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Nanomaterials for the cleaning and pH adjustment of vegetable-tanned leather

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Abstract Leather artifacts in historical collections and archives are often contaminated by physical changes such as soiling, which alter their appearance and readability, and by chemical changes which occur on aging and give rise to excessive proportion of acids that promote hydrolysis of collagen, eventually leading to gelatinization and loss of mechanical properties. However, both cleaning and pH adjustment of vegetable-tanned leather pose a great challenge for conservators, owing to the sensitivity of these materials to the action of solvents, especially water-based formulations and alkaline chemicals. In this study, the cleaning of historical leather samples was optimized by confining an oil-in-water nanostructured fluid in a highly retentive chemical hydrogel, which allows the controlled release of the cleaning fluid on sensitive surfaces. The chemical gel exhibits optimal viscoelasticity, which facilitates its removal after the application without leaving residues on the object. Nanoparticles of calcium hydroxide and lactate, dispersed in 2-propanol, were used to adjust the pH up to the natural value of leather, preventing too high alkalinity which causes swelling of fibers and denaturation of the collagen. The treated samples were characterized using scanning electron microscopy, controlled environment dynamic mechanical analysis, and infrared spectroscopy. The analytical assessment validated the use of tools derived from colloid and materials science for the preservation of collagen-based artifacts.

1 Introduction

Leather has been used by mankind since ancient times, yet the preservation of this collagen-based material represents a challenge for conservators worldwide [1, 2]. Leather objects are made from mammalian skin and hide, which are then transformed into a material that is resistant to mechanical abrasion, microbial attack, and heat. This is achieved through the tanning process, where either vegetable tannins or salts of metals (e.g., chromium, aluminum) are commonly applied to improve the stability of collagen. In particular, tannins have been used since Neolithic time to retard decay, and vegetable-tanned leather was indeed one of the most important materials in Western and Mediterranean Europe until the end of the nineteenth century, when chromium mineral tanning gradually came into use [3, 4]. Tannins form a complex with collagen, mainly through hydrogen bonds between free amino side groups of the collagen protein and hydroxyl groups from the polyphenolic tannin molecules, as well as through hydrophobic bonds [5]. Moreover, covalent bonds can be formed via quinoid structures between the protein and aromatic carbon in the molecules of condensed tannins, i.e., polymeric flavonoids consisting of flavan-3-ol (catechin) unit.

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Leather artifacts in historical collections and archives are often contaminated by soiling, salts, biocontamination, or coatings that alter their appearance and readability. Moreover, degradation is promoted by the presence of acidic compounds, coming either from manufacturing processes (tannery) or from the absorption of air pollutants such as sulfur and nitrogen oxides. Acidity increases the rate of hydrolysis of bonds within the collagen structure, reducing the polymer's structural integrity and eventually turning collagen into a gelatin colloidal solution. The tanning agents break down in turn under oxidative and acid hydrolytic conditions, forming products that can promote the degradation of collagen [1, 6].

The traditional materials and methods used for the cleaning and pH adjustment of collagen-based works of art involve risks to the artifacts, owing to the sensitivity of both collagen and the collagen–tannin complex to the action of solvents, water-based formulations, and alkaline chemicals. In fact, traditional moist-/water-based cleaning treatments of leathers that exhibit low pH (<3) and shrinkage temperature ($T_s < 35$ °C) may cause shrinkage or gelatinization of the collagen. Likewise, both pre-gelatinized and gelatinized collagen fibers are very sensitive to water even at room temperature [7]. Organic solvents are less likely to cause detanning of leather, although their use can involve toxicity issues and the risk to dehydrate or degrease the leather, jeopardizing its structure and properties.

Alternatively, oil-in-water (o/w) microemulsions are systems where low quantities of organic solvents are dispersed as stable nanosized droplets in a continuous aqueous phase. These systems exhibit high efficacy in removing layers of grime and aged coatings (varnishes, adhesives, etc.), and their low solvent content reduces the environmental impact. In fact, throughout the last decade, nanostructured fluids have been widely tested for the cleaning of mural paintings, and the results obtained have demonstrated that these systems are a valid alternative to the use of neat solvents [8–11]. However, for their use on water-sensitive substrates, o/w microemulsions must be confined within a retentive network that allows their controlled release, so as to grant the removal of unwanted layers without excessive wetting of the surface. For this purpose, o/w microemulsions have been recently used where they have been loaded in chemical hydrogels for the selective removal of adhesives from canvas paintings [12]. In particular, chemical gels formed by semi-interpenetrating (IPN) poly(2-hydroxyethyl methacrylate) (pHEMA) and polyvinylpyrrolidone (PVP) have emerged as advantageous systems for cleaning works of art, because they exhibit high retentiveness and optimal mechanical properties that allow their feasible application and their removal without leaving residues [12, 13].

Based on these results, we decided to test and report here the use of semi-IPN pHEMA/PVP gels loaded with an o/w nanostructured fluid for the cleaning of collagen-based artifacts.

As a matter of fact, the removal of dirt, salts, surface coatings, or patinas is also important because it facilitates the access to collagen fibers by any treatment applied successively to the artifact, such as the application of chemicals for the pH control of collagen.

Traditional methodologies for pH adjustment show several limitations, and their effectiveness on substrates such as vegetable-tanned leather has been debated. Alkaline aqueous solutions are risky owing to the aforementioned sensitiveness of collagen to water. Moreover, any excess alkalinity in the form of free, highly mobile hydroxide ions could damage either the collagen fibers [14] or the collagen–tannin complex in leather. On the other hand, salts of lactate and citrate have been shown to provide some protection against acidic degradation of leather during both natural aging in heavily polluted environments and strong accelerated aging, and it was found that treatment of new leathers with buffer salts can be effective without causing blooming [15]. It must be noticed that the pH of aqueous solutions of calcium lactate can range ca. from 6 to 8, which is milder and less risky to collagen than to strong alkalis. Moreover, lactic acid was used in the past for leather decalcination also because it favors the penetration of tannins [16].

