IL NUOVO CIMENTO **44 C** (2021) 22
DOI 10.1393/ncc/i2021-21022-9

COMMUNICATIONS: SIF Congress 2020

Nanoscale characterization of the fibrillar networking in collagen-based cultural heritage artefacts

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received 1 February 2021

Summary. — Parchment and leather artefacts are frequently involved in biodeterioration processes. Ionizing radiations represent a valid alternative to the chemical biocide normally employed to stop these deterioration processes. In this work, the use of the X-rays irradiation has been tested to evaluate its possible application to inhibit the microbial growth on parchment and leather samples. Two microscopic techniques, namely SEM and AFM, have been used to obtain information on the effects eventually induced by the treatment on the collagen matrix, helping to identify the threshold dose for which the treatment results effective and safe for parchment and leather cultural heritage objects.

Introduction

Collagen-based materials are frequently present in cultural heritage artefacts. In particular, parchment and leather have been employed for centuries for different uses such as writing supports, book covers, wallpapers and clothes.

Traditionally, as described by Pliny the Elder in his *Naturalis Historia* (Book XIII, 21), parchment was first produced during the Hellenistic period (323–31 B.C.) in *Pergamon*, Asia Minor. Besides the legend, the new material was invented to be used as a substitute of papyrus, whose monopoly of production was held by Alexandria in Egypt. More realistically, during the Late Antiquity, the Egyptian production of papyrus ceased making it progressively less available [1]. In the early Middle Ages, the use of parchment as a writing support took hold in particular for the transcription of the classics and the production of the codices of the Bible. More generally, following a widespread regression in the degree of literacy due to the particular political conditions, parchment became the ideal writing support for the European autarchic society and, more specifically, for a

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new kind of book, the *codex*, that was replacing the common form of *volumen* made with papyrus sheets [1-3]. Parchment was the only writing support employed in the western world at least until the invention of printing (XV century) and the diffusion of paper, a cheaper writing support more suitable to be printed [4].

With respect to parchment, leather has been used for centuries and for numerous applications in the history of human mankind. A very particular employment of leather is its use as wallpaper to enrich stately buildings. This application can be dated back to ancient times and still employed at least until the eighteenth century, when its use slowly declined being replaced by fabrics and then printed papers. A very impressive use of leather wallpaper is present in the rooms of *Palazzo Chigi* of Ariccia (Rome), an ancient residence of princes in the roman countryside. Several rooms in the building are enriched with sumptuously decorated corams of the seventeenth century. These leather panels are impressed with decorative motifs, gilded and silvered following the pattern of the so-called "cordovani", due to the manufacturing process imported from Cordova, Spain. The building constitutes a rarity in this genre also because the panels are still in their positions in the rooms [5].

Parchment and leather are both derived from animal skin like goat, sheep, cow and others. The fundamental difference lies in the manufacturing processes employed for their producion which characterize their peculiarities and, even more, their resistance to the denaturation processes they are involved in.

The peculiar manufacturing processes and, in particular, the drying process through tensioning, make the parchment different from leather or alum-tawed skin. The stretching of the pelt produced during the drying step induces a reorganization of the fibre network in the dermal layer (the only histological layer left after the treatments) giving a laminar structure in which the fibre network is permanently fixed [6-8].

Differently, for leather is the tanning process, part of the manufacturing step, that converts the protein of the raw skin into a stable and imputrescent material. Until the second half of the eighteen century, this process was made thanks to the so-called vegetable tanning. The tannins extracted from different kind of plants and vegetables are able to stop the putrefying process inducing the tanning in the pelt [9].

Like most of collagen-based materials, parchment and leather are frequently involved in bio-deterioration caused by microorganisms. In particular, fungi and bacteria cause structural and chromatic alterations inducing hydrolysis of the collagen fibres, modifying the parchment inorganic components, producing pigments and organic acids causing chromatic alteration or discolouration of the supports [10, 11] or inducing the partial detachments and delaminations of the surface layers [12, 13]. Such kind of produced damage is the result of different microbial species and, in particular, it depends on the enzymes they release, the simultaneous action of different species and their eventual succession on the parchment substrate [13-18].

