

Combining a hydrogel and an electrochemical biosensor to determine the extent of degradation of paper artworks

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Received: 30 December 2011 / Revised: 13 February 2012 / Accepted: 16 February 2012 / Published online: 13 March 2012
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Abstract Paper-based artworks are among the most valuable assets for transmission of knowledge. Historical paper is composed of different polysaccharides (e.g. cellulose), binders, and glues. During aging all of these components undergo several degradation processes, as a result of external and intrinsic causes, and these can compromise the state of conservation of the document. In this work, application of a new biotechnological strategy for paper artefact preservation is reported. By making use of innovative and non-invasive materials, for example appropriate hydrogels, in combination with selective electrochemical biosensors, it is possible to simultaneously verify the degradation condition of the paper artwork and then to efficiently clean it, while monitoring the process of removal of both pollution and degradation products. In this paper, we focus on specific examples in which such techniques have been applied to paper artworks and that illustrate the advantages and potential of this biotechnology compared with the traditional paper-cleaning methods currently in use.

Keywords Cultural heritage · Paper artworks · Rheoreversible gel · Electrochemical biosensor · Clean up · Degradation process

Introduction

The analytical tools and methods made available as a result of applied biotechnology have so far found scant use in

other fields of knowledge, including the conservation of cultural heritage materials, for example stone, paper, paintings, textiles, or glass [1–3]. Among these materials, paper-based artwork is probably one of the most difficult media to restore, because of its complexity and fragile structure. The main paper component is cellulose, a polysaccharide made up of β -glucose units linked together by $\beta(1 \rightarrow 4)$ glycosidic bonds. [4]. Protein materials, for example casein, and animal and fish glue, are also incorporated into paper artworks as adhesives and ingredients of binding media [5, 6]. Structural changes as a result of ageing lead to a decrease in the stability of the material, reduction of its strength, and a change in colour, frequently because of hydrolytic processes [4, 7]. Restoration of paper artworks has, in general, received less attention than that of other artefacts, because this support is more difficult to treat, owing to its inherent fragility, the type of degradation processes occurring, and the complexity of different materials present. Moreover the use of foreign substances, for example cellulose ethers or adhesives, can alter the optical characteristics of paper, especially its opacity, frustrating efforts to limit as far as possible any interference with the original appearance of the paper [5].

Therefore, any intervention for this support requires very skilled craftsmanship, combined with experience in several disciplines. Despite these difficulties, much has been accomplished in the study of new methods for the cleanup and restoration which are non-invasive and easier to implement.

In the process of paper restoration, very important steps are estimation of the state of conservation of the paper, cleaning of the sheets (i.e. removal of dull patina which could be of different origin), optimization of the humidity of the page, and glue removal [6]. A cleaning process, because may cause irreversible damage, must be performed

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selectively, by removal of successive layers of deposits and with possibly different treatments in the several areas; simultaneously, one must evaluate whether the cleaning process is necessary and also determine its duration. Moreover, commonly used solvents (organic or not) may cause several problems, for example swelling and dissolution of some components, and these may also be harmful to the users [8, 9].

Recently, to overcome these problems, innovative methods were proposed: application of new cleaning gels, sometimes with cleaning agents entrapped in their matrices, and use of electrochemical biosensors to monitor properties reflecting the status of the paper materials [10]. In particular, because of the high retentive power and viscosity of gel systems, penetration of the art substrate by the liquid is significantly reduced, thus minimizing damage [8, 11, 12]. Using such a system, a more rational process can be undertaken with less risk of damage.

Use of electrochemical biosensors to study paper degradation

One of the most frequent forms of paper deterioration is biodegradation caused by moulds, bacteria, and other higher organisms that bring about biochemical or enzymatic processes. These attacks reduce the pH of the paper and make it fragile. Two of the agents responsible for microbial attacks are the fungi *Trichoderma reesei* and *Aspergillus niger*, which produce significant amounts of extracellular

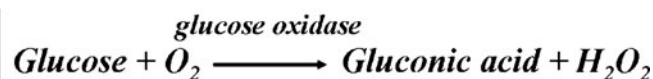
enzymes, called “cellulases” [13]. This family of enzymes reacts with the cellulose chains, either at their termini (exoglucanases) or internally (endoglucanases), giving rise to several hydrolysis products [14, 15]. Because glucose is one of the main metabolites produced by these cellulases, it is possible to monitor the extent of enzymatic degradation of cellulose-based materials by measuring the amount of glucose produced. An interesting new application is monitoring of the status of conservation of paper material by measuring the glucose produced after simulated attack by hydrolytic cellulase-producing fungi, by use of a disposable glucose biosensor coupled to a flow-injection analysis (FIA) system [10, 16]. This analytical system is a well-known tool in food and clinical analysis [17, 18] which has also proved useful in this application to conservation.