Based on these considerations, in the present study we opted for the application of calcium lactate in the form of nanoparticles dispersed in a nonaqueous medium, for pH adjustment. The use of nanoparticles for the deacidification of works of art has been explored in the last decade, and alkaline earth hydroxides dispersed in short-chain alcohols have been used on cellulose-based artworks such as inked paper, canvas, and waterlogged archaeological wood. The particles grant the safe neutralization of acids and leave mild alkaline buffers against recurring acidity [17–20]. For instance, calcium hydroxide nanoparticles have been used to stabilize the pH of paper and canvas around 7–8, which is an optimal value for cellulose-based artifacts. However, in the case of collagen substrates the pH needs to be adjusted to lower values. Therefore, dispersions of calcium lactate nanoparticles in 2-propanol were prepared here for the first time and used either as pure or as mixed with calcium hydroxide nanoparticles to obtain a milder alkaline system. In fact, the goal was to tune the treatment so as to reach a final pH of ca. 4.5, which is the "natural" acidic value of leather objects in stable conditions, avoiding too high pH values such as 7 or more. The nanoparticles dispersions were characterized using scanning electron microscopy (FE-SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), dynamic light

scattering (DLS), and turbidimetry measurements through UV–Visible spectroscopy.

The hydrogel, o/w nanostructured fluid, and nanoparticles dispersions were all tested on both modern and historical leather samples.

In order to monitor the effect of the cleaning and pH adjustment treatments on the artifacts, a set of analytical techniques was used, including attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), pH measurements, SEM, and controlled environment dynamic mechanical analysis (DMA-RH).

2 Experimental details

2.1 Chemicals

Lactic acid (90 %, Sigma-Aldrich, impurities of residual water), ethanol absolute (99.9 %, Fluka), 2-propanol (≥99.8 %, Sigma-Aldrich), and metal granular calcium (99 %, Aldrich) were used for the syntheses of nanoparticles and the preparation of nanoparticles dispersions. Sodium dodecyl sulfate (SDS) (≥99 %, Sigma-Aldrich), 1-pentanol (≥98.5 %, Merck), ethyl acetate (ACS reagents, ≥99.5 %, Sigma-Aldrich), and propylene carbonate (99 %, Sigma-Aldrich) were used for the preparation of the EAPC o/w nanostructured fluid. 2-Hydroxyethyl methacrylate (HEMA) (97 %, Sigma-Aldrich), polyvinylpyrrolidone (PVP) (average Mw ≈ 1300 kDa, Sigma-Aldrich), α,α′-azoisobutyronitrile (AIBN) (98 %, Fluka) and *N*,*N*-methylene-bis(acrylamide) (MBA) (99 %, Fluka) were used for the synthesis of the pHEMA/PVP hydrogels. Water was purified by a Millipore MilliRO-6 Milli-Q gradient system (resistivity >18 MΩ cm).

2.2 Preparation and characterization of nanoparticles dispersions, o/w nanostructured fluid, and hydrogel

Calcium hydroxide nanoparticles were synthesized via a solvothermal process as reported in the literature [20]. This synthetic method lets us obtain colloidal calcium hydroxide already dispersed in ethanol.

Calcium lactate nanoparticles were synthesized starting from the aforementioned $Ca(OH)_2$ nanoparticles dispersions. Four milliliters of lactic acid was gradually added to the $Ca(OH)_2$ dispersion (500 mL, 10 g/L) and magnetically stirred in a flask at 25 °C for 1 h. The obtained slurry was sonicated and centrifuged, and then dried in oven at 110 °C for 72 h, under a N_2 atmosphere. The obtained powders were finally suspended in 2-propanol (4 g/L). The choice of 2-propanol as a dispersing medium for the nanoparticles is due to the fact that this solvent might be less prone to induce alterations of collagen as compared to ethanol because of the lower polarity, and some alteration of the leather surface was observed during spot tests using ethanol as opposed to 2-propanol.

The mixed calcium hydroxide–calcium lactate dispersions were prepared by dispersing $Ca(OH)_2$ powders (obtained by drying the $Ca(OH)_2$ nanoparticles dispersion in oven) in 2-propanol and mixing them with a calcium lactate nanoparticles dispersion in the same solvent. For practical applications, the hydroxide/lactate ratio was set to 2:1 (w/w, 2 g/L calcium hydroxide, 1 g/L calcium lactate).

SEM analysis on dried nanoparticles dispersions were carried out with a FEG-SEM ΣIGMA (Carl Zeiss, Germany), using an acceleration potential of 5 kV and a working distance of 5.3 mm.

FTIR analysis on the dried dispersions was carried out using a BIORAD FTS 40-PC spectrometer with a resolution of 4 cm^{-1} and 32 scans. The spectral range was 4000–400 cm^{-1}. Pellets were prepared by finely grinding and mixing the powders with KBr.

XRD on the dried dispersions was carried out using a XRD Bruker New D8 Da Vinci. The source is a Cu tube ($\lambda = 1.5418$ Å, 40 kV, 40 mA). Data were collected from 5° to 50° 2θ with a step size of 0.03° and a count time of 38.4 s. The XRD spectrum was analyzed using the database ICCD PDF-2.

Dynamic light scattering (DLS) measurements were taken directly on the nanoparticles dispersions with a 90Plus Particle Size Analyzer (Brookhaven Instrument Corporation). The light scattered from the sample was collected at 90° with the incident 659-nm laser light radiation. The measurements were taken at 25 °C. Particle size distributions were obtained from the fitting of the measured autocorrelation functions using the NNLS (nonnegatively constrained least squares) method [21].

Turbidimetry measurements were taken directly on the nanoparticles dispersions to evaluate their stability, using a Varian Cary 100 Bio Spectrophotometer, equipped with a Peltier Multi-block. The absorbance of the dispersion at 600 nm was measured as a function of time. Absorbance was assumed proportional to the system turbidity: The decrease in absorbance over time is due to particles aggregation and settling. Measurements were taken at 25 °C, using sealed quartz cuvettes with an optical path of 1 cm.

The EAPC o/w nanostructured fluid was prepared as reported in the literature [22]. The fluid is named EAPC after the two solvents contained in the system, i.e., ethyl acetate (EA) and propylene carbonate (PC).

