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MORPHOLOGICAL CHARACTERISATION OF RED BLOOD CELLS IN HUMAN BLOODSTAINS ON STONE: A SYSTEMATICAL SEM STUDY

ABSTRACT: Mammalian erythrocytes or red blood cells (RBC) have been previously reported as forming part of residues on prehistoric implements. On the basis of the Principle of Actualism, several thick smears of human blood were obtained on chert. After increasing lengths of storage time span (1–36 months), the bloodstains were micromorphologically studied via scanning electron microscopy. Results revealed, in all the smears, the presence of an erythrocyte acme-zone with packed RBC, as well as negative replicas and moon-like shapes. Morphologies were found to be time-independent, and furthermore those erythrocyte acme-zones with packed RBC to be thick-bloodstain characteristic.

KEYWORDS: Scanning electron microscopy (SEM) – Organic residues – Bloodstains – Haemotaphonomy – Stone tools – Prehistory – Experimental archaeology

INTRODUCTION

Since a long time ago, the presence of morphologically preserved mammalian erythrocytes (red blood cells, RBC), as forming part of bloodstains, has been detected in prehistoric implements (Loy 1983). This preservation has been even reported for assigned ca 2-Myr-old Oldowan tools (Loy 1998). A smear may be defined as the result of a causal relationship, in which a physical contact (the cause) produces a trace (the effect). This causality – implicit in the Locard's Principle of Exchange, that is usually summarised as "every contact leaves traces" – agrees with such a presence of blood residues on (ancient) archaeological tools, as well as on (modern) forensic evidence implements.

Vertebrate blood is a suspension of cells (erythrocytes, leukocytes or white blood cells, and thrombocytes or platelets) in a fluid medium (plasma). Unlike the other vertebrates, in mammals erythrocytes are anucleate (Jain 1986: 527). Due to this lack of nucleus, the typical mammalian RBC are shaped as biconcave discs (discocytes). Based on SEM stereo micrographs, it has been found that the normal-sized erythrocytes (normocytes) in humans have a mean diameter of 7.49 μm (LeBlond, Shoucri 1978). According to Lewis (1970: 19), the normal value of erythrocyte count ($RBC \cdot L^{-1}$) in human whole blood averages $5.4 \cdot 10^{12}$ in males and $4.8 \cdot 10^{12}$ in females.

The presence of RBC in a smear is evidence for blood (Fiori 1962: 246–247, López 1953: 19–22), and an appropriate instrument for the microstructural characterisation of materials is the scanning electron microscope (SEM). Moreover, from the point of view of blood confirmation in ancient smears, SEM facilities are usually more available than molecular biology laboratories to either palaeobiologists and bioarchaeologists.

Actuopalaeontology and experimental archaeology are both based upon the Lyell's Principle of Actualism, which is usually summarised as "the present is the key to the past". Therefore, and because an experimental framework is necessary to all palaeosciences, I have been studying via SEM the variations in RBC morphology in bloodstains on either lithic and non-lithic substrates. In a previous paper, I have already reported several morphologies of preserved human RBC in mainly thin and ultrathin aged bloodstains

on chert, stainless steel and greywacke (Hortolà 1992a). In this paper, I report a systematic SEM study of human RBC in thick bloodstains on the same type of chert, a lithic material of main interest to prehistoric technology.

MATERIALS AND METHODS

Several thick smears of human blood from the same individual were obtained at one time on the fracture surface of white chert fragments from the same source. Chert was selected as bloodstain substrate due to be considered as one of the rocks preferred by prehistoric man as material for stone tools (Semenov 1964: 34–35). After puncture of the left-hand forefinger with a sterile surgical blade, smearing was carried out by the mechanism of "precipitation" (gravitational dribbling) from a small height ($h \approx 2 \cdot 10^{-2}$ m) while the blood extravasation was mechanically forced by finger pressure. The bloodstains were then dried in the sun at the open air without wind during 3 hours, and, finally, they were stored indoors under non-sterile and fluctuating room conditions, for increasing lengths of time ranging from 1 to 36 months (*Table 1*). Confirmation of the smear as being thick was done on the basis of presence of raised plaques with polygonal macrocracking. Diachronically, each sample was coated with gold by a SEM Coating Unit E 5000 sputter coater (Polaron Equipment Ltd., Watford, U.K.), and then micromorphologically studied by examining it using a Stereoscan 120 scanning electron microscope (Cambridge Instruments Ltd., Cambridge, U.K.) at a low accelerating voltage (10 kV) to decrease electrostatic charge. Micrographs were obtained using a (high-sensibility) T-max 400 ISO professional film (Kodak Ltd., Hemel Hempstead, U.K.).

