**

112 *Design of the response surface methodology*

113 The Whatman paper samples (d=18 cm) were treated with CNCs/PHMG of
114 different concentrations in the early stage of this study. The processed paper was
115 dried naturally in the air and then transferred to a vacuum drying oven (0.08 MPa,
116 50 °C) for 12 h. The weight percentage gain was 2.30% (± 0.59) on average.

117 A response surface methodology (RSM) was used to investigate the effect of the
118 concentrations of CNCs (A) and PHMG (B) on paper's mechanical properties.

119 The experimental design factors and horizontal pieces were shown in Table 1. The
120 tearing index (Y) of the treated paper samples was taken as the response value.
121 Equation (1) was used to calculate the enhanced percentage (P) of tearing strength:

$$122 \quad P = \frac{Y_{treated} - Y_{untreated}}{Y_{untreated}} \times 100 \quad (1)$$

123 Where $Y_{treated}$ and $Y_{untreated}$ are the tearing index of treated and untreated samples,
124 respectively.

125 According to the Central Composite Design method (CCD) provided by design-
126 Expert 8.0.6 statistical software, thirteen experimental runs were required, which
127 included five replicates of the central run (Jalali and Sobati 2017) .

128

Table 1 Experimental design factors and levels

Factors	Name	Units	Low	High	-alpha	+alpha
A	CNF	%	0.1	0.5	0.017153	0.582843
B	PHMG	%	0.1	0.5	0.017153	0.582843

129 The quadratic polynomial regression equation obtained by using the design-Export
130 software to correlate the strengthening process of paper:

$$131 \quad Y = b_0 + b_1A + b_2B - b_{12}AB - b_1^2A^2 - b_2^2B^2 \quad (2)$$

132 Where Y is the predicted response (i.e., the tearing index); A, B are coded levels of
133 independent variables; the coefficients of linear effect, quadratic effect and
134 interaction effect are respectively represented by b_i, b_{ii}, b_{ij} (Wang et al. 2015).

135 *The repair process of ancient book*

136 The CNCs/PHMG suspension was sprayed evenly onto the paper (17.5×14 cm)
137 published in 1954 at the optimum ratio that derived from the RSM. 10ml of the
138 suspension was used to spray twice on paper with an interval of 20 min (Bergamonti
139 et al. 2020). The processed paper samples were naturally dried at room temperature
140 for 6~7 h and then transferred to a vacuum drying oven (0.08 MPa, 50 °C) for 12 h.
141 The weight percentage gain of samples was 2.70% (± 0.113) on average.

142 *Artificial aging experiment*

143 According to the standard of GB/T 22894-2008, the aging experiment of the
144 treated and untreated paper was carried out to simulate the natural aging process and
145 evaluate the anti-aging performance. The samples were aged under the conditions of
146 80 °C and 65% RH for certain days.

147 **Characterization and measurements**

148 *Mechanical tests*

149 The properties of paper samples were tested under the certain condition of 25 °C,
150 50% RH after balancing moisture for 24h (ISO1871990). Ten samples were tested for
151 each group and each sample was measured in parallel five times, the results were
152 averaged.

153 Tensile strength of paper (15×100×0.08 mm) were determined by universal testing
154 machine (INSTRON 3300) (30mm/min) accordance with ISO 1924-2:2008 standards.
155 Tearing strength were measured by the instrument (L&W009, Sweden) according to
156 GB/T 455-2002. The size of the samples was (63±0.5) mm×(50±2) mm. In order to
157 ensure the accuracy of the test, paper samples were selected according to GB/T-450
158 to avoid wrinkles and other defects. The zero-span tensile strength measurements on
159 samples were carried out according to GB/T 26460-2011 standards with the
160 instrument (L&W CE062, Sweden) (25±2 N/s). Paper samples were tested in parallel
161 ten times with a width of 63 mm and a length of 40~195 mm. Folding Endurance
162 measurements were performed accordance with GB/T 457-2002 by a double fold
163 instrument (MIT/U21B, America, applied force=1.0 KN). Paper samples were cut to
164 130 mm×15 mm.

165 *Brightness test*

166 The Brightness of paper was determined by L&W Elrepho070 whiteness meter
167 according to ISO 11475-2017 standard. Taking 5 areas of each sample for
168 measurement. CIE L * a * b * system was used to evaluate the color change of the

169 paper, ΔE represent the color difference, calculation formula as shown in the equation
170 (3):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

172 *SEM and XRD analysis*

173 The scanning electron microscope (SEM, Merlin) was used to observe the
174 morphology of paper. The X-ray diffractometer (X' pert Powder) was used to
175 analysis the X-ray diffraction (XRD) of samples. The crystallinity (CrI) of cellulose
176 can be calculated by equation (4):

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (4)$$

178 Where I_{am} and I_{002} are the intensity of the diffraction peaks of $2\theta=18.0^\circ$ and
179 $2\theta=22.5^\circ$, respectively.