For these reasons and from the ancient times, during the centuries different treatments have been employed to try to stop the infection and to preserve animal derived cultural heritage artefacts [19]. At present, different chemical treatments are employed as biocide, most of which result to be extremely toxic and unsafe for the operator and the artefact, as in the case of ethylene oxide fumigation [20, 21]. Aqueous treatments, like the ones based on benzalkonium chloride, cannot be used on parchment or leather because of their hygroscopic nature. The use of UV radiation, frequently employed for its fungicidal and bactericidal properties, is on the other hand, strictly banned in the field of cultural heritage. UV radiation results to be effective against fungi in their active growth phase (with a maximum efficiency in the range 230–275 nm), but it induces the

depolymerization of the cellulosic and peptide chains accelerating the organic material ageing processes [22].

Alternatively, the employment of γ ionizing radiation is being investigated for the decontamination of library and archival materials damaged by micro and macro organisms and, in particular, in the disinfection of paper [23-27]. Recent studies also concern the investigation of the possible damage induced by disinfection by irradiation on the collagen structure [28-30].

In this work, the results obtained by a new irradiation approach, based on the employment of X-ray radiation to be used for parchment and leather disinfection, are presented [31]. The effects caused by exposition to increasing X-ray radiation doses on parchment and leather substrates have been investigated by means of two different microscopic techniques: Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM). In particular, in the case of parchment, the combination of these two techniques enables the investigation of both morphological and structural changes related to different doses of the X-rays employed, highlighting morphological variations from the micro- and meso-scale (in the case of SEM), to the nanoscale (thanks to AFM) [32-37].

For both biodeteriorated parchment and leather, the use of X-ray irradiation as an alternative method to chemical biocides has been evaluated and, at the same time, a study of their effect on the collagen matrix of these different substrates has been characterized by means of different analytical techniques. In recent researches [31, 38, 39], thanks to the employment of traditional techniques, such as the Fourier Transformed Infrared spectroscopy (FTIR), the eventually induced hydrolysis and gelatinization processes has been evaluated for different irradiation doses. Similarly, the use of a new opto-thermal method, namely the Light Transmission Analysis (LTA) [40], has helped in the characterization of the deterioration effects of the irradiation, allowing the analysis of alterations in the so-called hydro-thermal stability of the collagen molecule within parchment and leather. The presented results are part of this multidisciplinary research regarding the evaluation of the effectiveness of the disinfection treatment by X-rays irradiation on infected collagen-based substrates and of the effects eventually induced in the collagen matrix [41].

1. – Materials and methods

1˙1. *Irradiation system.* – In order to inhibit the microbial growth and, thus, the biodegradation of collagen-based artefacts, a high-energy X-ray beam produced by the REX (Removable Electrons to X rays) source, was used. The facility, developed at the ENEA laboratories in Frascati (Rome), allows the use of volumetrically penetrating radiation (photons and electrons) without the safety risks of the radioactive sources conventionally employed in this field. REX is based on a 5 MeV S-band electron linear accelerator equipped with an interchangeable X-ray converter. The instrumentation can deliver radiation with intensity and fluence modulated for specific purposes and it allows to perform online dosimetry [42, 43]. For this work, in order to provide a uniform X-ray distribution over large treatment areas, a tungsten converter specifically designed has been used.

1˙2. *Samples preparation and irradiation.* –

Parchment. – In order to evaluate the possible applicability of the X-ray radiation, as a disinfection treatment, the characterization of the damage eventually induced in

the collagen fibres within parchment, as well as their capability to inhibit the microbial growth on the biodeteriorated collagen based artefact, is crucial.

For this purpose, a series of samples cut off from the same sheet of a modern parchment has been irradiated at increasing radiation doses and then analysed in order to characterize any effects of the radiation on the structural stability and integrity of the collagen molecules (0, 50, 200, 350, 500, 700, 1000, 2000, 3000 Gy).