The pHEMA/PVP gel was synthesized by free radical polymerization of HEMA monomer and the crosslinker MBA in a water solution containing linear PVP, as reported in the literature [13]. Hydrogel synthesis was carried out in

molds to obtain flat hydrogel films having 2 mm thickness. After polymerization, hydrogels were washed and placed in containers filled with distilled water. The water was renewed twice a day for 7 days to remove any residue of unreacted monomer and the free PVP. Then, the gels were loaded with the EAPC o/w fluid through immersion for 12 h before application on the artifacts.

2.3 Applicative tests on modern and historical samples

The gel, nanostructured cleaning fluid, and nanoparticles dispersions were tested on both modern and historical leather samples. The modern sample (M1) was a vegetable-tanned (sumac) leather made from calfskin. Leaves from sumac shrub (*Rhus coriaria*) were among the most important vegetable source for tanning leather [1]. The sample was tested as received. The first historical sample (H1) was the cover of a Luther Bible (1749 AD). The second historical sample (H2) was the cover of a *Missale Romanum* (Roman Missal, 1725 AD). The historical samples exhibited surface deposits of dirt, salts, or waxy (or lipid) materials, which needed to be removed.

The cleaning tests were carried out by applying sheets of the pHEMA/PVP gel, loaded with the EAPC fluid, directly on the surface of the samples. Application time was 20 min. During application, the gel was covered with a foil of Mylar®, to prevent evaporation of the volatile components of EAPC. After the application, the gel was removed, and no further mechanical action was carried out on the sample surface. Then, a gel simply loaded with water was applied for 20 min on the cleaned area to remove residues from the nonvolatile components of EAPC (i.e., SDS surfactant). The presence of gel residues over the surface was checked with ATR-FTIR performed on the leather samples after the application and removal of the gels. A Thermo Nicolet Nexus 870 FTIR spectrometer equipped with a Golden Gate diamond ATR was used. The spectra were obtained from 128 scans with 4 cm^{-1} of optical resolution, in the 4000–650 cm^{-1} range. For each sample, five spectra (from five different spots) were collected.

For each leather sample, the nanoparticles dispersions were applied on several areas of ca. 2 × 2 cm^2, dripping 30 μL in 2–3 steps over each spot, with a Gilson P200 pipette.

Surface pH measurements on leather samples were taken in situ using a WTW pH 3310 SET 2 pH meter, equipped with a SenTix®41 electrode. Each measurement spot (1 × 1 cm^2) was damped with a droplet of water using a pipette. After 2-min extraction, the electrode was placed on the surface and the pH of the water film was measured. The pH value was obtained as the average between 2 measurements (being the leather surface highly water sensitive, it was not possible to carry out numerous measurements on each spot).

Surface examination of the leather samples before and after treatments was made using a FEG-SEM (Philips XL30 FEG-SEM–FEI, Eindhoven, Netherlands). For each leather sample, a small piece (0.5 mm^2) was cut and fixed, with the grain side up, on a standard SEM Al stub and gold–palladium sputter coated at 20 mA and 1.25 kV for a minute and half (Palaron E5000 sputter coater). The images were obtained at 5 keV acceleration voltage and a spot size of 3 μm at three different locations for each sample.

Controlled environment dynamic mechanical analysis (DMA-RH) was used to evaluate the mechanical properties of the leather samples under selected conditions of RH using the Triton 2000 DMA together with the Triton Technology humidity generator and controller. DMA-RH measures the variation in elastic (or storage) modulus E' and inelastic (or loss) modulus E'' and changes in viscoelasticity with changes in RH, as moisture is taken in and lost by the material at a programmed rate. The samples were measured in tension, and this also gave a measure of the sample displacement (D %) under a selected strain and relative humidity. All the analyses were carried out on samples from a well-defined and small area for each leather object (modern leather, historical leathers). The same sample was not analyzed by DMA-RH before and after treatment; however, the samples came from the same location in the hide, and all were measured in the head to tail direction. Samples (15 mm × 5 mm) were pre-dried for 24 h before measurement and then mounted in the tensile mode in the DMA analyzer. The sample chamber was set to 20 % RH, and the sample remained at 20 % RH for at least 30 min. The RH was then increased at 1 %/min until it reached 80 % RH. More than one sample was measured (from same location and in the head to tail direction), and variability in calculated slope (D %/RH) was ±5 %.

3 Results and discussion

3.1 Characterization of the nanomaterials

The calcium hydroxide nanoparticles synthesized via a solvothermal process and used for the tests are hexagonal platelets that exhibit a bimodal size distribution, with one population having a mean hydrodynamic diameter of ca. 80 nm, and the second population having a mean of ca. 220 nm. The thickness of particles is 20–30 nm. The platelets are highly crystalline, the crystalline form being portlandite [20].

Calcium lactate was synthesized in the form of irregular particles with an average dimension of 400–500 nm as

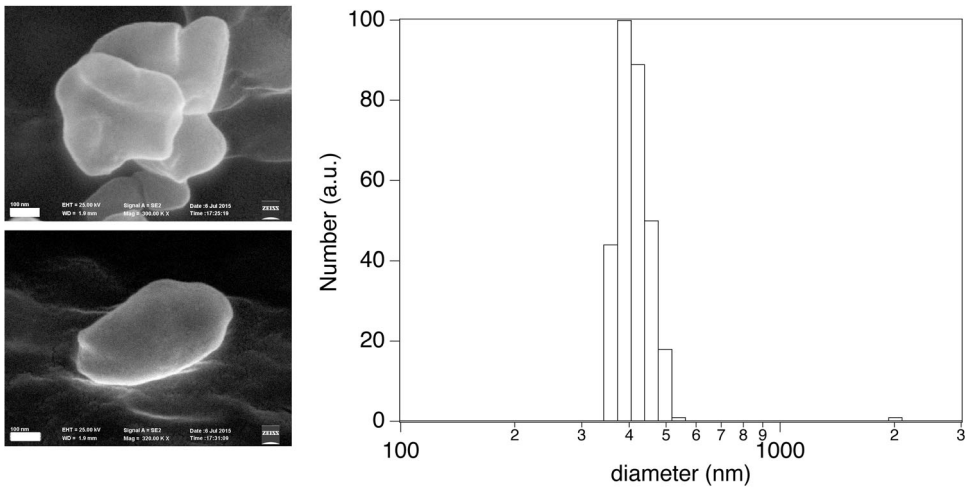

Fig. 1 *Left* SEM image of calcium lactate nanoparticles (dry powders). *Bar* is 100 nm. *Right* DLS analysis of a dispersion of calcium lactate nanoparticles in 2-propanol