180 *anti-mold test*

181 The mold-resistant experiments of paper samples were carried out by Guangdong
182 Microbiological testing Center according to the GB/T 24346-2009 and AATCC 30-
183 2013III. The paper samples were treated with four kinds of mixed mold (Aspergillus
184 Niger, Trichoderma Viride, Penicillium Funiculosum, Chaetomium Globosum) for 28
185 days and a single Aspergillus Niger with a cycle of 7 days, respectively. The original
186 paper samples were blank.

187 Results and Discussion

188 The preparation of CNCs/PHMG suspension

189 In this study, PHMG combined with CNCs was supposed to apply for the
190 preservation and enhancement of ancient books. The CNCs/PHMG complex
191 solutions were prepared by mixing CNCs suspensions in various dilute concentrations
192 with PHMG. The concentration of CNCs and PHMG had influence on the mechanical
193 properties of paper (Yi et al. 2017). In order to investigate this interaction, RSM was
194 used to simulate the experiment to obtain the optimum treatment conditions, so as to
195 achieve the superior improvement in the mechanical strength of paper.

196 Model fitting and adequacy checking

197 Design of the experiment and results of the response surface are shown in Table 2.
198 Equation (5) represents the quadratic multiple regression equation, which is used to
199 fit the experimental data of tearing index of paper patterns.

$$200 \quad Y=43.58+0.56A+0.054B+9.11AB-14.53A^2-10.30B^2 \quad (5)$$

201 Where Y is the tearing index ($\text{mN}\cdot\text{m}^2/\text{g}$); A , B are the content (%) of CNCs and
202 PHMG, respectively. The parameters have increasing and decreasing effects on the
203 response value Y , which can be seen from the symbols and values of the coefficient
204 (Jalali and Sobati 2017).

205 Table 2 Experimental design for response surface analysis and corresponding experimental data

Run	Coded variable levels		Response Y
	A	B	
1	0.10	0.50	5.66
2	0.02	0.30	5.58
3	0.30	0.30	7.21
4	0.50	0.50	6.54

5	0.50	0.10	5.52
6	0.30	0.30	7.57
7	0.30	0.30	7.06
8	0.30	0.30	7.31
9	0.30	0.30	7.20
10	0.30	0.02	6.17
11	0.30	0.58	6.04
12	0.10	0.10	6.48
13	0.58	0.30	5.79

Table 3 represents analysis of variance (ANOVA) of regression equation, that is a statistical method for detecting differences between sample groups. As can be seen from the Table 3, the smaller P-value and the larger F-value confirm the exists of significant corresponding coefficients (Wang et al. 2015). The values of "Prob > F" less than 0.0500 imply that the model is significant and the curvature is present. The interaction term of AB is significant ("Prob > F" is 0.0014), indicating the remarkable interaction of the concentration of CNC_s (A) and PHMG (B) on the tearing strength of the samples.

Table 3 Analysis of variance of regression equation

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F
Model	6.21	5	1.24	38.05	<0.0001
A-CNF	5.885E-003	1	5.885E-003	0.18	0.6838
B-PHMG	3.261E-005	1	3.261E-005	9.993E-004	0.9757
AB	0.85	1	0.85	25.94	0.0014
A ²	3.95	1	3.95	121.11	<0.0001
B ²	2.06	1	2.06	63.03	<0.0001
Residual	0.23	7	0.033		
Lack of fit	0.084	3	0.028	0.78	0.5641
Pure Error	0.14	4	0.036		
Cor Total	6.44	12			

The coefficient of determination of the proposed correlation are shown in Table 4. The regression model has high level of confidence and good adaptability in the

217 experimental data (>96%) (Jalali and Sobati 2017), which is confirmed by the high
 218 value of the variation coefficient, the adjustment coefficient ($Adj.R^2=0.9392$) and the
 219 low value of the coefficient of variation ($CV=2.79\%$). The correlation coefficient R^2
 220 of this model is 0.9645, which means that there are the obvious correlation of this
 221 regression model and the data cannot be explained only account for 3.55%
 222 (Peydayesh et al. 2017). The 2.79 of the CV% confirmed the better reproducibility of
 223 the results (Rabelo et al. 2014).