Leather. – Similarly to what has been done for the parchment, a preliminary research has been carried out to characterize the damage eventually induced by X-rays on the collagen molecule within leather, in order to determine their possible applicability as a disinfection treatment also in the case of biodeteriorated leather artefacts. In this case, the samples have been extracted from the leather wallpapers of *Palazzo Chigi* in Ariccia and, therefore, they are originals ancient samples.

The images presented were acquired on both the untreated sample and on a series of samples extracted from the same leather fragment after be treated with different irradiation doses (750 Gy, 1000 Gy and 5000 Gy). As mentioned before, due to the fact that the hair side of the samples is richly decorated, only the flesh side can be analysed, since it leaves the collagen fibres exposed.

1˙3. *Microscopic characterization*. –

AFM characterization of the parchment. – The analysis were conducted with a Bruker Dimension Icon AFM, assisted by a Nanoscope V controller, and through a Bruker Multimode AFM, equipped with a Nanoscope III controller, in both using the so-called "tapping" operating mode. The instruments were equipped with Bruker RTESP 300 probes, with a resonance frequency of about 300 kHz. The acquisitions were made on areas ranging from 1 μm to 10 μm with a scanning speed of about 0.5–1.5 Hz on the so-called "flesh" side of the membranous sheet. The possibility to analyse the samples without surface treatment (as occurs for example in electron microscopy) makes the technique particularly useful in the field of diagnostics for cultural heritage.

SEM characterization of parchment and leather. – The microscopic analysis was performed using a Zeiss Auriga Field Emission Scanning Electron Microscope (FE-SEM) on parchment samples treated with a 30 nm coating of Cr, in order to prevent sample's charging under the electron beam. The images presented were acquired with an acceleration voltage of 3 keV, using magnifications from 1 kX to 50 kX for parchment and 1 kX to 5 kX in the case of leather samples, on both the untreated sample and on the samples treated with different irradiations doses.

2. – Results and discussion

2˙1. *Irradiated parchment*. –

SEM characterization of parchment. – The SEM micrograph recorded on the untreated sample (fig. 1(A)) shows the appearance of a parchment in good preservation condition with the typical ordered fibrillar networking. The fibrils are grouped in bundles, discontinuities or gelatinized areas are not visible and the typical periodic structure of the fibril is clearly appreciable.