shown by SEM analysis. The DLS analysis carried out on the particles dispersion in 2-propanol confirmed the presence of a main population with a size of 350–500 nm and a small portion of micrometric particles, consistently with SEM data (see Fig. 1). The FTIR spectrum of the dried dispersions shows all the characteristic peaks of calcium lactate: a broad band centered at 3360 cm^{-1} (νOH), and peaks at 2990, 2936, 2885 cm^{-1} ($\nu_s CH_3$, $\nu_{as} CH_3$, νCH), 1583 cm^{-1} ($\nu_{as} CO_2^-$), 1485, 1470, 1431 cm^{-1} ($\delta_{as} CH_3$, $\delta_{as} CH_3$, $\nu_s CO_2^-$), 1388, 1368, 1318 cm^{-1} (δ_{AL}OH, $\delta_s CH_3$, δC–H), 1270 cm^{-1} (δC–H), 1130 cm^{-1} ($\rho CH_3 + \nu_{AL}$C–O), 1090, 1047 cm^{-1} (ν_{AL}C–O), 930 cm^{-1} (rCH_3), 864 cm^{-1} (νC–CO_2^-), 778 cm^{-1} (δCO_2^-), 551 cm^{-1} (wCO_2^-), 434 cm^{-1} (rCO_2^-) [23, 24]. The band at 667 cm^{-1} is assigned to the Ca–O mode. The peak at 3642 cm^{-1} is characteristic of calcium hydroxide (νOH). Consistently, the XRD spectrum shows the peaks of crystalline $Ca(OH)_2$ (*portlandite*), as well as broad bands that indicate the presence of amorphous domains (see Fig. 2). Therefore, we hypothesize that the preparation method led to the formation of particles with a crystalline core of calcium hydroxide, and a shell consisting of amorphous calcium lactate. Turbidimetry measurements showed that the absorbance of the nanoparticles dispersion at 600 nm did not decrease significantly in the first 2 h after preparation. Moreover, no significant particles aggregation was observed even after days following the preparation of the dispersion, with only minimal aggregation that was reversible upon slight mechanical agitation.

Mixing the calcium hydroxide and calcium lactate nanoparticles dispersions (see Sect. 2.2) did not hinder their stability.

The o/w nanostructured fluid EAPC has been fully characterized in previous studies. The system's water content is ca. 73 %, the rest being solvents (ethyl acetate, propylene carbonate), surfactant SDS, and 1-pentanol (as a co-surfactant). A small-angle neutron scattering study recently detailed the structure of this system, showing that EAPC is neither a "classical" microemulsion (i.e., where the solvents are only found as confined in nanosized droplets dispersed in the aqueous phase) nor a simple micellar solution. In EAPC, both ethyl acetate and propylene carbonate are partitioned between the continuous phase (water) and the dispersed droplets, which also accounts for the

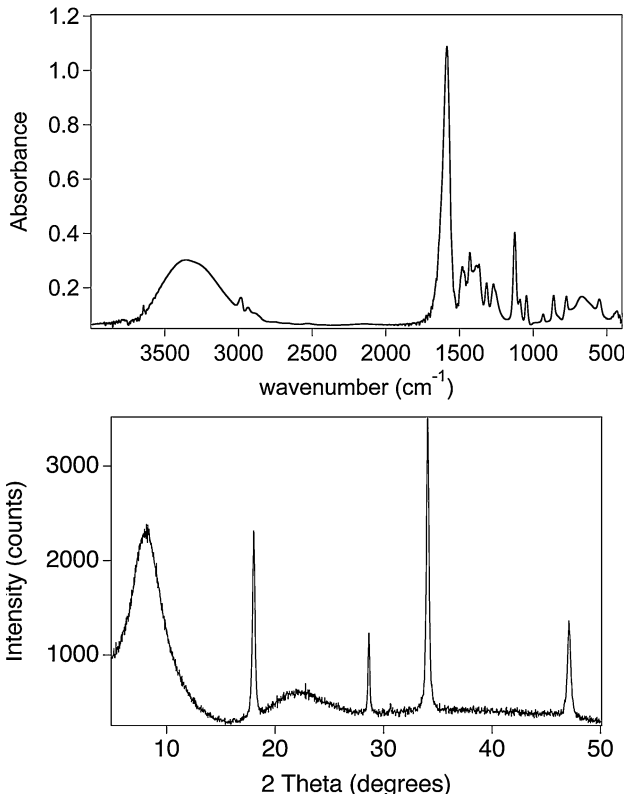

Fig. 2 FTIR spectrum (*top*) and XRD pattern (*bottom*) of calcium lactate nanoparticles (dry powders)

versatility and the effectiveness of this fluid in removing different types of coatings from the surface of works of art [22].

Three formulations of semi-IPN pHEMA/PVP chemical hydrogels have also been characterized in the literature [12, 13]. The formulations differ in the monomer/crosslinker and pHEMA/PVP ratios, which result in different porosities (both macro-porosity and micro-porosity), equilibrium water content, and water release rate. The formulation selected for this study ("H58") represents a balance that was deemed optimal for application on collagen-based artifacts. The formulation with the highest release rate could be risky in that it might cause excessive wetting, which is detrimental to collagen. The formulation with the lowest release rate was not selected as it might prove too slow in removing hydrophilic surface dirt or other unwanted layers.

3.2 Application of the nanomaterials to leather

Sample M1 (see Fig. 3) is vegetable-tanned modern leather that was tested exclusively to investigate the effects of the treatment with the H58 gel (loaded with EAPC) and with dispersions of alkaline nanoparticles in 2-propanol on collagen in pristine conditions, i.e., one that did not undergo natural aging. The gel was applied on the sample grain side, i.e., the external side normally exposed to aging and pollution. Then, the mixed calcium hydroxide–calcium lactate nanoparticles dispersion was applied by dripping. The FEG-SEM analysis of the sample shows that the nanoparticles are homogeneously distributed on the leather surface following the treatment (see Fig. 4).