224 Table 4 Statistical analysis of regression equation error

Statistics project	value	Statistics project	value
Std.Dev.	0.18	R-Squared	0.9645
Mean	6.47	Adj R-Squared	0.9392
C.V.%	2.79	Pred R-Square	0.8719

225 The normal probability plot, the plot of residual versus predicted values, and the
 226 chart of the actual values of the tearing index versus the predicted data are shown in
 227 Fig. 1a-c, respectively.

228 The points are approximately located on a straight line in Fig. 1a, which proves the
 229 normal distribution of the residual. As can be seen from the Fig.1b, the distribution of
 230 these points is random and the location is in the range of ± 3.0 , which confirmed the
 231 adequacy of the empirical model (Rabelo et al. 2014). Fig.1c displays that the points
 232 are clustered near the diagonal, which proves the good correlation between
 233 experimental and predicted values. In conclusion, the model with effectiveness and
 234 usability can effectively predict the enhancement effect of the tearing strength of
 235 paper.

Fig. 1. Evaluation of RSM correlation for the paper's tearing index ($\text{mN}\cdot\text{m}^2/\text{g}$). (a) The normal probability distribution of residuals, (b) Distribution of residual versus predicted values, and (c) Chart of predicted value and actual value

Interaction between the independent parameters

Fig.2 showed the response surface diagrams of the interaction between operating parameters (the concentration of CNC_S and PHMG) and the tearing index of paper. As can be observed, the tearing strength of treated paper improved with the increase of CNC_S concentration (0.10~0.30wt%) and then declined in the concentration between 0.30~0.50wt%. The effect of PHMG on the reinforcement of paper also showed the same trend. This trend can be attributed to the higher concentration of CNC_S with the higher viscosity, more solution retained and attached to the paper, which significantly improved the strength of paper. The decreasing trend can be explained by the fact that CNC_S can only improve the mutual binding force between fibers, but not increase the fiber length. What's more, PHMG may limit the improvement effect of CNC_S on the mutual binding force due to the existence of -

252 NH_2 , which prevent the formation of hydrogen bonds (Liu et al. 2014; Zhong et al.
 253 2013). The optimal concentration of nanocellulose on ancient books repair was about
 254 0.3wt%, higher concentration did not bring a significant improvement in tearing
 255 strength, indicate that the strengthening effect of CNCs on aged paper is not linearly
 256 increasing. The similar trends was also found in the work of Yi et al. (2017).

257 RSM suggested the optimal conditions of the above two parameters in the
 258 improvement of tearing strength. According to the response surface diagrams and the
 259 evaluation of the regression model, the predicted tearing index of 7.27 and the
 260 enhanced percentage of 44.53% could be achieved using the suspension of CNCs and
 261 PHMG (0.3wt%:0.3wt%). The above prediction results were verified by carrying out
 262 three repeated experiments (Jalali and Sobati 2017). The tearing index was as high as
 263 7.31, with an average increase of 42.5%, which was basically consistent with the
 264 predicted percentage of tear strength enhancement, proving the applicability and
 265 reliability of the model.

266
 267 Fig.2. The interaction between the concentration of CNCs and the density of PHMG on the
 268 enhancement of tearing strength of paper samples

269 Application of CNCs/PHMG treatment on old paper samples

270 Nanocellulose was applied to repair and strengthen old paper due to its superior

271 advantages such as unique properties that similar to natural cellulose and large
272 number of hydrogen bonds formed with paper fibers (Xiao et al. 2020).

273 *Performance comparison of treated and untreated paper*

274 In this work, we combined PHMG and nanocellulose to improve the ancient
275 literature strength at the optimal ratio of 0.3wt%: 0.3wt%, which was obtained by
276 RSM above.

277 Table 5 showed the results of chromaticity and mechanical properties of paper
278 documents before and after treatment. The original paper had a tearing index, a zero-
279 span tensile strength and a folding endurance of 3.61 mN·m²/g, 75.0 KN/m and 5
280 times. In contrast, mechanical properties of samples were improved after treated with
281 CNC_S/PHMG, with a significant increase (1.78 times) in folding strength and an
282 obvious improvement (29.93%) in tearing strength, which further proves the
283 applicability of the response surface model. What's more, the tensile strain of the
284 treated sample tested on a universal material testing machine increased from 1.42% to
285 2.08% with an increase ratio of 46.62%, which can be observed from the Fig.3.
286 Meanwhile, the tensile strength increased by 1.10 times from the original 15.57 MPa
287 to 17.15 MPa. The excellent mechanical properties further illustrated the considerable
288 enhancement effect of CNC_S on ancient books, which is consistent with the previous
289 research (Bergamonti et al. 2020). Nanocellulose with the similar structure to paper
290 fibers can form crossing-linking effect, strengthen the bonding network and act as the
291 adhesive between cellulose fibers (Zhang et al. 2016). The enhancement effect of
292 CNC_S on ancient literature may be ascribed to the formation of a large number of
293 hydrogen bonds between the fibers (Kukharensko et al. 2014). What's more, the -NH₂
294 group of PHMG may also form chemical bonds with the -OH group in paper (Salam
295 et al. 2013).