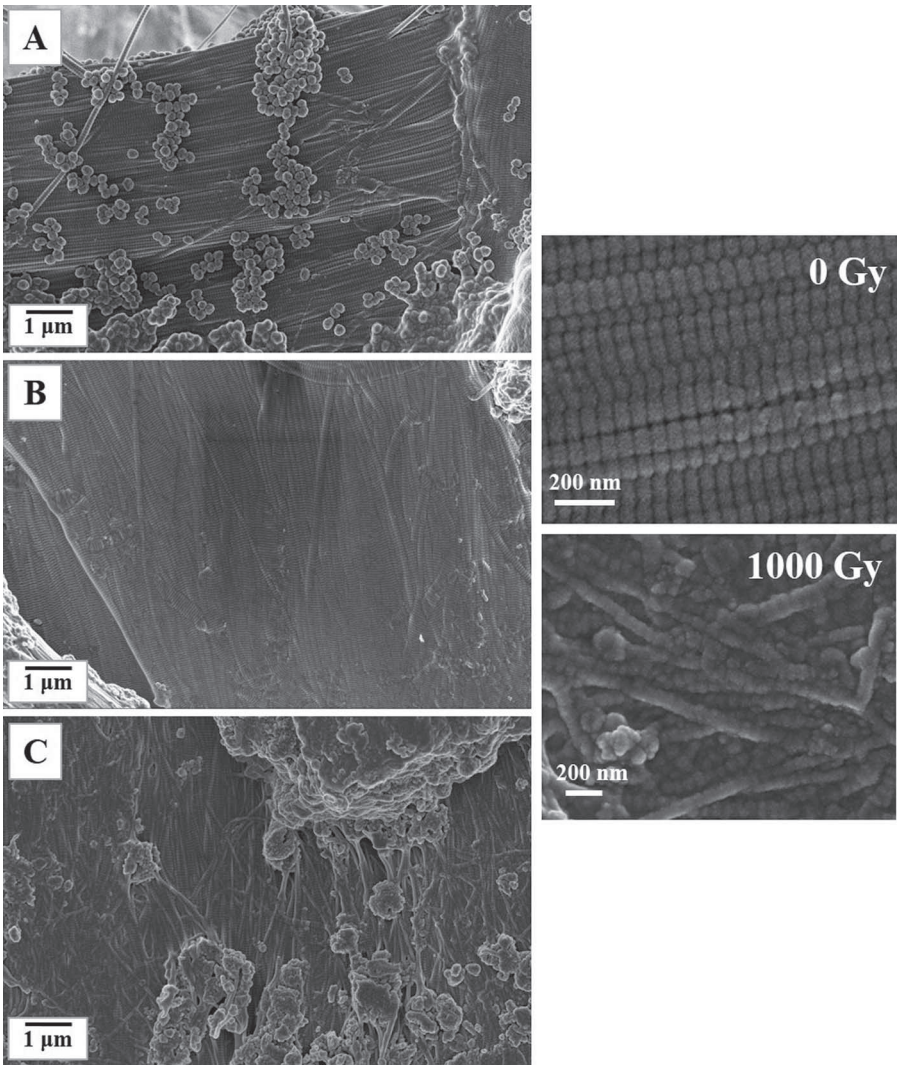

Fig. 1. – SEM micrographs of parchment samples treated with different X-ray doses: untreated (A), 350 Gy (B), 1000 Gy (C). The regular annular periodicity of the fibrils is clearly persisting till the sample treated with low radiation doses (350 Gy). Alterations can be found at higher doses of irradiation as it is possible to see in the images of the sample treated with the maximum dose. Large areas of amorphous and gelatinized structures can be observed and a general alteration of the periodic and ordered structure of the fibrils can be recognized especially in the images at high magnifications.

The same ordered and well-preserved fibrillar structure remain visible in the sample irradiated at the relatively low dose of 350 Gy (fig. 1(B)). In a preliminary research [31], this dose was found to constitute the threshold for the onset of disinfection effectiveness by X-rays irradiation. The maintenance of the typical surface aspect recorded in the untreated sample confirms that for doses up to 350 Gy the treatment can be safely employed, since no further deterioration processeses in the collagen of the parchment substrate are induced.

On the contrary, SEM images obtained on the irradiated sample with larger doses (in the presented case 1000 Gy) alterations in the networking structure can be appreciated, showing the effects of the treatment. Slight damages of the ordered fibrillar structure, such as the altered dimensions, fragmentation and bifurcations of the fibrils and areas of partial unrolling and gelatinization, starts to become recognizable.

Comparing the fibrillary networking at higher magnification, the alterations in the ordered structure of the irradiated collagen fibrils with respect to the non-irradiated one are easily appreciable especially in the irregularity of the peculiar annular periodicity along the fibril of the collagen molecule, the so-called D-spacing.

AFM characterization of parchment. – The topographic images recorded by AFM result to be very useful in the characterization of the preservation conditions of the fibrillar structure of collagen before and after the irradiation. In the untreated sample it is possible to recognize the typical morphological aspect of a well-preserved parchment matrix (fig. 2(A)). The fibrils appear intact within well aligned bundles and, similarly to what observed by means of SEM analysis, the so-called period D is found to be well maintained up to the irradiation threshold dose of 350 Gy (fig. 2(B)), resulting in both cases in a value of 67 nm. Confirming the previously described analysis, beyond the threshold dose damaged fibrils start to appear and the induced deteriorating effect of the treatment appears clearly visible. More specifically, the fibrillar structure results altered and a progressive decrease in the persistence of the fibre networking can be detected. The lack of areas showing an evident fibrillar networking suggests the presence of gelatinized areas where the collagen molecule results to be completely deteriorated and its triple-helical structure fully deconstructed (figs. 2(C) and (D)) [28].

