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Case study

Analysis of polychromy binder on Qin Shihuang's Terracotta Warriors by immunofluorescence microscopy

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ABSTRACT

Qin Shihuang's Terracotta Warriors is one of the major discoveries in the archaeological history of the world. It has become a key issue to identify the composition of polychrome binder of Qin's terracotta warriors in understanding their traditional painting technology and deterioration mechanism to provide conservation strategies. Previous instrumental analysis proposed that the binder in Qin's polychrome samples might be egg. In this work, specific fluorescence, generated by the ovalbumin antibody-egg white interaction was observed on Qin's samples under immunofluorescence microscopy (IFM). Our results demonstrate how specific and sensitive the IFM method is to analyze the organic substances in precious artworks.

1. Introduction and research aim

Colorful painting layers on the surface of heritage objects were modulated by pigments and binders. Common binders in ancient China were mainly natural organic compounds, such as eggs, animal glue and plant gum. Here binder is the fixative for the pigments that could be preserved for a long time and play a decisive role in the stability of colors [1]. So it is important to identify the composition of binders to understand the painting technique and support the conservation work.

Instrumental analysis is the most frequently used method to identify the organic binders in ancient samples, such as gas chromatography-mass spectroscopy (GC-MS) [2–4], high performance liquid chromatography (HPLC) [1,5–8], FTIR spectroscopy [9–13] and Tandem mass spectroscopy [14]. Wei et al. used GC-MS to analyze Western Han's (206 B.C–8 A.D) polychomy terracotta army and found that animal glue was the binding medium [4]. Bonaduce et al. analyzed the amino acids in Qin's samples by GC-MS. Comparing the relative contents of eleven amino acids and considering the features of low levels of proline and the missing hydroxyproline, they proposed that the binder might be egg [2].

Immunofluorescence microscopy combines the high specificity and sensitivity of proteins with the accuracy of microscopic tracing and begins to be systematically used in cultural heritage [15–20]. Several groups have shown that the IFM results were convincing [21–23]. In this study, the ovalbumin was used as markers for the identification of model polychromy samples and real Qin's samples in order to identify egg white.

2. Experimental

Standard samples were prepared according to Qin's techniques. Taking egg white for example, mix egg white and water in a volume ratio of 1:1, then mix with pigment in a ratio of 1:1, and then smear the mixture on a soil layer. The cross-section preparation steps are reported in Table 1. All the samples were analyzed after one month of natural aging. The real Qin's samples were collected from the excavation site of terracotta warriors. These polychrome samples fell off from the fragment of a pottery figure's armor labeled as T23G9:10.

The IFM observation procedures are illustrated in Table 2. All the IFM tests were performed at least three times on each sample.

Polyclonal anti-ovalbumin antibody and monoclonal anti-collagen antibody were purchased from Abcam in the UK. Alexa Fluor 594 anti-rabbit IgG antibody and Alexa Fluor 488 anti-mouse IgG antibody were purchased from Invirogen-MP. Bovine serum albumin was purchased from Sigma-Aldrich. Phosphate-buffered saline solution (PBS, 150 mM NaCl, 5.2 mM Na_2HPO_4,

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1.7 mM KH_2PO_4, pH 7.4, 0.2% Tween 20) was used to dilute antibodies and for washing steps. 5% BSA in PBS was used as a blocking solution. Azurite $Cu_3[CO_3]_2(OH)_2$ and malachite ($Cu_2(OH)_2CO_3$) were purchased from Beijing Rock Color Sky Elegance. Eggs and gelatin were purchased at local supermarket and Jiangsu Jiang Si Xutang, respectively. Acrylic resin (ethyl α-cyanoacrylate) for sample embedding was purchased at local market.