296 In addition, the surface tension and the binding force brought by water evaporation
 297 can promote the combination of CNCs and paper fibers, make the fiber more tightly
 298 by filling the pores, so as to achieve the enhancement on the paper literature, which
 299 can also be confirmed by SEM images (Fig.4a). It can be seen the presence of lots of
 300 pores and the loose dispersion of fiber, which would have a negative effect on the
 301 strength of the paper. In contrast, the images of treated paper show a dense structure
 302 and a smooth surface that filled the space among the fibers with nanocellulose (Zhang
 303 et al. 2016), which is of great significance for the enhancement of paper's strength.

304 The XRD was used to evaluate the effect of CNCs/PHMG treatment on fibers
 305 crystalline structure. It can be seen a slight change in peak intensity of paper samples
 306 before and after treatment. The CrI of paper fibers that calculated by formula (4) were
 307 61.94% (UT) and 64.90% (T), which indicate that the treatment did not cause an
 308 obvious decline in the degree of the order for paper cellulose and had little impact on
 309 the fiber.

310 Table 5 Performance comparison of untreated paper (UT) and the sample(T) treated by CNCs/PHMG

Paper sample	Tearing strength (mN·m ² /g)	The zero-span tensile strength (KN/m)	Folding Endurance (times)	Brightness	Chromatic aberration (ΔE)	Tensile strain (%)
UT	3.61±0.07	75.00±4.25	5.00±0.14	39.42±0.27	—	1.42
T	4.69±0.28	79.30±1.80	5.33±0.04	39.15±1.55	0.47	2.08
Increased ratio (%)	+29.92%	+5.74%	+17.12%	−0.68%	—	+46.62%

311 Note: In the table, “+” represents the percentage of increase and “−” represents the ratio of decrease

Fig.3. Tensile force vs. percentage tensile strain for treated and untreated paper

In the process of ancient books restoration, attention should also be paid to minimize the impact on their appearance (He et al. 2019). The whiteness of samples was tested to determine changes on the surface. These results showed a slight decrease in whiteness of 0.68% and a small value in color difference ΔE of 0.472. A faint decrease in brightness is not obvious to the human eye, which can be observed from the photos shown in Fig.S2, indicating that this treatment of ancient books is appropriate.

Fig. 4. (a) SEM images of these paper samples treated (T) and untreated (UT)

Fig. 4. (b) SEM-EDS images of the paper sample treated by CNCs/PHMG

Fig. 5. XRD images of treated (T) and untreated (UT) paper samples

Comparison of anti-aging property

In order to better assess the stability and anti-aging properties of the treatment with CNCs/PHMG suspension, paper samples were subsequently artificially aged for a certain time in the same conditions.

Fig.6 showed a similar decline trend in mechanical properties of the treated paper and untreated group after 3, 5, 7 and 9 days of aging, in which the folding

strength of the untreated paper declined sharply. The ancient paper's aging rate was faster within the aging time of 0~5 days, which mainly due to the acid substance that produced in the aging process degraded cellulose, reduced the degree of polymerization of fiber, thus result in a rapid decline in fiber strength(Erhardt 1995). The strength of treated sample was much better than its untreated aged counterpart, which indicates the obvious protective effect against the properties decline of this treatment. What's more, the treated paper samples still exhibited the same good zero-span tensile strength as the paper untreated unaged that can be observed from 7 days of aging. The zero-span tensile strength reflects the fiber strength (Zeng 2012). The fiber strength of the treated aged paper samples is higher than that of the untreated aged counterpart, which further indicates the stability of the treatment by CNCs/PHMG.

Fig.6. The comparison of treated and untreated paper samples after aging on folding endurance (A); tearing index(B) and zero-span tensile strength(C); Enhanced ratio comparison: folding endurance (a), tearing strength(b) and zero-distance tensile strength(c)

The XRD pattern of the paper fibers before and after accelerated aging is shown in Fig.S1, which can be found in supporting information. The CrI of treated and untreated samples changed slightly, indicating that the treatment method has subtle influence on the fiber structure even after a certain time of aging.