In the example reported here, an FIA system with a screen printed electrode modified with glucose oxidase enzyme (GOx) (details are given in Fig. 1), was used to monitor the amount of the glucose produced during hydrolysis of cellobiose. This substance is a product of the degradation of cellulose by cellulase and is a limiting factor for enzymatic activity. FIA coupled with biosensor amperometric detection was used to measure the H_2O_2 , produced by the immobilized GOx, (more details are given in Refs. [19–21]). With this system (flow rate $350 \mu\text{L min}^{-1}$, detection limit $8 \times 10^{-4} \text{ mol L}^{-1}$, correlation coefficient 0.995, sensitivity $30 \text{ nA mmol}^{-1} \text{ L cm}^{-2}$, repeatability, as RSD, 4% ($n=3$, $5 \times 10^{-3} \text{ mol L}^{-1}$), it has been possible to measure the amount of the glucose produced by the fungal attack with satisfactory sensitivity and to estimate the paper quality

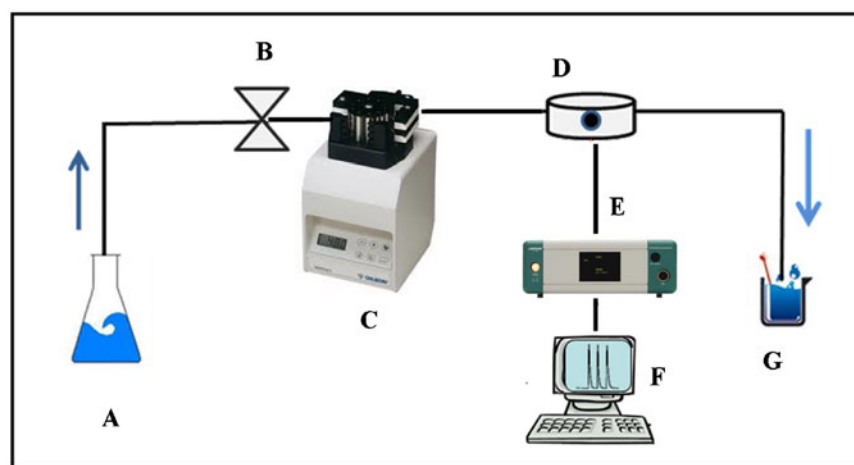
Fig. 1 Determination of the glucose produced by fungal attack on old paper by use of flow-injection analysis (FIA) with an integrated electrochemical glucose biosensor. 1, screen printed electrode (SPE); 2, enzymatic reaction catalysed by glucose oxidase; 3, FIA system: A, carrier buffer; B, loop and injection valve; C, peristaltic pump; D, thin-layer flow-through cell (which contains the biosensor); E, potentiostat; F, computer; G, waste



1. Screen printed electrode



2. Enzymatic reaction catalysed by glucose oxidase



3. Flow Injection Analysis system (FIA) with integrated electrochemical glucose biosensor.

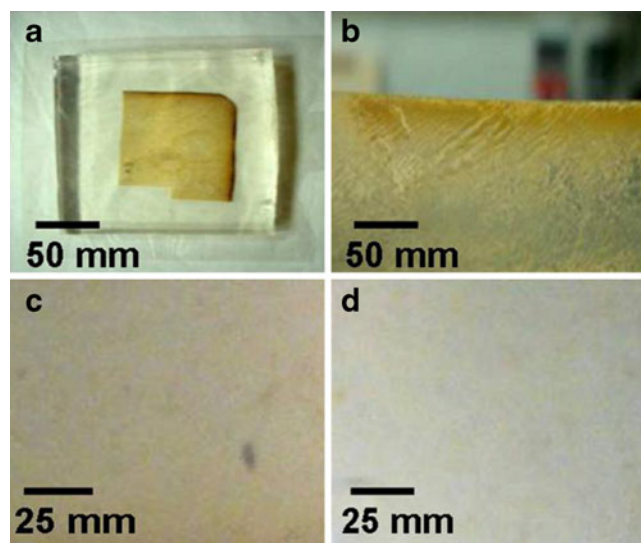


Fig. 2 **a.** Application of gellan gel to paper samples. **b.** Cross section of gellan gel 1 h after application to a paper sample. Adsorbed paper-degradation products and pollutants are clearly visible. **c** and **d.** Comparison of paper sample before (**c**) and after (**d**) treatment with the gel. The effects of the gel cleaning treatment are apparent from the evident lightening of the colour of the sample of paper

