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A portable device for on site detection of chicken ovalbumin in artworks by chemiluminescent immunochemical contact imaging

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ABSTRACT

The development of user-friendly devices for on site analysis and characterization of painting materials is one of the most challenging objectives in the diagnostics for cultural heritage. Thanks to the specificity of antigen–antibody reactions, immunological methods have been already successfully applied for the detection of proteins and for their localization within painting stratigraphies. Moreover, by combining the advantages of the immunological techniques with the high detectability offered by chemiluminescence detection, it has been possible to achieve good analytical performance and very low detection limits. This work was aimed at developing a portable analytical device for the detection of chicken ovalbumin (a protein found in egg tempera and in egg-based protective varnishes) in painting samples employing ready-to-use analytical cartridges and a thermoelectrically-cooled CCD camera as a chemiluminescence detector. The protein was extracted from using a simple procedure and revealed by a non-competitive immunoassay with chemiluminescence contact imaging detection. The assay was simple, fast and suitable for the detection of ovalbumin in small samples. This analytical system provided positive identification of ovalbumin in samples obtained from both fresh or artificially aged paint reconstructions and historical paintings. It might be easily employed in different contexts (such as small museums, restoration laboratories or even on site) by restorers to obtain prompt information during restoration actions, such as cleaning operations. In perspective this device could be also employed for the detection of other proteinaceous and organic painting components.

1. Introduction

The prompt identification of painting materials and the diagnosis of their state of conservation are of outmost importance for addressing urgent restoration issues, selecting proper conservation strategies and monitoring restoration activities. Moreover, on site diagnostic campaigns may also allow for a preliminary documentation of chemical heterogeneity of paintings, providing identification of areas to be submitted to further studies (e.g., stratigraphic analysis). Restorers would thus benefit from simple, cheap and rapid analytical tools suitable to perform on site preliminary tests and thus support decision regarding the most suitable restoration operations. Indeed, the new generation of restorers possesses a deeper knowledge on scientific methods, thanks to the introduction in their educational programmes of scientific subjects such as chemistry, biology and geology. Thus, the role of restorers may be actually compared to that of primary care physicians because their experience and wide knowledge on the painting techniques may allow them to plan autonomously the restoration interventions.

Nowadays, the need for user-friendly portable analytical systems has been recognized as an urgent issue in fields such as public health and environmental monitoring, and miniaturized analytical devices based on different biospecific recognition reactions (e.g., enzyme, immuno and nucleic acid hybridization reactions) are widely used in bioanalytical chemistry, clinical diagnostics, forensic sciences, feed and food safety assessment, toxicology and pharmacogenomics [1–3]. Unfortunately, this research subject is till now almost unexplored in the field of conservation science and the setting up of portable devices is one of the most challenging objectives in the diagnostics for cultural heritage.

The reliable identification of proteins and other organic substances (oils, gums, etc.), often present in limited amount in paintings and other artworks, still represents an issue in analytical studies aimed at revealing working practices, dating artworks and supporting restoration actions. Indeed, these substances, used since ancient times as binders, varnishes and adhesives [4], are characterized by a wide chemical variety, and undergo significant physicochemical changes with aging.

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Until now, the characterization of organic substances has been commonly performed by chromatographic and proteomic techniques, which require complex sample treatment procedures and expensive instrumentation and can be applied only in well-equipped laboratories [5–15]. In addition, several non-destructive diagnostic techniques such as mid- and near-infrared reflectance spectroscopy, Raman spectroscopy, NMR mouse spectroscopy, and terahertz (THz) spectroscopy [16–20] can be applied on site. However, they again require expensive instrumentation and expert scientists for both performing the analysis and interpreting the results. In addition, spectroscopic techniques present limitations related to selectivity and sensitivity, especially in the investigation of organic components (i.e., they do not discriminate among materials belonging to the same class of organic substances).

In the last decade, immunological methods have been deeply evaluated as alternative approaches for the detection of proteins in paintings. Thanks to the high specificity of antigen–antibody reactions, these methods have been used for the identification of proteins using enzyme-linked immunosorbent assays (ELISAs) [21–23] and for their localization within paint cross sections [21,24,25]. Among other detection techniques, chemiluminescence (CL) imaging has been used for the localization of proteins by single and multiplexed immunoassays, offering high detectability and avoiding interferences caused by autofluorescence of painting components [26–28]. Chemiluminescence detection has been also implemented in miniaturized analytical devices, providing low detection limits, wide dynamic ranges and assay rapidity with simple instrumentation [29–32]. For example, we have recently described a portable system for CL bioassays [33] based on a thermoelectrically-cooled CCD camera and contact imaging detection and reported its application for detection and genotyping of parvovirus B19 [34].