353 The whiteness and chromatic aberration (ΔE) of aged paper was also examined
 354 which is showed in Fig.7, detailed data can be found in Table S1. It can be seen the
 355 same decreasing trend of treated and untreated paper samples in brightness with the
 356 aging time extended, which may be due to the oxidation reaction of the paper fibers
 357 during the damp-heat aging process, resulting in the generation of colored aldehyde
 358 group (Fan and Guo 2018). The treatment method had little impact on the surface of
 359 paper samples with only a slight difference in brightness (UT 35.11%, T 34.94%)
 360 even aged for 9 days.

361

362 Fig.7. The brightness of treated and untreated paper samples after aging

363 **Anti-mold property**

364 In addition to the enhancement of strength, improving the mildew resistant
 365 property is also an important content need to be paid attention to in the preservation
 366 of ancient literatures. Guanidine antibacterial agents (PHMG, PHMG-Cl etc.) exhibit
 367 excellent antimicrobial effects against a wide range of strains (Mathurin et al. 2012;
 368 Oule et al. 2008). Paper obtained by dip-coating with modified guanidine-based
 369 particle aqueous dispersion (PHGH-PPGDE) exhibits 100% antibacterial rate against
 370 Escherichia coli (Wei et al. 2017).

371 To investigate the anti-mildew effect and the stability, the biocidal activity on four
372 mixed mold (*Aspergillus Niger*, *Trichoderma Viride*, *Penicillium Funiculosum*,
373 *Chaetomium Globosum*) and a single *Aspergillus Niger* were verified on paper
374 treated by CNCs/PHMG and the original paper with a cycle of 28 days and 7 days,
375 respectively. Fig.8 showed the result of 7-days anti-*Aspergillus Niger* activity.
376 Obvious fungal growth can be seen on the untreated paper, indicating the lack of
377 antifungal activity for ancient literature. In contrast, a significant biocidal activity on
378 treated sample was observed, with the generation of a mold proof circle and no mold
379 growth in the surface which confirmed the importance of PHMG in mold-proof
380 treatment. Similar results were found in the study of Liu et al. (2014), the paper
381 coated with DCMC-GH exhibit remarkable bacteriostatic effect on *Escherichia coli*
382 and *Staphylococcus aureus*, with inhibition zone of 16mm and 10mm, respectively.
383 The photos of the 28-days anti-mixed fungus results were showed in Fig.9. We also
384 observed the paper surface to see the growth state of mold by a polarizing microscope
385 at a magnification of 50 times. It can be seen small amounts of fungus on the surface
386 of treated paper, whereas the original sample with more mold growth and even
387 generated colonies that distributed in rows. Detailed antibacterial analysis and test
388 results showed in the report, which can be found in supporting information. As shown
389 in the report, with the treatment of PHMG a stronger antifungal activity can be
390 observed, mold is rarely and the traces of growth less than 10% in the treated paper
391 sample.

392 As can be seen from the above, such a superior antifungal property was attributed
393 to the evenly disperse of PHMG on paper surface, which can be observed from the
394 SEM-EDS in the Fig.3b. The PHMG with strongly positive charges can quickly bind
395 to the surface of the negative fungus, damage the cell membrane, thus achieve the
396 inhibition of the mold growth (Zhang 1999). The anti-mold test results of 7-days in
397 the single fungi and 28-days in four mixed mold fungus confirmed the significant

398 mildew proof effect and long-term effectiveness of CNCs/PHMG treatment, which is
399 of great significance for the preservation and protection of ancient books.

400

401 Fig.8. The 7-days anti-Aspergillus Niger effect of paper samples:(1) A UT (untreated) (2) B T (treated)

Fig.9. The 28-days anti- mixed mold effect of paper samples:(1)A untreated (2)B treated, and the surface photo of the paper observed by polarized light microscope (scale bar=20μm)

405 Conclusion

406 The protection of paper documents is very important in the area of cellulosic
407 material. Polyhexamethylene guanidine (PHMG) and CNCs were used to achieve the
408 enhancement and mold-proof of ancient books. RSM was employed to optimize the
409 experimental conditions to get the optimal ratio of the compound solution for paper
410 reinforcement. Mechanical tests demonstrated the enhancement of paper treated by
411 CNCs/PHMG, with 1.78 times improvement of the folding strength, which was
412 mainly caused by the interaction between CNCs and paper fibers. Meanwhile, the
413 treated paper with slight change on the whiteness still maintained good mechanical
414 properties after a period of aging. In addition, excellent biocidal activity was
415 observed on treated paper. To the final summary, this treatment approach gives the
416 paper excellent anti-aging performance and superior anti-mildew effect with the
417 significant improvement of the mechanical properties, which is conducive for the
418 long-term preservation and protection of ancient books.