With respect to the SEM analysis, the AFM enables also the investigation of the texture of the sample along the fibrils, allowing to obtain a confirmation of the deterioration caused by the radiation and its effects on the collagen molecule [28, 30, 31]. The AFM line profiles extracted for each selected sample show that up to 350 Gy the typical fibrillar profile is preserved. It is characterized by a well-defined anular periodicity, and the D-period values remain close to the ones recordable in untreated collagen ($\simeq 65$ nm). The persistence of such features up to certain irradiation doses suggests that no radiation effects on the molecule are induced by the treatment and confirms the safeness of the X-ray irradiation. At least up to the doses investigated in this work. In the case of samples treated with doses exceeding 500 Gy, peculiar effects starts to be appreciable mainly in the alterations of the single fibril periodicity as in the case of the sample irradiated at 750 Gy (fig. 2(C)) were the fibrils present D values lower than 60 nm. The effect starts to be noticeable in the sample treated with high doses of irradiation (1000 Gy and more) where it results difficult even to extract the fibril profile because of the inhomogeneity of the surface (fig. 2(D)). When it is recognizable an intact fibril, the D-spacing results strongly altered and inhomogeneous with respect to the values recorded for the non-deteriorated collagen.

2˙2. *Irradiated leather.* –

SEM characterization of leather. – Similarly to the research carried on the parchment substrates, SEM analysis has been employed in order to evaluate morphological changes in the fibres networking in irradiated leather samples with the aim of evaluating the possible application of the disinfection treatment also in the case of this kind of artefacts.