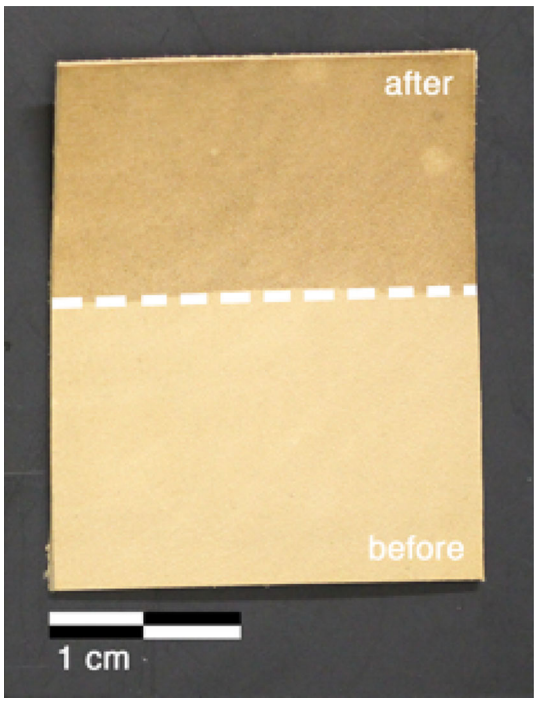

Fig. 3 M1 sample (modern leather, vegetable tanned, 10 cm × 15 cm) before (*bottom*) and after (*top*) treatment with a H58 gel (loaded with EAPC) and a mixed calcium hydroxide–calcium lactate nanoparticles dispersion in 2-propanol

The applied quantity was finely tuned to adjust the pH from 3.6 up to 4.5, which is considered to be an optimal value for leather substrates. It must be noticed that when only calcium lactate nanoparticles were used (i.e., not mixed with calcium hydroxide), significantly larger quantities of dispersion or highly concentrated dispersions were needed to reach the ideal pH values (ca. 4.5), which might not be feasible in practical applications, for instance due to the risk of forming white veils on the surface.

ATR-FTIR was carried out before and after the treatment with the H58 gel and the nanoparticles (see Fig. 5). The spectrum of the untreated sample shows the following bands, assigned either to collagen or to tannins: 3414 cm^{-1} (νOH tannins), 3279 cm^{-1} (νN–H), 3075 cm^{-1} (δNH_2 overtone), 2921, 2852 cm^{-1} ($\nu_{as}CH_3$, ν_sCH_3, ν_sCH_2), 1712 cm^{-1} (νC=O tannins), 1650 cm^{-1} (amide I), 1621 cm^{-1} (aromatic ring stretch vibrations, tannins), 1548 cm^{-1} (amide II), 1513 cm^{-1} (aromatic ring skeletal vibration, tannins), 1448 cm^{-1} (δCH_2, δCH_3, aromatic ring stretch vibrations, tannins), 1320 cm^{-1} (ν_sC–O, tannins), 1174 cm^{-1} (νC–O, tannins), 1112, 1077 cm^{-1} (ethereal C–O asymmetric stretching, tannins), 1030 cm^{-1} (νC–O, tannins) [25–28]. The bands at 923, 910, 867, and 755 cm^{-1} are also ascribed in the literature to the presence of tannins and might be assigned to aromatic C–H out-of-plane and C–H rocking vibrations. The bands at 2950 cm^{-1} (νCH) and the shoulder at 1736 cm^{-1} (νC=O) might be ascribed to the presence of waxy or lipid material, which can be found as a finish layer on leather.

In the spectrum of the treated sample, the band at 3642 cm^{-1} (νOH, $Ca(OH)_2$) confirms the presence of the alkaline nanoparticles. The increased intensity of bands at 3011 cm^{-1} (not clearly observable before treatment) and 1467 cm^{-1} (νC–H aromatics, tannins) [25] suggests that the treatment resulted in the partial removal of a surface finish coating originally laid over the tanned leather surface. This is also suggested by the discoloration (darkening) of the sample following the treatment (see Fig. 3). It must be noted that in this case the aesthetic alteration due to the finish removal is not of concern, as the model leather sample was treated exclusively to check the compatibility between the applied nanomaterials and the collagen, as verified through FTIR and DMA-RH (see below).

The amide II band is hidden by two peaks at 1577 ($\nu_{as}CO_2^-$, calcium lactate) and 1542 cm^{-1} (ν_{as}COO carboxylate), which can be ascribed to the presence of calcium lactate nanoparticles and of carboxylate groups, the latter