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422 References

- 423 Amornkitbamrung L et al. (2015) Polysaccharide stabilized nanoparticles for deacidification and
424 strengthening of paper. RSC Advances. 5:32950-32961. <https://doi.org/10.1039/c4ra15153d>
425 Bergamonti L et al. (2020) Ag-functionalized nanocrystalline cellulose for paper preservation and
426 strengthening. Carbohydr Polym. 231:115773. <https://doi.org/10.1016/j.carbpol.2019.115773>
427 Brogden KA (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev
428 Microbiol. 3:238-250. <https://doi.org/10.1038/nrmicro1098>
429 Dai L, Liu R, Hu LQ, Si CL (2017) Simple and green fabrication of AgCl/Ag-cellulose paper with
430 antibacterial and photocatalytic activity. Carbohydr Polym. 174:450-
431 455. <https://doi.org/10.1016/j.carbpol.2017.06.107>

432 Dupont AL, Lavédrine B, Cheradame H (2010) Mass deacidification and reinforcement of papers and
 433 books VI – Study of aminopropylmethyldiethoxysilane treated papers. *Polymer Degradation*
 434 *and Stability*. 95:2300-2308.<https://doi.org/10.1016/j.polymdegradstab.2010.09.002>
 435 Erhardt D, & Mecklenburg, M. F. (1995) Accelerated vs natural aging: effect of aging conditions on
 436 the aging process of cellulose. . *MRS Online Proceeding Library Archive*.
 437 352:352.<https://doi.org/10.1557/PROC-352-247>
 438 Fan H, Guo M (2018) The Preservation and Repair of Ancient Books. *China Pulp and Paper*. 8:61-
 439 65.<https://doi.org/10.11980/j.issn.0254-508X.2018.08.011>
 440 Feiertag P, Albert, M., Ecker - Eckhofen, E. - M., Hayn, G., Hönig, H., Oberwalder, H.W., Saf, R.,
 441 Schmidt, A., Schmidt, O. and Topchiev, D. (2003) Structural characterization of biocidal
 442 oligoguanidines. . *Macromolecular Rapid Communications*,. 24:567-
 443 570.<https://doi.org/10.1002/marc.200390085>
 444 He B, Lin Q, Chang M, Liu C, Fan H, Ren J (2019) A new and highly efficient conservation treatment
 445 for deacidification and strengthening of aging paper by in-situ quaternization. *Carbohydr*
 446 *Polym*. 209:250-257.<https://doi.org/10.1016/j.carbpol.2019.01.034>
 447 Isca C, Di Maggio R, Pajares Collado N, Predieri G, Lottici PP (2018) The use of polyamidoamines for
 448 the conservation of iron-gall inked paper. *Cellulose*. 26:1277-
 449 1296.<https://doi.org/10.1007/s10570-018-2105-8>
 450 Jalali MR, Sobati MA (2017) Intensification of oxidative desulfurization of gas oil by ultrasound
 451 irradiation: Optimization using Box–Behnken design (BBD). *Applied Thermal Engineering*.
 452 111:1158-1170.<https://doi.org/10.1016/j.applthermaleng.2016.10.015>
 453 Jia H, Hongli, Zhu, Yuchen, & Chen, et al. (2013) Highly transparent and flexible nanopaper
 454 transistors. *ACS Nano*. 7(3):2106-2113.<https://doi.org/10.1021/nn304407r>
 455 Konkol NR, McNamara CJ, Hellman E, Mitchell R (2012) Early detection of fungal biomass on
 456 library materials. *Journal of Cultural Heritage*. 13:115-
 457 119.<https://doi.org/10.1016/j.culher.2010.05.002>
 458 Kukhareenko O et al. (2014) Promising low cost antimicrobial composite material based on bacterial
 459 cellulose and polyhexamethylene guanidine hydrochloride. *European Polymer Journal*.
 460 60:247-254.<https://doi.org/10.1016/j.eurpolymj.2014.09.014>
 461 Li J, Ye Y, Xiao H, He B, Qian L (2017) Microwave Assisted Preparation of Antimicrobial Chitosan
 462 with Guanidine Oligomers and Its Application in Hygiene Paper Products. *Polymers (Basel)*.
 463 9<https://doi.org/10.3390/polym9120633>
 464 Liu K, Xu Y, Lin X, Chen L, Huang L, Cao S, Li J (2014) Synergistic effects of guanidine-grafted
 465 CMC on enhancing antimicrobial activity and dry strength of paper. *Carbohydr Polym*.
 466 110:382-387.<https://doi.org/10.1016/j.carbpol.2014.03.086>
 467 Manetti F et al. (2009) Synthesis of new linear guanidines and macrocyclic amidinourea derivatives
 468 endowed with high antifungal activity against *Candida* spp. and *Aspergillus* spp. *J Med*
 469 *Chem*. 52:7376-7379.<https://doi.org/10.1021/jm900760k>
 470 Mathurin YK, Koffi-Nevry R, Guehi ST, Tano K, Oule MK (2012) Antimicrobial activities of
 471 polyhexamethylene guanidine hydrochloride-based disinfectant against fungi isolated from