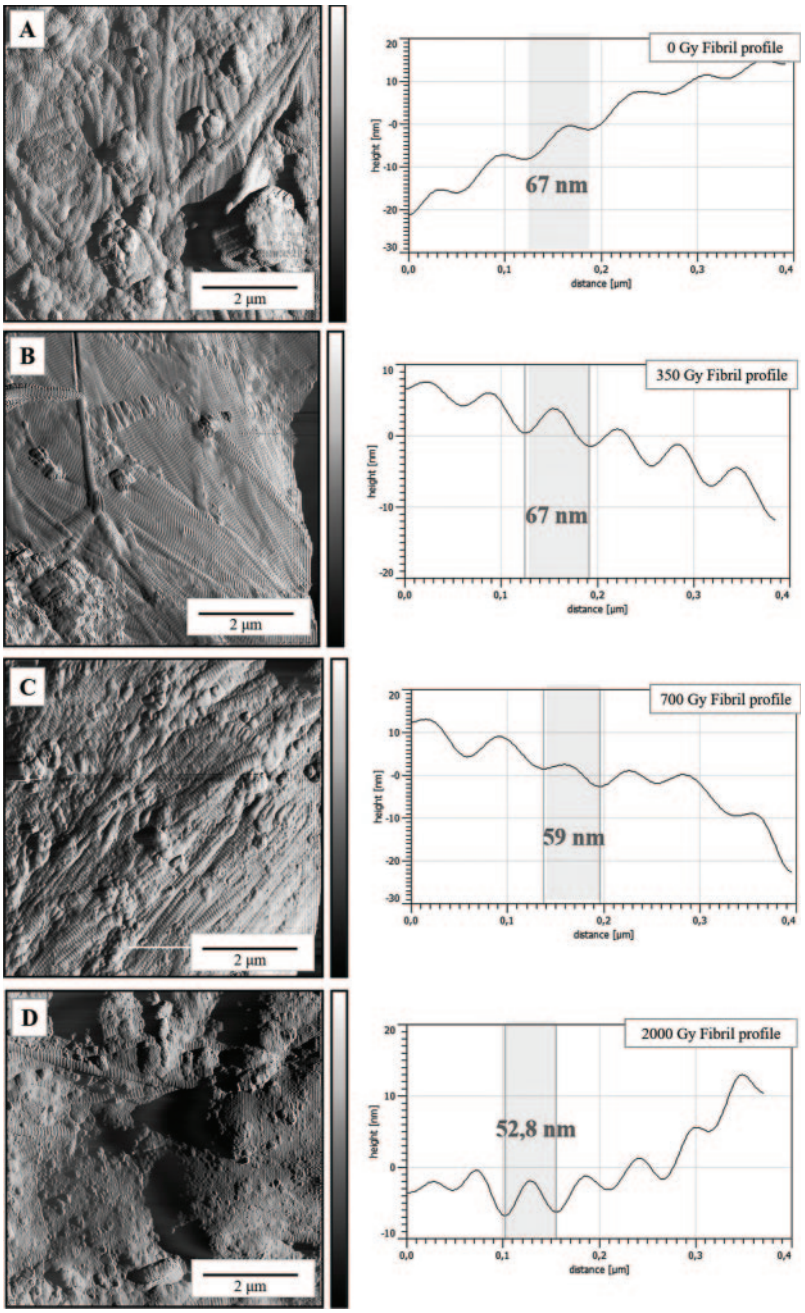

Fig. 2. – AFM topographic images and fibril profiles extracted of different irradiated parchment samples: untreated (A), 350 Gy (B), 700 Gy (C) and 2000 Gy (D). The regular periodicity of the fibrils is maintained at low irradiation doses while at increasing ones the regular periodicity of the fibrils is strongly altered (the well-defined periodicity of the fibrillar structure and its characteristic D-spacing is altered) and large areas of amorphous and gelatinized structures are recognizable on the sample surface.

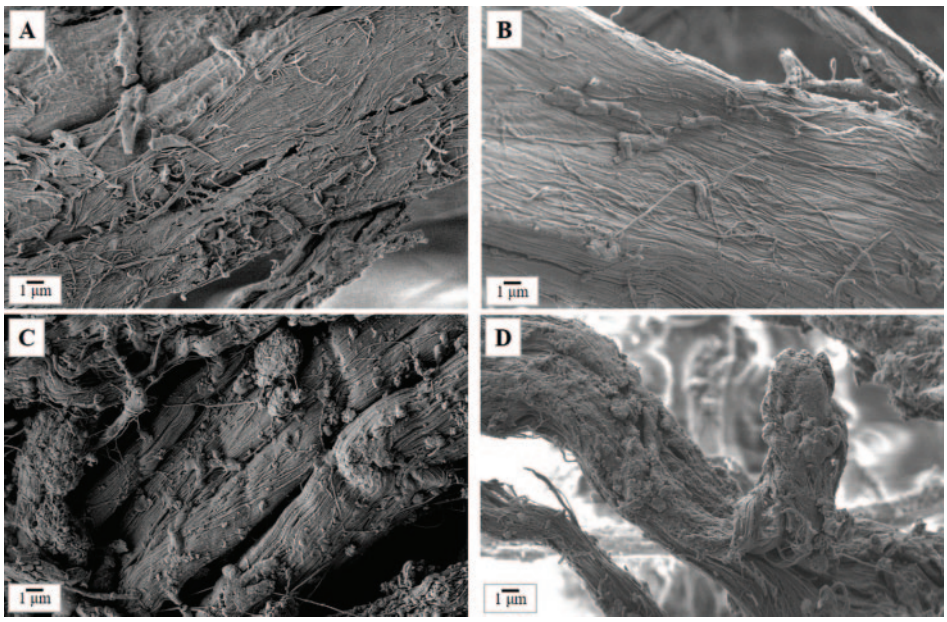

Fig. 3. – SEM micrographs of the untreated sample (A), of the leather irradiated with a dose of 750 Gy (B), with a dose of 1000 Gy (C) and with the maximum dose of 5000 Gy (D). For the samples irradiated with doses up to 1000 non-significant alterations in the surface morphology of irradiated samples can be detected with respect to the untreated one. Differently, in the sample irradiated with the maximum dose of 5000 Gy, the deterioration induced by the treatment results clearly visible in the appearance of the fibres bundles that appear reduced in their dimension, frayed and fragmented.