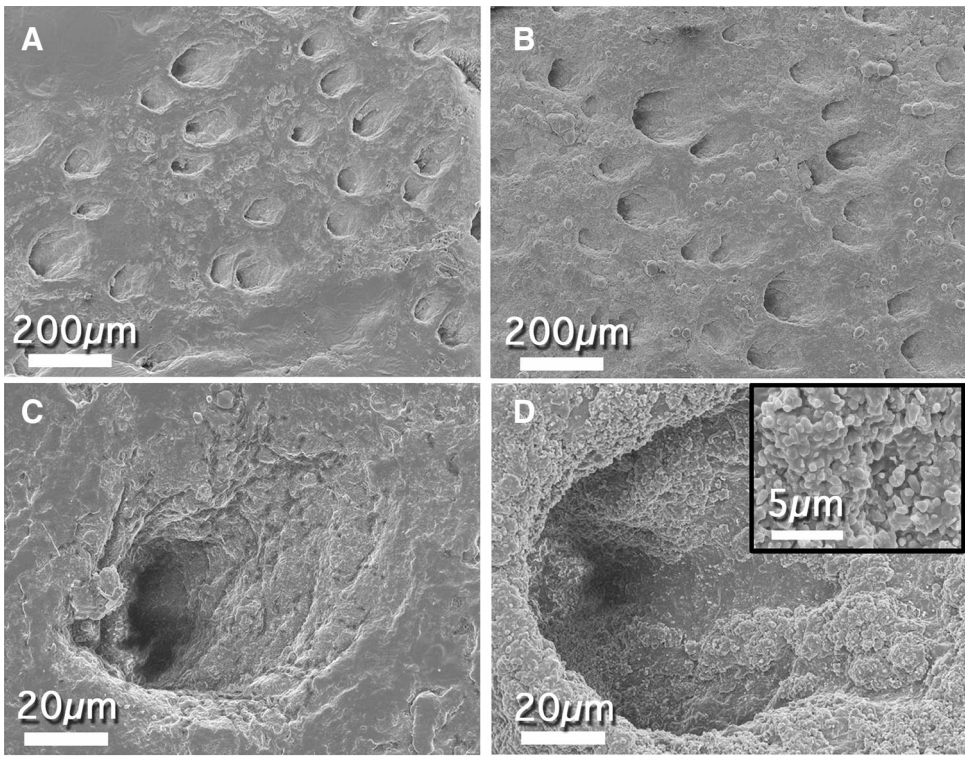

Fig. 4 FEG-SEM images of the M1 sample (modern leather, vegetable tanned). **a**, **c** Before treatment with nanomaterials. **b**, **d** After treatment with a H58 gel (loaded with EAPC) and a mixed calcium hydroxide–calcium lactate nanoparticles dispersion in 2-propanol. The *inlet* in panel **d** shows a detail of the nanoparticles distributed on the leather surface

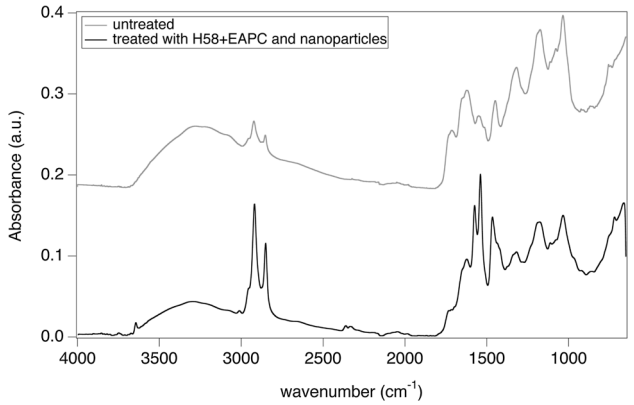

Fig. 5 ATR-FTIR spectrum of the M1 leather sample. *Top* untreated sample. *Bottom* sample treated with a H58 gel (loaded with EAPC) and then with a mixed dispersion of calcium lactate–calcium hydroxide nanoparticles in 2-propanol

possibly formed following the partial neutralization of acids present in the tannins.

No bands ascribable to SDS (1084 cm^{-1}, $\nu_s SO_2$) or to the H58 gel (1724 cm^{-1}, νC=O of HEMA) are observable in the spectrum, confirming that the application of the gel loaded with EAPC did not leave any residue (as detectable via FTIR) on the surface of the sample.

DMA-RH analysis was used to obtain the rate of sample displacement (*D* %) with RH, a useful damage marker for collagen-containing materials. The procedure was

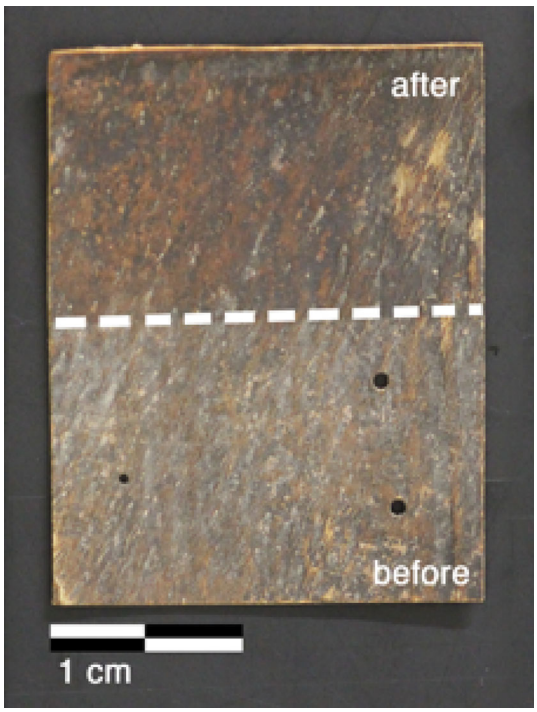

Fig. 6 H1 sample (Luther Bible cover, 1749 AD) before (*bottom*) and after (*top*) treatment with the pHEMA/PVP H58 gel (loaded with EAPC) and a mixed calcium hydroxide–calcium lactate nanoparticles dispersion in 2-propanol. The area on the *bottom* shows the dull grayish surface patina of dirt, salts, and pollutants. The area on the *top* shows the removal of the patina to reveal the underlying heterogeneous leather surface