cocoa beans and reference strains of bacteria. *J Food Prot.* 75:1167-1171.<https://doi.org/10.4315/0362-028X.JFP-11-361>

Oule MK et al. (2008) Polyhexamethylene guanidine hydrochloride-based disinfectant: a novel tool to fight meticillin-resistant *Staphylococcus aureus* and nosocomial infections. *J Med Microbiol.* 57:1523-1528.<https://doi.org/10.1099/jmm.0.2008/003350-0>

Peng Y, Duan C, Elias R, Lucia LA, Fu S (2018) A new protocol for efficient and high yield preparation of cellulose nanofibrils. *Cellulose.* 26:877-887.<https://doi.org/10.1007/s10570-018-2112-9>

Peydayesh M, Bagheri M, Mohammadi T, Bakhtiari O (2017) Fabrication optimization of polyethersulfone (PES)/polyvinylpyrrolidone (PVP) nanofiltration membranes using Box–Behnken response surface method. *RSC Advances.* 7:24995-25008.<https://doi.org/10.1039/c7ra03566g>

Piantanida G, Pinzari F, Montanari M, Bicchieri M, Coluzza C (2006) Atomic Force Microscopy Applied to the Study of Whatman Paper Surface Deteriorated by a Cellulolytic Filamentous Fungus. *Macromolecular Symposia.* 238:92-97.<https://doi.org/10.1002/masy.200650613>

Pinto F, Maillard JY, Denyer SP, McGeechan P (2010) Polyhexamethylene biguanide exposure leads to viral aggregation. *J Appl Microbiol.* 108:1880-1888.<https://doi.org/10.1111/j.1365-2672.2009.04596.x>

Piovesan C, Fabre-Francke I, Paris-Lacombe S, Dupont A-L, Fichet O (2018) Strengthening naturally and artificially aged paper using polyaminoalkylalkoxysilane copolymer networks. *Cellulose.* 25:6071-6082.<https://doi.org/10.1007/s10570-018-1955-4>

Ponce-Jimenez MDP, Toral, L. D. , & Fornue, E. D. (2003) Antifungal protection and sizing of paper with chitosan salts and cellulose ethers. part 1, physical effects. *Journal of the American Institute for Conservation.* 41(3):243.<https://doi.org/10.2307/3179921>

Qian L, Guan Y, He B, Xiao H (2008) Modified guanidine polymers: Synthesis and antimicrobial mechanism revealed by AFM. *Polymer.* 49:2471-2475.<https://doi.org/10.1016/j.polymer.2008.03.042>

Rabelo SC, Andrade RR, Maciel Filho R, Costa AC (2014) Alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis and fermentation of sugarcane bagasse to ethanol. *Fuel.* 136:349-357.<https://doi.org/10.1016/j.fuel.2014.07.033>

Salama A, Hasanin M, Hesemann P (2020) Synthesis and antimicrobial properties of new chitosan derivatives containing guanidinium groups. *Carbohydr Polym.* 241:116363.<https://doi.org/10.1016/j.carbpol.2020.116363>

Sarantopoulou E, Kollia Z, Gomoiu I (2006) Preventing biological activity of *Ulocladium* sp spores in artifacts using 157-nm laser. *Applied Physics A.* 83:663-668.<https://doi.org/10.1007/s00339-006-3554-8>

Simona Margutti GC, Paolo Calvini, Enrico Pedemonte (2001) Hydrolytic and oxidative degradation of paper. *Restaurator.* 22(2):67-83.<https://doi.org/10.1515/REST.2001.67>

Thakkar P, Passic, Zhu, Kholodovych, & Welsh, et al. (2009) Specific interactions between the viral coreceptor cxcr4 and the biguanide-based compound nb325 mediate inhibition of human

512 immunodeficiency virus type 1 infection. *ANTIMICROB AGENTS CHEMOTHER.* 53(2)(-
513):631-638.