SEM micrographs of the non-irradiated sample (fig. 3(A)) show an intact fibres networking of the collagen. Despite being specimens coming from an original and ancient leather artefact, long and ordered bundles of fibres can be recognized showing well preservation conditions of the artefact. Comparing the untreated sample with the images recorded on the leather irradiated at 750 Gy (fig. 3(B)) and 1000 Gy (fig. 3(C)), slight differences start to be appreciable in the sample treated with the dose of 1000 Gy, where some areas show a disordered structure due to a partial unwinding of the fibrils. Since these areas constitute the onset of a gelatinization process, it has been possible to identify this dose as the threshold value.

As expected, the micrograph recorded on the sample irradiated at the maximum dose of 5000 Gy (fig. 3(D)) shows an extremely deteriorated collagen matrix visible in a completely different surface morphology with respect to the non-irradiated sample. The total lack of the bundled organization of the fibres is clearly the main effect of the treatment: the remaining bundles have lost the ordered fibrillary structure, their dimension is heavily reduced and the recognizable fibres appear frayed and fragmented.

3. – Conclusions

In this work, a brief review of the employment of advanced microscopic techniques in the characterization of collagen-based cultural heritage artefacts has been presented. It has been shown the capability of both SEM and AFM to help in the evaluation of

alterations in the fibrillar networking eventually induced by the exposition of the matrix to the X-ray irradiation employed as a disinfection treatment, alternative to the chemicals normally used in the treatment of bio-deteriorated parchment and leather. The images obtained have demonstrated the evolution of the deterioration of the fibrillar network with the increase of the irradiation doses, mainly showing the altered morphology of the surface of the irradiated samples and the alterations in the typical periodicity of the single fibril, as in the case of the D-spacing analysis performed by means of AFM. These results are part of a wider study started few years ago and still in progress. The aim is to characterize all the deterioration processes eventually induced by the radiation on the collagen molecule within parchment and leather, in order to propose an alternative method for the disinfection of collagen-based cultural heritage.

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This research was partially supported by the project ADAMO B86C18001220002 of the Excellence Centre at the Lazio Technological District for Cultural Heritage (DTC). I would like to especially thank Dott. Monia Vadrucci of Enea (Frascati) for letting me participate to this research and Prof. Giovanni De Bellis of Sapienza University of Rome, for giving me the possibility to learn the use of advanced microscopic techniques.

REFERENCES

[1] Agati M., *The Manuscript Book, A Compendium of Codicology* (L'Erma di Bretschneider, Roma) 2017.
[2] Tanasi M., in *Chimica e Biologia Applicate Alla Conservazione negli Archivi,* Saggi **74** (Pubblicazioni degli archivi di stato) 2002.
[3] Cavallo G., *Libri Editori e Pubblico nel Mondo Antico* (Editori Laterza, Roma-Bari) 2004.
[4] Febvre L. *et al.*, *La Nascita del Libro* (Laterza, Roma) 1985.
[5] Contadini A., in *Arte Veneziana e Arte Islamica: Atti del Primo Simposio Internazionale sull'Arte Veneziana e l'Arte Islamica*, edited by Grube E. J. (Edizioni l'Altra Riva, Venezia) 1989.
[6] Kennedy C. *et al.*, *Restaurator*, **24** (2003) 61.
[7] Haines B., in *Conservation of Leather and Related Materials*, edited by Kite M., Thomson R. (Elsevier, Oxford) 2006.
[8] Clarkson C., *Pap. Conserv.*, **16** (1992) 5.
[9] Kite M. *et al.*, *Conservation of Leather and Related Materials* (Elsevier, Oxford) 2006.
[10] Pinzari F. *et al.*, in *Historical Technology, Materials and Conservation: SEM and Microanalysis,* edited by Meeks N. *et al.* (Archetype Publications, London) 2012.
[11] Piñar G. *et al.*, *Microb. Ecol.*, **69** (2015) 118.
[12] Migliore L. *et al.*, *Collectanea Arch. Vaticani*, **102** (2016) IX.
[13] Migliore L. *et al.*, *Sci. Rep.*, **7** (2017) 9521.
[14] Karbowska-Berent J. *et al.*, *The Role of Streptomycetesin the Biodeterioration of Historic Parchment* (Nicholas Copernicus University Press, Totun) 2000.
[15] Kowalik R., *Restaurator*, **4** (1980) 200.
[16] Polacheck I. *et al.*, *Mycopathologia*, **106** (1989) 89.
[17] Mercuri F. *et al.*, *J. Cult. Herit.*, **31** (2018) 53.
[18] Cicero C. *et al.*, *Int. Biodeter. Biodegr.*, **134** (2018) 76.
[19] Gallo A., *Le Malattie del Libro. Le Cure ed i Restauri* (Forni, Calderara di Reno) 2006.
[20] Craig R., *Pap. Conserv.*, **10** (1986) 27.
[21] Kowalik R., *Restaurator*, **4** (1980) 99.
[22] *Paper Conservation Catalog* (American Institute for Conservation Book and Paper Group, Washington D.C.).