TABLE 1. Microclimatic conditions of the storage room, per bloodstain sample. Values according to measurements taken at different hours, on an average of every 3 days except for BS1, whose average was every 2 days. Legend: BS = bloodstain sample, according to its age in months; M = maximum; m = minimum; RH (%) = relative humidity, in percent; t (°C) = temperature, in degrees Celsius.

BS	t_m (°C)	t_M (°C)	RH_m (%)	RH_M (%)
1	27	31	39	46.5
2	27	32.5	38	56
3	25	32.5	38	70
6	12	32.5	38	81
12	11	32.5	38	83
18	11	34	38	84
24	11	34	38	84
36	11	34	38	84

RESULTS AND DISCUSSION

A high inter-sample uniformity at both the macromorphological and the micromorphological levels was revealed.

Concerning the whole bloodstains, from the naked eye – either before and after gold coating – to low SEM magnification they displayed the typical aspect of crack-individualised arisen scales (*Figure 1*); the observed variability in smear pattern would have originated by individual differences between each smear-substrate topography. Moreover, at the naked eye – after gold coating – the whole bloodstains clearly evidenced two distinguishable areas: a central depression, and a medial elevation surrounding it. By using the SEM, discrimination between these two areas was difficult; however, at high SEM magnification a third area – not clearly distinguishable at the naked eye – was detected: a peripheral depression external to the medial elevation. Such a three-area bloodstain structure agrees with previous optical microscopy observations of experimental blood smears (Balthazard *et al.* 1939, cited in López 1953: 9–16).

Concerning the RBC, at high SEM magnification they displayed the following morphological characteristics. An erythrocyte acme-zone with packed RBC, visible in the cracks mainly as cup-shaped cells (stomatocytes), was evidenced in the (central and medial) thicker areas (*Figure 2*). Out of these areas, in the (peripheral) thinner ones, two different morphologies, as exemplified in the remainder illustrations, were evidenced: negative replicas at the thin-crack bottoms (*Figure 3*), and moon-like shapes at the smear boundaries (*Figure 4*). In these moon-like shapes, a wide range of RBC visible diameter was clearly evidenced (*Figure 4*, *cf.* 1M *vs.* 24M); from experience, this high size variability is not related to ageing but to bloodstain drying phenomena.

Negative replicas and moon-like shapes were analogous to those previously observed in either primarily (stone-knapping accidental, experimental) or secondarily (forensic) thin/ultrathin samples (Hortolà 1992a,b, 1994, 2001) and thus, because they share presence in both thick and thin/ultrathin bloodstains, they may not be considered as thick-bloodstain characteristic.

By storing the samples indoors, the microclimatic variations were mitigated in order to simulate "natural-like" environmental conditions similar to those which could be found in a cave. Although there were microclimatic differences between the samples aged less than 18 months, the fact that the temperature and relative humidity ranges for the samples of 18–36 months were the same (temperature range = 11–34°C, relative humidity range = 38–84%) allows to consider that the environmental conditions between these samples have been nearly homogeneous. Furthermore, provided that all the smears were obtained at one time from blood of the same individual and on the same type of substrate, it may be considered that in such samples only the time factor has significantly varied.