514 Wang L (2006) Ancient Book Restoration—the Burden is Heavy and the Road Ahead is Long.
515 *Journal of Library and Information Sciences in Agriculture.* 08:89-
516 90.<https://doi.org/10.13998/j.cnki.issn1002-1248.2006.08.031>

517 Wang L, Guo H, Chen X, Chen Q, Wei X, Ding Y, Zhu B (2015) Optimization of the catalytic
518 hydrogenation of terebinth by a Ni-based catalyst. *Catalysis Science & Technology.* 5:3340-
519 3351.<https://doi.org/10.1039/c4cy01704h>

520 Wei D, Li Z, Wang H, Liu J, Xiao H, Zheng A, Guan Y (2017) Antimicrobial paper obtained by dip-
521 coating with modified guanidine-based particle aqueous dispersion. *Cellulose.* 24:3901-
522 3910.<https://doi.org/10.1007/s10570-017-1386-7>

523 Xiao G, Wang Y, Zhang H, Zhu Z, Fu S (2020) Dialdehyde cellulose nanocrystals act as multi-role for
524 the formation of ultra-fine gold nanoparticles with high efficiency. *Int J Biol Macromol.*
525 163:788-800.<https://doi.org/10.1016/j.ijbiomac.2020.07.057>

526 Yi X, Yan Z, Zhang M, Long K (2017) Study on the Consolidation of Aged Literature's Paper by
527 Nanocellulose. *Journal of Cellulose Science and Technology.*
528 25<https://doi.org/10.16561/j.cnki.xws.2017.03.05>

529 Yuri Kobayashi TS, & Akira Isogai (2015) Aerogels with 3d ordered nanofiber skeletons of liquid-
530 crystalline nanocellulose derivatives as tough and transparent insulators. *Angewandte Chemie*
531 *International Edition.* 53(39):10253-10253.<https://doi.org/10.1002/anie.201406635>

532 Zeng X, Fu, S. , Retulainen, E. , & Heinemann, S. (2012) Fibre deformations induced by different
533 mechanical treatments and their effect on zero-span strength. *Nordic Pulp and Paper Research*
534 *Journal.* 27(2):335-342.<https://doi.org/10.3183/NPPRJ-2012-27-02-p335-342>

535 Zhang M (2020) Research of Paper Documents Reinforcement Technology at Home and Abroad.
536 *China Pulp and Paper.* v.39:76-82.<https://doi.org/10.11980/j.issn.0254-508X.2020.03.011>

537 Zhang W et al. (2016) Paper reinforced with regenerated cellulose: a sustainable and fascinating
538 material with good mechanical performance, barrier properties and shape retention in water.
539 *Journal of Materials Chemistry A.* 4:17483-17490.<https://doi.org/10.1039/c6ta07681e>

540 Zhang Y, Jiang, J. , & Chen, Y. (1999) Synthesis and antimicrobial activity of polymeric guanidine and
541 biguanidine salts. . *Polymer.* 40(22):6189-6198.[https://doi.org/10.1016/S0032-3861\(98\)00828-3](https://doi.org/10.1016/S0032-3861(98)00828-3)

542

543 Zhong LX, Peng XW, Yang D, Cao XF, Sun RC (2013) Long-chain anhydride modification: a new
544 strategy for preparing xylan films. *J Agric Food Chem.* 61:655-
545 661.<https://doi.org/10.1021/jf304818f>

546

Figures

Figure 1

Evaluation of RSM correlation for the paper’s tearing index ($\text{mN}\cdot\text{m}^2/\text{g}$). (a) The normal probability distribution of residuals, (b) Distribution of residual versus predicted values, and (c) Chart of predicted value and actual value

Figure 2

The interaction between the concentration of CNCS and the density of PHMG on the enhancement of tearing strength of paper samples

Figure 3

Tensile force vs. percentage tensile strain for treated and untreated paper

Figure 4

(a) SEM images of these paper samples treated (T) and untreated (UT) (b) SEM-EDS images of the paper sample treated by CNCS/PHMG

Figure 5

XRD images of treated (T) and untreated (UT) paper samples

Figure 6

The comparison of treated and untreated paper samples after aging on folding endurance (A); tearing index(B) and zero-span tensile strength(C); Enhanced ratio comparison: folding endurance (a), tearing strength(b) and zero-distance tensile strength(c)

Figure 7

The brightness of treated and untreated paper samples after aging

Figure 8

The 7-days anti-*Aspergillus Niger* effect of paper samples:(1) A UT (untreated) (2) B T (treated)

Figure 9

The 28-days anti- mixed mold effect of paper samples:(1) A untreated (2)B treated, and the surface photo of the paper observed by polarized light microscope scale bar=20μm

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