Based on this previous research, the present work was aimed at developing a user-friendly "point-of-need" device for the immunological detection of ovalbumin (a protein often used as painting fixative or binding medium). The device was tailored for the on site analyses of artistic samples taking into account of the following points: (i) the number and amount of samples may be extremely limited, (ii) the complexity of samples may affect the immunochemical results, (iii) the solubility of organic substances often decreases with aging, thus proper extraction procedures have to be optimized, and (iv) the analytical protocol should be easily applied by restores. Thanks to the employment of ready-to-use analytical cartridges no specific conditions such as cleanliness or temperature control are required (apart from the storage of antibodies in portable refrigerators). Moreover, results are easily readable since the detection of the target protein is simply associated to the emission of light. This device can be thus considered an important step towards the development of portable analytical systems overcoming the drawbacks related to the complex instrumentation now employed for on site investigations.

To assess the applicability of the system to the real conservation practice, its performance has been evaluated on fresh mock-ups, artificially aged paint reconstructions and historical samples. In addition, different ancient paintings in which the target protein was not present were also investigated to evaluate possible interferences. Encouraging results have been obtained for both positive and negative samples, suggesting that this device could represent a valid analytical tool that would allow restorers to perform the analysis during the restoration workshop, thus enabling a "point-of-need" approach and reducing response time and cost of analysis.

2. Materials and methods

2.1. Reagents

Polyclonal anti-ovalbumin antibody produced in rabbit (capture antibody, 3.6 mg mL^{-1}), monoclonal anti-ovalbumin antibody produced in mouse (primary antibody, 10.0 mg mL^{-1}), horseradish peroxidase (HRP)-conjugate polyclonal anti-mouse IgG antibody produced in goat (secondary antibody, 1.3 mg mL^{-1}), HRP-conjugate polyclonal anti-rabbit IgG antibody produced in goat (0.68 mg mL^{-1}), ovalbumin from chicken egg white, non-fat dried milk, Silane-prep silanized microscope slides (26 × 76 mm^2) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). The Silgard 184 PDMS prepolymer and the curing agent were obtained from Dow Corning (Midland, MI). The SuperSignal ELISA Femto CL substrate for HRP was purchased from Thermo Fisher Scientific, Inc. (Rockford, IL). Rabbit glue, linseed oil, gypsum ($CaSO_4 \cdot 2H_2O$) and inorganic pigments – blue smalt (potassium glass colored with cobalt(II) salts), hematite (Fe_2O_3), white lead ($(PbCO_3)_2 \cdot Pb(OH)_2$), minium ($Pb_3O_4$), and malachite ($Cu_2CO_3(OH)_2$) – were obtained from Zecchi (Florence, Italy).

2.2. Standard samples

Paint reconstructions were prepared according to ancient painting treatises [35]. Briefly, a gypsum preparation ground was obtained by applying a mixture of gypsum (190 g) and rabbit glue (10 g dissolved in 150 mL of hot water) onto a wood panel. After drying, layers of inorganic pigment and binding medium (i.e., egg, rabbit glue, or linseed oil) were painted onto the gypsum layer. The pigment/binding medium ratio was chosen to obtain a homogenous mixture suitable for application as a thin paint layer. For the egg tempera layers, the ratios between pigment and binding medium – prepared according with the traditional recipe by mixing egg white, yolk, and water in a 1:1:1 (w/w) ratio – varied from 4:1 to 4:3 (w/w) depending on the grinding size of the pigment. Paint reconstructions were artificially aged by heating for 3 months at 40–50 °C and 85% relative humidity.

2.3. Historical paint samples

Three historical samples were investigated in order to establish the suitability of the system for the characterization of real artworks. The first sample (sample SG1) was collected from a canvas painting representing *The Saint John*, belonging to a private collection and dated around the second half of 1600. The second case study (sample AM7) was a wall painting attributed to Giuseppe Milani (1719–1798) and located in the dome of the Abbey of S. Maria del Monte, Cesena (Italy), dated to 1773–1774. The last sample (sample SP1) was collected from a painted wood panel located in San Bartolomeo Church, Formigine (Italy) and representing *The Virgin of the Rosary*, which has been attributed to Vincenzo Spisano (1595–1662), named *Spisanelli*. Preliminary characterization of historic samples in order to detect proteinaceous materials was carried out by micro-infrared analysis in ATR mode. Spectra were acquired using a Nicolet iN10 MX Fourier transform infrared (FTIR) microscope equipped with a mercury cadmium telluride detector cooled with liquid nitrogen (Thermo Fisher Scientific, Waltham, MA). Data were processed using the OMNIC Picta software.

2.4. Analytical device

The analytical device (Fig. 1) employed a disposable reaction cartridge consisting of a silanized microscope glass slide with an array of immobilized anti-ovalbumin antibody spots coupled with a 5 mm-thick PDMS fluidic element. The fluidic element was prepared by mixing PDMS prepolymers with the curing agent in a 10:1 (w/w) ratio. The mixture was degassed under vacuum for 30 min, then poured in the casting master and incubated overnight at 25 °C to allow PDMS polymerization [36]. According to the design of the casting master, three parallel channels (35.0 mm length, 2.0 mm width, about 0.05 mm height) were engraved in the final fluidic element, each of them connected to a 100-μL inlet reservoir for loading samples and reagents. To immobilize the anti-ovalbumin capture antibody the microscope glass slide was previously activated according to a literature protocol [37] by soaking for 2 h at room temperature in a 2.5% (v/v) solution of