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Fungal biosorption of silver particles on 20th-century photographic documents

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ABSTRACT

Microbial deterioration is a common problem in photographic collections and is considered a major cause of loss of documents. However, few studies so far have been addressed to biological damage on these materials. Several species of naturally occurring fungi can cause infections on the gelatin-silver emulsion of both positive and negative photographic material, producing defacement and loss of mechanical and aesthetical properties of the objects. In this study a particular phenomenon, spontaneously caused by fungi on 20th-century photographic films and positive supports, was documented by means of variable pressure scanning electron microscopy (VP-SEM) combined with electronic dispersion spectroscopy (EDS). This technique allowed the observation of entire, unaltered films without metallisation thus with a not invasive approach. The ability of some fungi to alter the distribution of silver crystals in the gelatin emulsion was described thanks to a backscattered electrons detector that showed differences in the atomic number of the visualised objects, giving rise to an appreciable contrast in case of different chemical composition.

1. Introduction

Microbial deterioration of photographic materials is a frequent problem and represents a major cause of damage on historical visual documents. However, few studies so far have been addressed to biological damage on photographic collections (Abrusci et al., 2005, 2006 and references therein).

Photographic documents represent an important component of the historic and cultural heritage, because they have ethnographic, social and artistic values. Photographic positive and negative supports are composed of at least three components: a rigid support (plastic or paper), an image-forming material (black and white images are formed by metallic silver particles) and a binder that in 20th century documents is mainly based on gelatin. Gelatin-silver based positive prints and negative films were the dominant photographic process nearly from the period of their introduction in the 1880s until the 1960s. The gelatin silver or black-and-white print and films represented, in fact, a primary form of visual documentation in the 20th century. The process that brings to image formation is based on the light induced selective trapping of metallic silver in a gelatin emulsion (Gray, 1987). When small crystals (called grains) of silver salts such as silver chloride are exposed to light, some atoms of free metallic silver are liberated. These free silver atoms form the latent image. Films are developed using solutions that reduce silver halides in the presence of free silver atoms. Once development is complete, the undeveloped silver salts must be removed by fixing in ammonium thiosulphate or sodium thiosulphate, and then the negative or print must be washed in clean water (Gray, 1987). The final image consists of metallic silver embedded in the gelatin coating. Gelatin is a mixture of high-molecular-mass polypeptides produced from collagenous animal tissues that was used for all silver halide-based photographic materials. Gelatin is also a hydrocolloid and a polyelectrolyte which controls the growth of silver halide crystals and prevents coagulation. Gelatin is easily degraded by bacteria and fungi when the environmental conditions are suitable (Abrusci et al., 2005). Contamination of photographic and cinematographic materials by bacteria and fungi has been described also in their manufacturing phases (mainly by species of the genera *Bacillus* and *Pseudomonas*) (Stickley, 1986). Abrusci et al. (2006) isolated from cinematographic films both bacteria and fungi. Several fungal strains were associated to photographic and cinematographic films biodeterioration, mainly in the *Aspergillus* (i.e. *A. ustus*, *A. nidulans* and *A. versicolor*) and *Penicillium* genera. The chief limiting factor

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that determines fungal development on photographic materials is water. Spore germination only occurs when some water is available; for some fungal species a very small amount of water is sufficient to trigger the growth of a new mycelium (Samson et al., 2010). Biodegradation of photographic materials by moulds can affect the material integrity of the same because the fungus' hyphae penetrate the substrate. Damage can also occur as a result of enzymatic action. Moulds can produce a wide range of enzymes (proteinases, gelatinase, cellulases) which are able to destroy all the component materials, from gelatin, to paper or cellulose nitrate. Some fungal spots and stains that frequently occur on both positive and negative photographic materials result in the loss of image sharpness and in the defacement of its contours. The analysis of some 20th century films and positives by means of variable pressure scanning electron microscopy (VP-SEM) equipped with a backscattered electrons detector disclosed us a further role of fungi in biodeterioration processes occurring to photographs and films.