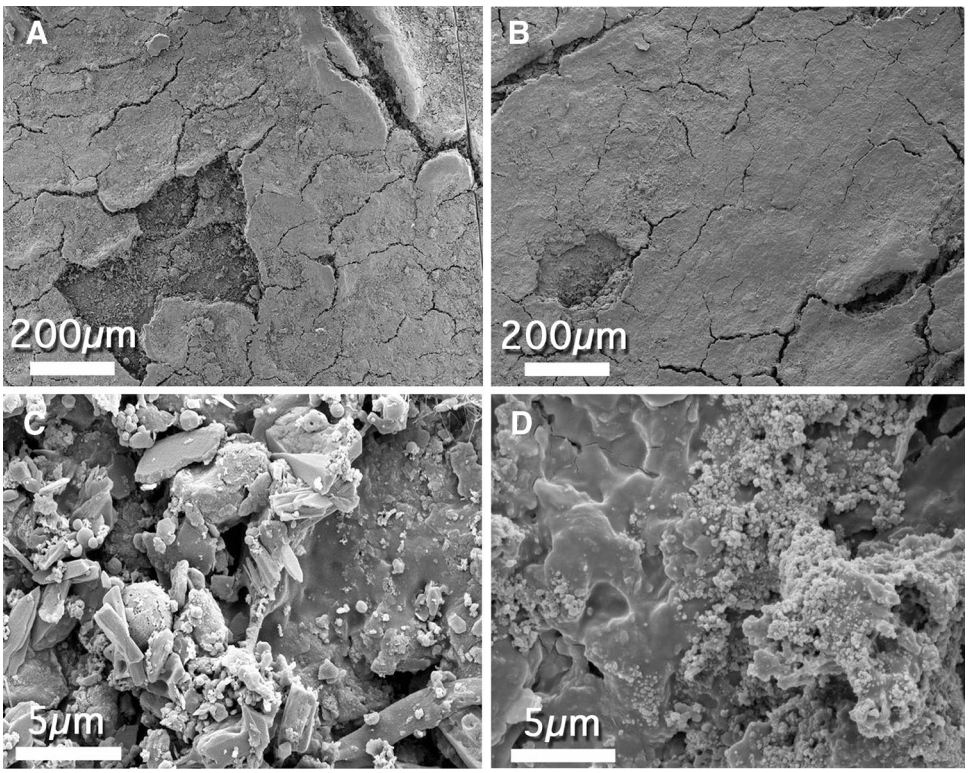

Fig. 7 H1 sample (Luther Bible cover, 1749 AD). **a**, **c** Before treatment. Panel **c** details the presence of salts, dirt deposits, and signs of biological contamination on the surface. **b**, **d** Images of the sample after treatment with the pHEMA/PVP H58 gel (loaded with EAPC) and a mixed calcium hydroxide–calcium lactate nanoparticles dispersion in 2-propanol. Panel **d** shows the reduction of salts, dirt, and biological contamination after the treatment, as well as the presence of nanoparticles on the surface

originally developed in the IDAP project for damage assessment of collagen in collagen-containing parchment samples [29]. There it was demonstrated that samples that underwent natural or accelerated aging exhibited a lower rate of displacement (expansion) than undamaged samples [29]. Elsewhere, a correlation has been demonstrated between the shrinkage temperature (T_s) and D % for thermally aged parchment samples. Lower T_s values and therefore lower D % values indicate damage to collagen [30]. Reduction in D % values was also correlated with

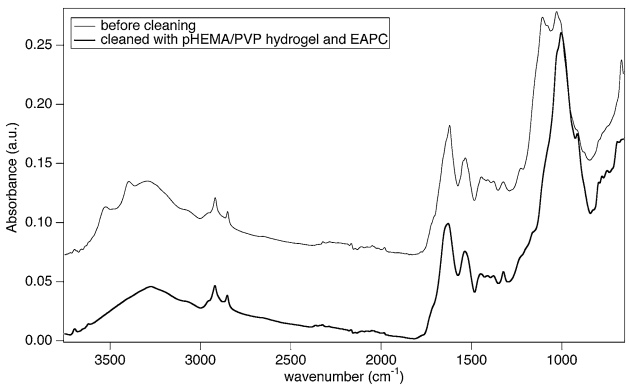

Fig. 8 ATR-FTIR spectrum of H1 sample (Luther Bible cover, 1749 AD). *Top* untreated sample. *Bottom* sample treated with a pHEMA/PVP H58 gel (loaded with EAPC)

Fig. 9 H2 sample (Roman Missal cover, 1725 AD) before (*bottom*) and after (*top*) treatment with the pHEMA/PVP H58 gel (loaded with EAPC) and a mixed calcium hydroxide–calcium lactate nanoparticles dispersion in 2-propanol. The area on the *bottom* shows the presence of surface dirt. The area on the *top* shows the removal of the *white–gray* surface patina of dirt enhancing the underlying leather surface

reduction in extent of coverage of sample with intact *D*-banding (as observed from AFM studies), which indicates loss of structure in the collagen fibrils [30].

DMA-RH data obtained on the M1 sample showed that there were no significant changes in the rate of displacement, before and after the treatment with gel and nanoparticles. Where minor changes were observed, they occurred in a direction opposite to that which typically occurs in aged samples.

The H1 sample (Luther Bible cover, 1749 AD) exhibited a grayish surface patina of gypsum, biological contaminants (spores and hyphae), and dirt deposits, which was removed using the H58 gel loaded with EAPC (see Figs. 6, 7). The presence of waxy or lipid surface material cannot be excluded, even though it was not possible to detect it clearly through FTIR.

In fact, using the gel simply loaded with water did not allow the effective removal of the patina. It is also possible to load the gel with ethanol or with ethanol/water mixtures [12]; however, cleaning tests carried out in the present work showed that the use of ethanol is detrimental to the leather surface, producing small cracks across the treated area.

The patina was removed applying the gel loaded with the EAPC fluid, with no additional mechanical action required. After the removal of the patina, the subsequent application of the mixed calcium lactate–calcium hydroxide nanoparticles dispersion adjusted pH from 4.0 to 4.6. It is important to notice that the surface patina must be removed prior to application of the nanoparticles dispersion to favor the penetration of particles and avoid the formation of white veils.

The ATR-FTIR spectrum of the sample before treatment (Fig. 8) clearly shows the absorption bands of gypsum at 3525, 3398 cm^{-1} (νOH), 1619 cm^{-1} (δOH), 1111 cm^{-1} (asymmetric stretching vibrations of SO_4^{2-}), and 669 cm^{-1} (symmetric bending of SO_4^{2-}) [31, 32]. These absorptions are no longer observable in the spectrum of the sample after treatment with the gel, while the bands of collagen and tannins can be clearly observed, which confirmed the effective removal of the surface patina. Also in this case, no bands ascribable to SDS (1084 cm^{-1}, $\nu_s SO_2$; 1227 cm^{-1} $\nu_{as} SO_2$; 1471 cm^{-1}, δCH_2) or to the H58 gel are observable in the spectrum.

The DMA-RH analysis on sample H1 showed that the rate of displacement (*D* %) versus RH is the same for the untreated sample and the sample treated with the gel and the nanoparticles, indicating that there is no further damage to the collagen resulting from the treatment.