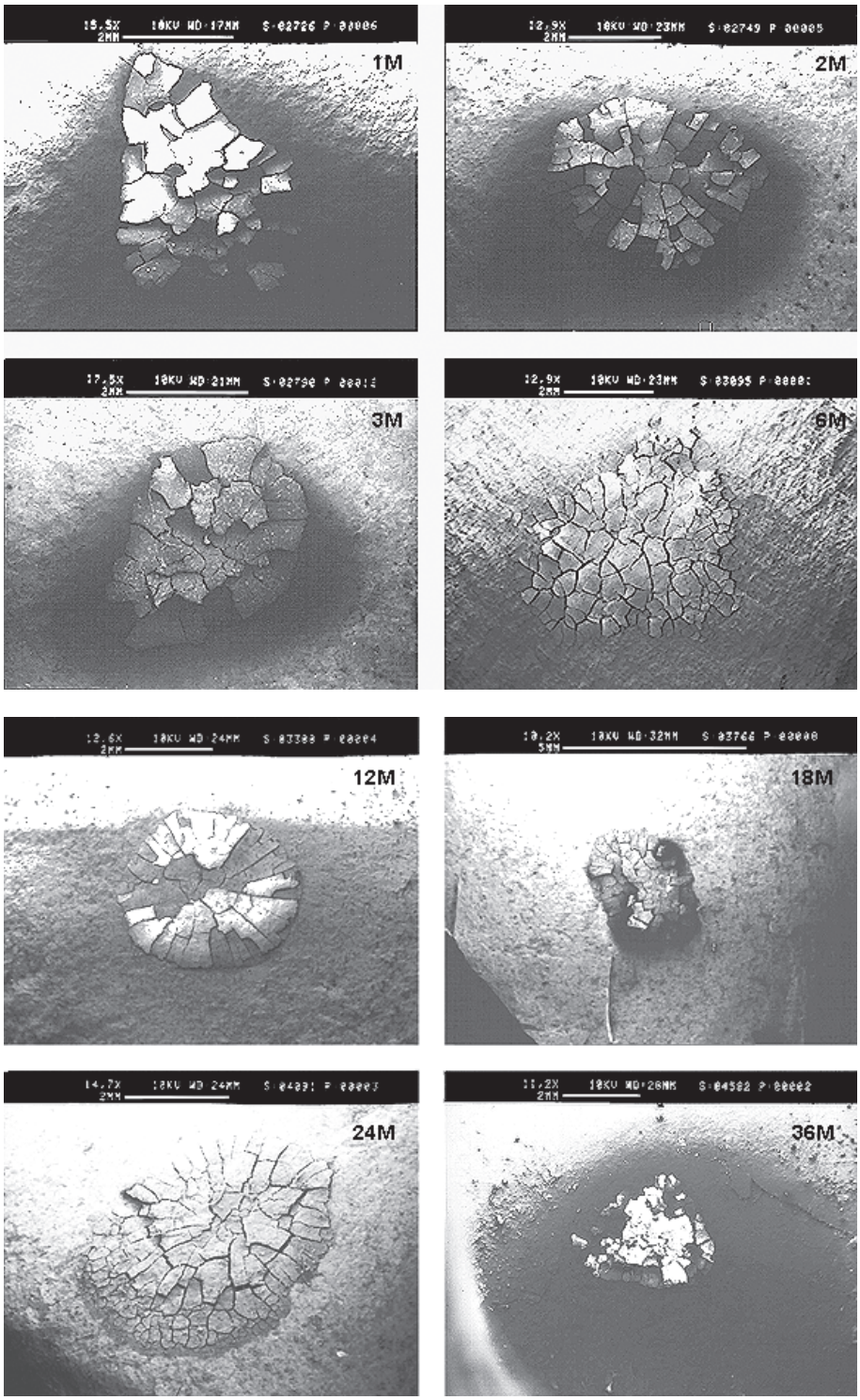

FIGURE 1. Aspect at low magnification of the whole bloodstains. Typical arisen scales, individualised by cracks, may be seen. Samples according to their age in months (M). Scale bars are 2 mm, except for 18 M (5 mm).

Whereas the burial of a sample provides a greater similitude to final taphonomic conditions, the unburied storage permits a more accurate evaluation of the possible influence of the meteorological variables on the diagenetical changes of a biological sample. At the same time, it permits a full watching of the sample evolution, minimising the possibility of cytomorphological misinterpretations during microscopic examination. Furthermore, assuming that burial provides a more favourable environment for bloodstain preservation (Loy 1983), these non-burial conditions are intended as some of those less favourable to the smear preservation, similar to the conditions which would affect a pre-buried archaeological bloodstain.