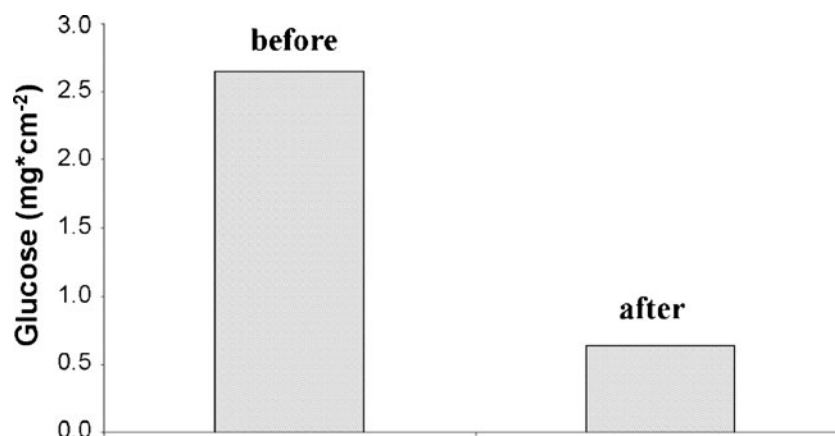
(composition, amount of lignin and additives) [16, 22]. The advantages of this approach consist in the small amount of sample necessary for the analysis and the possibility of performing on-line and continuous analysis, and using the instrumentation on site. Traditional approaches for assessing the conservation status of paper documents after a fungal attack were based on destructive measurement of physical–mechanical characteristics and empirical observations, for example the variation of the colour of the paper [4, 9].

Application of hydrogel for cleaning paper

The innovative hydrogel approach made use of materials used in food and biomedical science [23, 24]. One of these

is gellan, a high-molecular-weight polysaccharide, already in use as a gelling agent in biomedical and pharmaceutical science and in industry. This gel has also been in use since 2003 to clean wet paper artworks at the Istituto Centrale per il Restauro e la Conservazione del Patrimonio Archivistico e Librario (ICPAL, Rome, Italy) [25, 26]. It is a polysaccharide composed of units of β -glucose, α -rhamnose, and β -glucuronic acid which is capable of forming very transparent, thermo-reversible, viscoelastic and rigid gels that can gradually release the water contained within the polymer network [27]. Its application has been shown to be a valid alternative technique because it was able to remove degradation substances from graphic works without causing morphological changes in the paper. At the same time, it absorbs the water-soluble degradation products present. Finally, because of its viscoelastic properties, its application to and removal from artworks is fairly simple while enabling a localized cleaning operation. Because it is stiff and non-sticky, it can be peeled from a surface in one piece, leaving behind a minimum of residues. Several restoration projects on old paper have shown that gellan can be used to perform clean-up similar to immersion in a water bath, with the advantage of optimizing the solvent power of the water when it is released on the paper surface in a gradual, constant, and uniform way [28, 29]. Given its transparency it is easier to visually follow the process of removing coatings from the paper substrate to which it is applied (Fig. 2A, B), and lightening of the colour of the paper is evident from comparison of the same paper sample before and after gel application (Fig. 2C and D, respectively). By using gellan gel combined with an electrochemical biosensor, numerous samples can be analyzed continuously in a short time (1 h of application of the gel on the paper and 5 min for electrochemical analysis). Monitoring of the cleaning treatment showed that treatment for 1 h is sufficient to remove almost 80% of the glucose produced by fungal attack (Fig. 3). The dimensions of the gel to be used during cleaning treatment are a function of the size of the paper samples to be treated, but are subjective for each treatment. The criterion for

Fig. 3 Determination of glucose, produced by attack by *Trichoderma reesei* and *Aspergillus niger* fungi, by use of FIA combined with an electrochemical biosensor. Glucose measurement before and after treatment demonstrates the utility and effectiveness of the cleaning method