[23] Adamo M. G. *et al.*, *Restaurator*, **19** (1998) 41.
[24] Adamo M. G. *et al.*, *Restaurator*, **22** (2001) 107.
[25] Da Silva M. *et al.*, *Int. Biodeter. Biodegr.*, **57** (2006) 163.
[26] Magaudda G., *J. Cult. Herit.*, **5** (2004) 113.
[27] Otero D'Almeida M. L. *et al.*, *Radiat. Phys. Chem.*, **78** (2009) 489.
[28] Sendrea C. *et al.*, *Rev. Chim.-Bucharest-Original Ed.*, **68** (2017) 1535.
[29] Sendrea C. *et al.*, *Rev. Piel. Incaltaminte*, **15** (2015) 139.
[30] Nunes I. *et al.*, *Radiat. Phys. Chem.*, **81** (2012) 1943.
[31] Vadrucci M. *et al.*, *Appl. Radiat. Isot.*, **149** (2019) 159.
[32] Bozec L. *et al.*, *Biophys. J.*, **92** (2007) 70.
[33] Bartoletti A. *et al.*, *Insight*, **59** (2017) 265.
[34] Bozec L. *et al.*, *Biophys. J*, **101** (2011) 228.
[35] Larsen R. *et al.*, *Damage assessment of parchment: complexity and relations at different structural levels*, in *14th ICOM-CC Preprints*, Vol. **1** (James and James/Earthscan, London) 2005, pp. 199–208.
[36] Odlyha M. *et al.*, *e-PS*, **6** (2009) 138.
[37] Odlyha M. *et al.*, *Damage assessment of parchment at the collagen fibril level using atomic force microscopy and mechanical testing at the macro level*, in *17th ICOM-CC Triennial Conference Preprints, Melbourne, 15–19 September 2014, Paris* (International Council of Museums) 2014, art. 0607.
[38] Vadrucci M. *et al.*, *Parchment disinfection treatment by ionizing radiation*, in *2018 Metrology for Archaeology and Cultural Heritage (MetroArchaeo), Cassino FR, Italy* (IEEE) 2018, pp. 367–372.
[39] Vadrucci M. *et al.*, *Front. Mater.*, **7** (2020) 21.
[40] Cicero C. *et al.*, *Thermochim. Acta*, **676** (2019) 263.
[41] Vadrucci M. *et al.*, *Appl. Surf. Sci.*, **513** (2020) 145881.
[42] Borgognoni F. *et al.*, *Nucl. Instrum. Methods Phys. Res. B*, **406** (2017) 309.
[43] Vadrucci M. *et al.*, *Nucl. Instrum. Methods Phys. Res. A*, **930** (2019) 126.