2. Materials and methods

In this study cellulose nitrate negative films and gelatin-silver positive prints dated back to 1938–1940 and conserved at the "Archivio Ente EUR – Archivio Centrale dello Stato", Italy, were analysed by means of non invasive techniques. Prints and films underwent to a flooding event about 25 years ago and still appeared affected by moulds and other microbial stains (Fig. 1). The study was aimed at the documentation of the damage on the different structural components of the objects.

2.1. SEM-EDS observations

The study was conducted by observing photographic prints and films affected by discolourations and damaged areas with a variable pressure scanning electron microscopy (VP-SEM) combined with electronic dispersion spectroscopy (EDS).

SEM analysis was made using, in Variable Pressure mode, at 20 keV, an EVO 50 Scanning Electron Microscope produced by the Carl-Zeiss Electron Microscopy Group (Oxford, UK) fitted with detectors for both Electron Backscattered Diffraction (BSD) and Secondary Electron Scanning in Variable Pressure mode (VPSE). Chemical characterisation of the inorganic components of the positive and negative photographic materials was performed by means of electronic dispersion spectroscopy (EDS INCA Energy 250), which allows for an X-ray area scanning of what is brought into focus in SEM images, thereby creating a compositional map of the sample's surface (Goldstein et al., 2003). In the case study showed in this paper conventional ZAF correction (Goldstein et al., 2003) integrated into Oxford INCA 250 microanalysis package was applied to the spectrum dataset (Oxford Instruments). EDS analysis was made at 20 kV accelerating voltage with a tungsten filament.

Fig. 1. One of the negative gelatin-silver films dated back to 1938–1940 and conserved at the "Archivio Ente EUR – Archivio Centrale dello Stato", Italy, analysed during the survey.

VP-SEM permits the characterisation of sample surfaces without having to prepare them beforehand by means of metallisation. It therefore represents a micro-invasive methodology – in other words, a non-destructive technique. Samples were positioned entirely on non-invasive supports as large as the prints themselves, thereby allowing for the observation of entire objects without any, or in any event, very limited damage occurring.

The number of points analysed on each sample/area depended on the selected spot size (around 200 nm to obtain an acceptable X-ray Microanalysis detector dead time of around 20–25%) (Hall and Gupta, 1984). The interaction volume situated between the electron beam and the sample is not in fact a point, but instead a small volume: actual interaction corresponds to a spot on the sample's surface where the electrons penetrate it.

2.2. Culturing methods

The viability of fungi, actually visible on the objects at the naked eye, and eventual bacteria was tested using agar and broth culture media. Microbial structures sampled with cotton swabs and sterile needles were inoculated directly on to agar plates and the swabs were then immersed in sterile Czapek and gelatin broths (DIFCO, Becton Dickinson, USA). The media used to grow the inocula were Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA), which were prepared according to Samson et al. (2010) for fungi, and Nutrient Agar (5% peptone, 0.3% beef extract, 1.5% agar, pH adjusted to neutral) for bacteria (Pitt and Hocking, 1997). DG18 (dichloran–glycerol) media was also employed, with the objective of testing for the presence of xerophilic species. The growth of some fungal species can be favoured by culturing them in media that feature a low water activity. The culturing reagents employed were produced by DIFCO (Becton Dickinson, USA). All the inoculations were performed within the confines of a laminar flow hood so as to ensure sterility throughout the procedures. The plates were incubated at 26 °C and checked daily for colony development over a 30-day period (Pitt and Hocking, 1997).

2.3. Statistics

The EDS data were analysed using parametric *t*-Student test, for independent samples normally distributed, available in the XLStat 9.0 (Addinsoft, Paris) software package.

3. Results

3.1. SEM-EDS

The appearance of the blemished samples of Ag containing films and prints indicated that the fungal attack was associated with a biosorption of the Ag ions from the gelatin layer to the fungal hyphae. This can be observed in Fig. 2, where the fungal hypha with a bright appearance due to a high backscatter signal is indicated by the arrow "a". Biogenic accumulation of Ag coinciding with fungal and other microbial colonisation was documented (Fig. 2). The