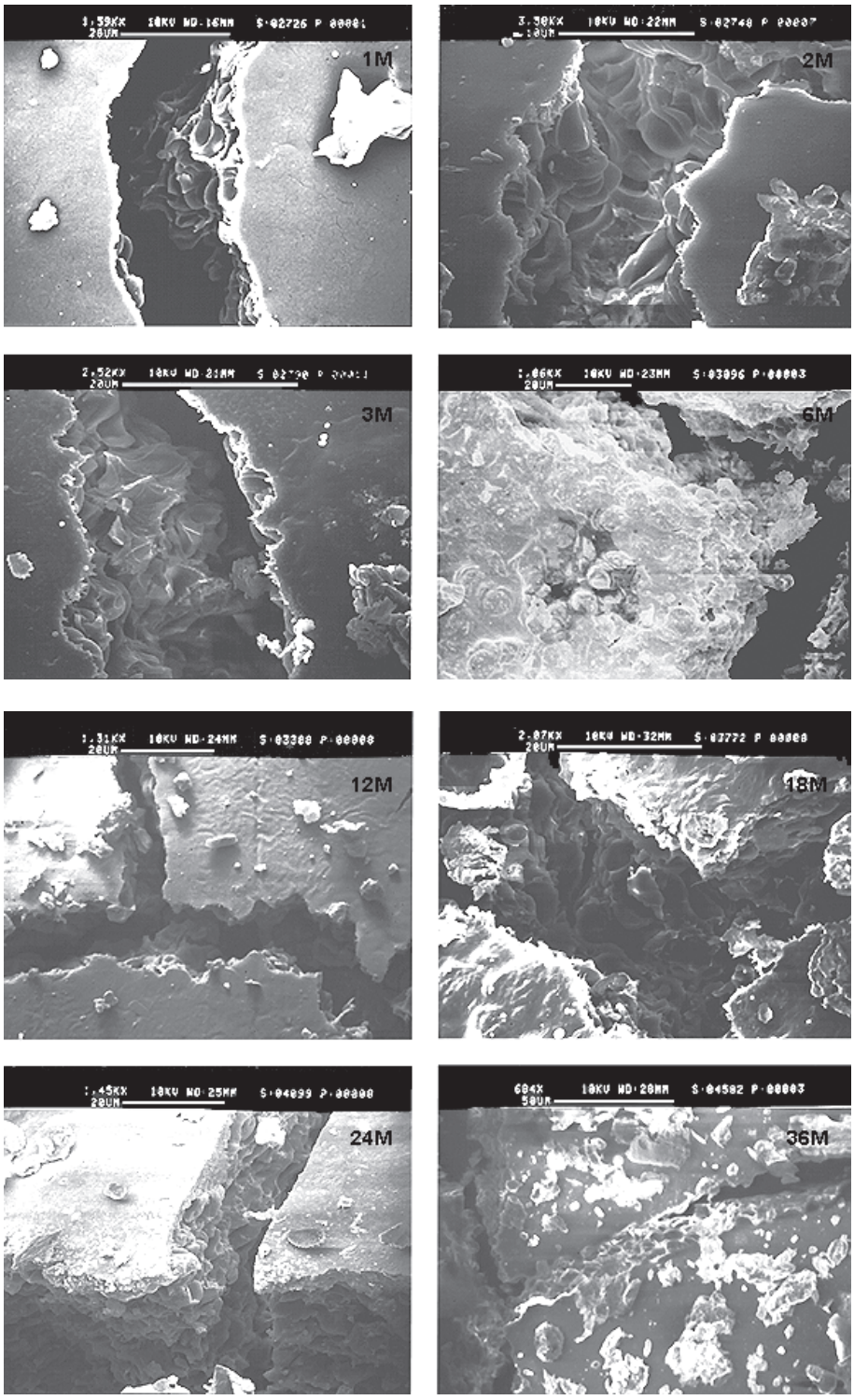

FIGURE 2. RBC acme-zones with packed erythrocytes in the central and medial smear cracks. In these structures, RBC mainly attained the stomatocyte morphology. Samples according to their age in months (M). Scale bars are 20 μm, except for 2 M (10 μm), and 36 M (50 μm).

CONCLUSIONS

At least moderate exposure to ultraviolet light from the sun and to air does not appear to be critical in the preservation of human RBC in bloodstains on stone. This is consistent with other previous observations, where non-human, artiodactylian bloodstain exposure to both sun and air was also done (Hortolà 1992b, 2001), and it does not agree with the previous statement that these factors lyses most of the RBC in a bloodstain (Loy 1983).

Moreover, provided that moon-like shapes and negative replicas are not found in physiological conditions, they are interpreted as two bloodstain-characteristic RBC morphologies, which would be due, respectively, to erythrocyte – plasma interaction when drying and to moulding by dried plasma matrix. Such RBC acme-zones