Optical images were collected at 100 × and 200 × magnifications using a continuous zoom stereomicroscope with a digital camera (Leica DC 300). The microscopic images of 100 × and

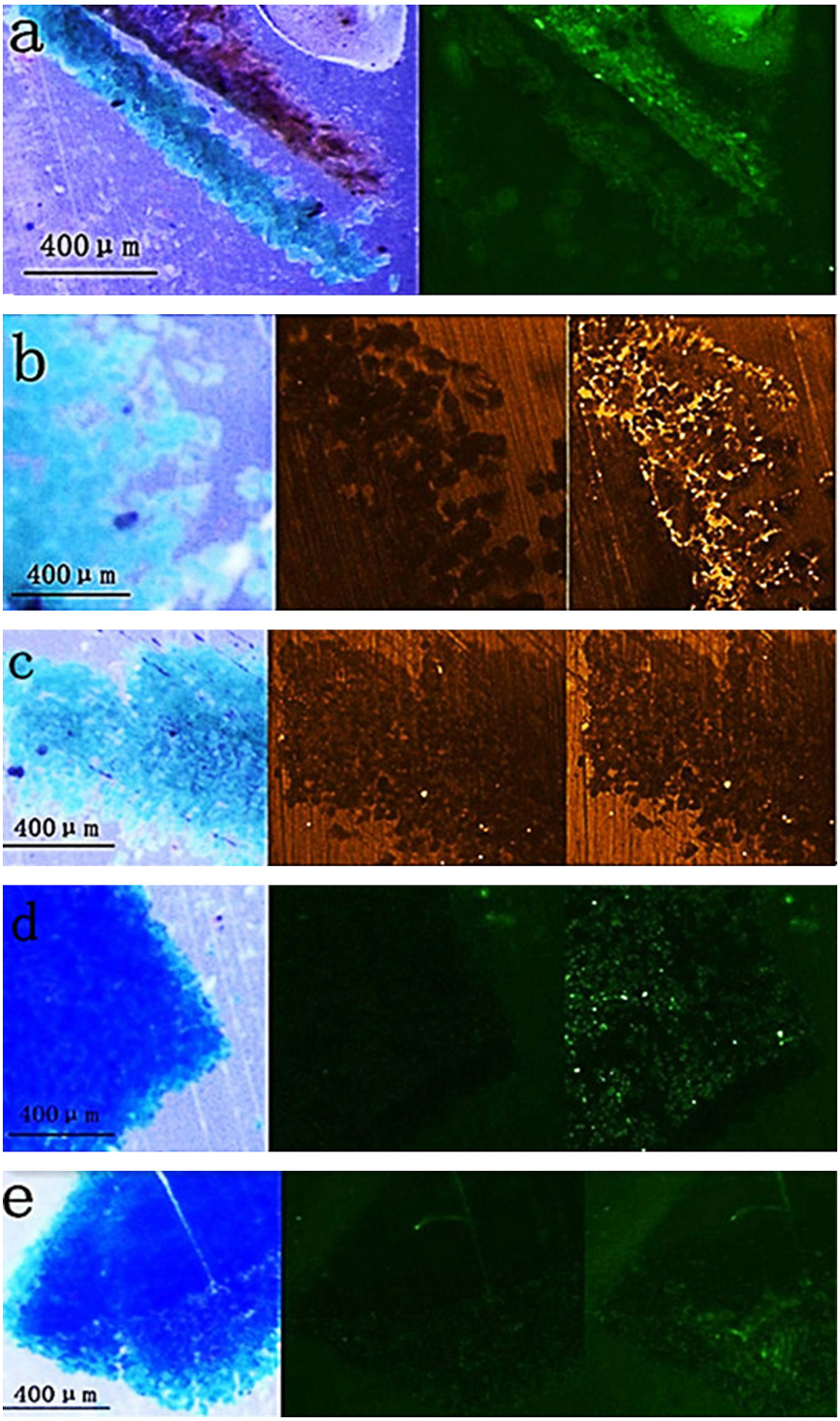

Fig. 1. (a) Stereomicroscopy (left) and IFM (right) images (100 ×) of egg white sample mixed with malachite, untreated with antibody; IFM staining for collagen and egg white model samples, from left to right: OM 100 × image, before and after IFM 100 × image; (b) positive results for egg white mixed with malachite; (c): negative results for egg white mixed with malachite; (d) positive results for collagen mixed with azurite; (e) negative results for collagen mixed with azurite.