Sample H2 (Roman Missal cover, 1725 AD) exhibits a white–gray surface patina of dirt, which was removed using the H58 gel loaded with EAPC (see Fig. 9). Also in this case, the use of the gel simply loaded with water was not effective, and the use of ethanol or ethanol/water mixtures proved detrimental to the leather surface. The FEG-SEM analysis showed a smoother surface following

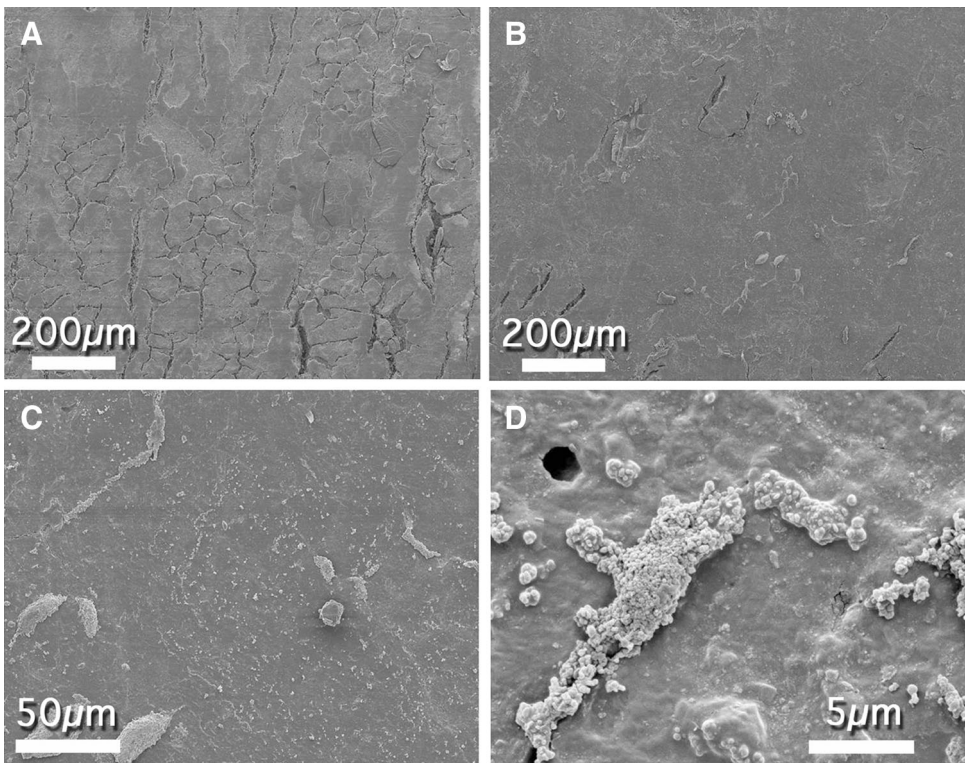

Fig. 10 FEG-SEM images of H2 sample (Roman Missal cover, 1725 AD). **a** Before treatment. **b–d** After treatment with the pHEMA/PVP H58 gel (loaded with EAPC) and a mixed calcium hydroxide–calcium lactate nanoparticles dispersion in 2-propanol. Panel **c** and **d** detail the presence of the particles on the surface

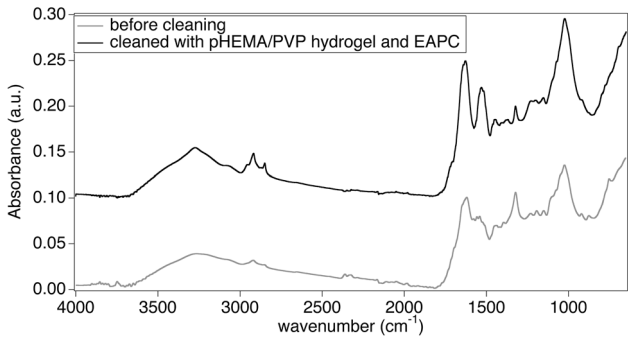

Fig. 11 ATR-FTIR spectrum of leather sample H2 (Roman Missal cover, 1725 AD). *Top* untreated sample. *Bottom* sample treated with a pHEMA/PVP H58 gel (loaded with EAPC)

the treatment, where dirt deposits are reduced and nanoparticles are distributed on the surface, in some cases also forming clusters (see Fig. 10).

In the ATR-FTIR spectrum of the sample after treatment with the H58 gel (Fig. 11), the increased intensity of bands at 2921 and 2852 cm^{-1} ($\nu_{as}CH_3$, ν_sCH_3, ν_sCH_2), and peaks at 1650 cm^{-1} (amide I), 1530 cm^{-1} (amide II), and 1029 cm^{-1} (νC–O, tannins) confirmed the removal of the surface patina that covered the original tanned leather surface.

After the removal of the patina, the subsequent application of the mixed calcium lactate–calcium hydroxide nanoparticles dispersion adjusted pH from 4.1 to 4.7. The removal of the surface patina favored the penetration of the particles, avoiding the formation of white veils.

DMA-RH data for sample H2 showed an increase in the measured rate of displacement over the RH range (40–60 %). The increase in the *D* % value for the treated samples as compared to the untreated one indicates an improvement in the mechanical properties and confirms that the treatment did not cause damage to the collagen.

4 Conclusions

Chemical hydrogels loaded with an o/w nanostructured cleaning fluid were used for the first time to remove surface patinas of dirt and salts (possibly including waxy or lipid material) from the surface of historical leather objects. The use of gels allows the controlled release of water-based cleaning fluids, avoiding damage to the collagen.

After the removal of the unwanted surface layers, it is possible to apply dispersions of alkaline nanoparticles (calcium lactate mixed with calcium hydroxide) to adjust the surface pH of leather to its typical value of ca. 4.5, avoiding excessive alkalinity that might be detrimental to collagen and the collagen–tannin complex.

Results from DMA-RH show that for the treated samples there is no decrease in the *D* %/RH parameter, indicating that there is no negative effect of the treatment on the state of the collagen.

In some cases, the increase in the *D* %/RH value shows improvement in the mechanical performance of the leather samples, as the frictional resistance to the movement of the fibers with moisture uptake is reduced.

The obtained results may open new perspectives in the conservation of collagen-based artifacts.

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