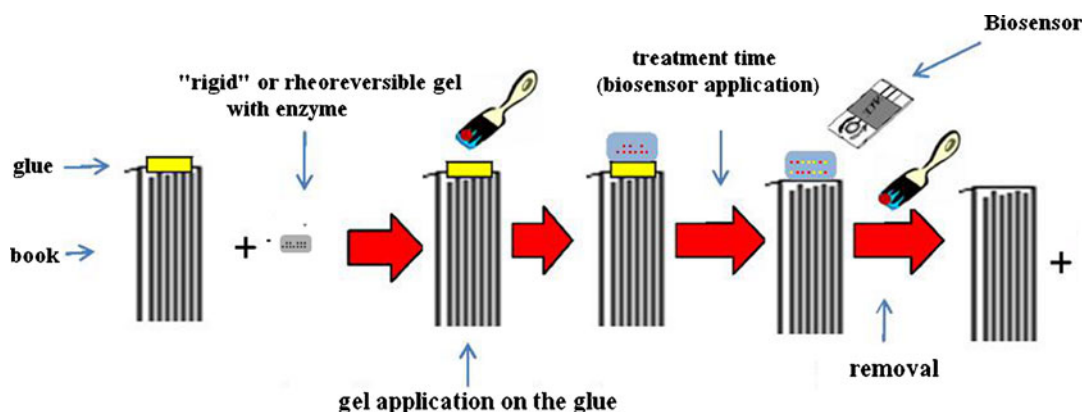


Fig. 4 Schematic representation of the gel ("rigid" or rheoreversible) systems (containing enzymes) used to remove glue

complete cleaning is when the glucose measurement does not change (minimum or zero).

Removal of old glue from artworks is very important for their preservation, because transformation of the glue as a result of ageing causes yellowing and an increase of the paper surface area. In recent years, substitution of traditional methods (i.e. use of solvents and scalpels to remove unwanted residues) with a cleaning process involving hydrolytic enzymes immobilized in hydrogels is a step forward. Application time is significantly reduced, because of the specific and targeted enzyme activity. For example, α -amylase enzymes, which hydrolyze starch glue immobilized in cellulose ether gels, have been successfully used since 1977 [30–32] to remove highly localized adhesive mountings in albums or books (Fig. 4). A recent innovative method made use of a rheoreversible gel [10] to support active hydrolytic enzymes on the adhesive surface, thus facilitating complete removal of the degradation products derived from the hydrolyzed glues. In this study the enzyme was entrapped in a physical hydrogel made up of α -cyclodextrin (α -CD) and the polymer poly(ethylene oxide) (PEO); a partial inclusion complex of PEO with the cyclodextrin leads to formation of a supramolecular hydrogel [33]. This kind of gel was chosen for several reasons: it is a physical gel and thus could be easily removed from the substrate simply by external triggering of the sol–gel transition by use of soft mechanical action. It is biocompatible and non-toxic, and thus safe for restoration staff. It can be prepared in a buffer compatible with the enzyme activity, because the properties of the gel are not sensitive to pH nor do they require the presence of specific ions. Thus the gel can be prepared in a buffer that is optimum for the chosen cleaning agents [34]. Moreover, molecules could be directly encapsulated into the hydrogel in situ at room temperature [35] such that the stability of temperature-sensitive agents, similarly to digestive enzymes, is not compromised. Because this gel can be easily transformed into a sol, it is easily removable from the artwork, unlike previously used gels (Fig. 4), for example

Wolber gels (e.g., Carbopol, a poly(acrylic acid)-based gel) still widely used in cultural heritage conservation and, in particular, for paper cleaning and enzymatic removal of starch paste. Burnstock and Kieslich, however, demonstrated that Carbopol residues remain on a painting surface even after washing with alcohol [36, 37]. Other gelators, for example high-molecular-weight cellulose derivatives, have been used mainly to gel enzymes and chelators in dilute aqueous solutions for paper cleaning [30, 31, 37]. In these cases, appropriate interface paper should be used to avoid direct contact between gel and artwork.

Outlook

In the field of restoration of paper artwork, efforts are required to optimise the procedures used for restoration. Electrochemical biosensors, which have been widely used in other areas of science, have great potential for evaluation of the extent of removal of unwanted substances during the clean-up, and gels have been shown to be highly effective in removing pollutants and degradation products. By selecting the appropriate enzyme to be immobilized, biosensors can be selective for the substances of interest. For example, the biosensor for glucose indicates the amount of glucose that is produced by the degradation of cellulose as a result of attack by fungus, and whether or not it has been removed during cleaning of the sample. In this way it is possible to know when the cleanup process is complete, avoiding lengthy and sometimes unnecessary applications of cleaning material. For example, in the removal of starch glue, the combination of an alpha-amylase biosensor and gel containing hydrolytic enzymes could enable conservation staff to determine when the removal process is effectively complete, and provide information about cleaning efficiency. A problem to confront is that hydrophilic gels (for example gellan) can be used to remove mainly hydrophilic molecules from paper artwork, whereas hydrophobic residues, for example dust,

could remain on the paper after treatment. This problem might be overcome by including solvents or surfactants in the hydrogel, a practice that has been used to clean paintings [38].

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