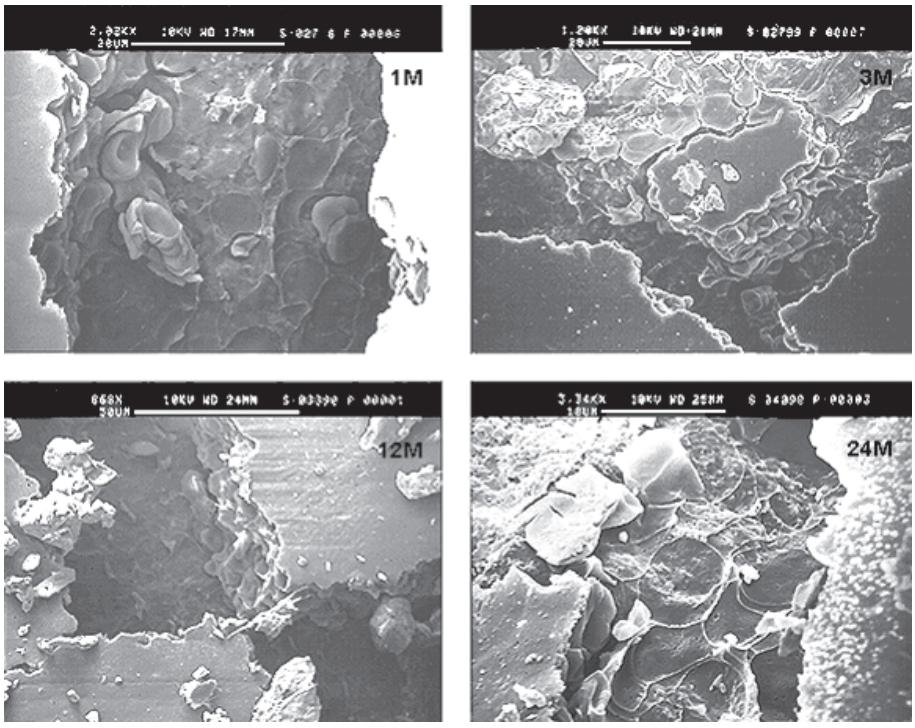

FIGURE 3. Examples of negative replicas at the bottom of thin cracks, in the smear periphery. Samples according to their age in months (M). Scale bars are 20 μm (1M, 3M), 50 μm (12M), and 10 μm (24M).

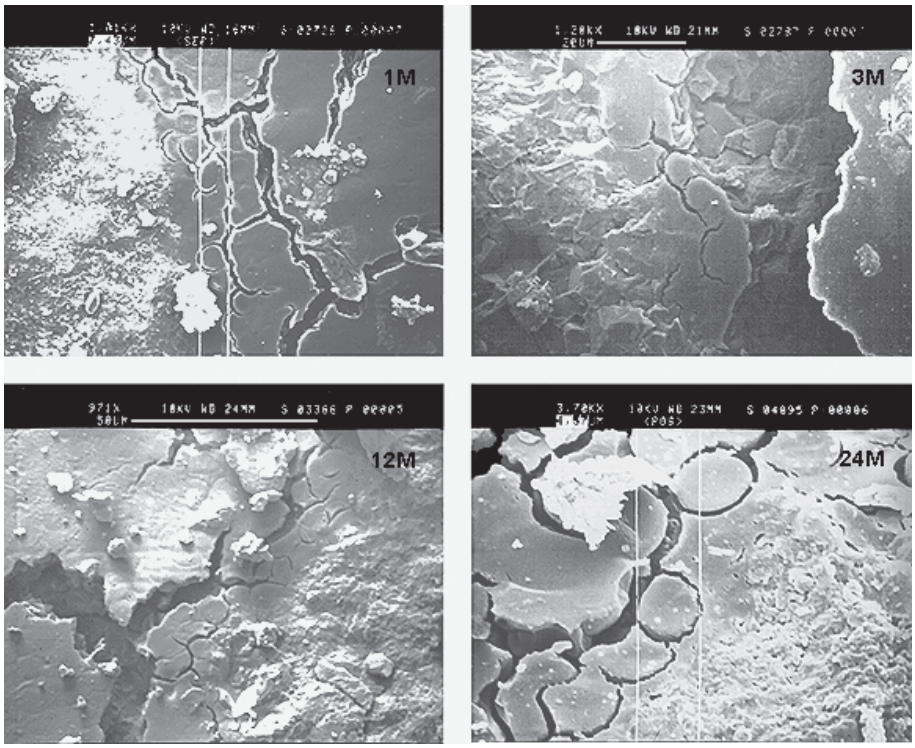

FIGURE 4. Examples of moon-like shapes in the (peripheral) smear boundaries. The white lines in 1M and 24M confine two moon-like shapes which evidence the wide range of RBC size variability that can be found in a bloodstain. Samples according to their age in months (M). White lines are 7.43 μm (1M), and 4.57 μm (24M); scale bars are 20 μm (3M), and 50 μm (12M).

visible in the thick-smear cracks mainly as stomatocytes demonstrated to be time independent, as were the smear-common negative replicas and moon-like shapes.

Prehistoric human bloodstains could mainly have originated by accidental slicing during stone tool manufacturing (Bahn 1987, Loy, Wood 1989). However, since indicia or even evidence of hominid cannibalism have been found (e.g., Defleur *et al*. 1999, Fernández-Jalvo *et al*. 1999, Marlar *et al*. 2000), the answer to the question on which characteristic RBC shapes may be expected in the presence of a thick bloodstain on a stone tool, when such indicia or evidence are pointed out, has clear applications to the knowledge of this aspect of hominid behaviour, and represents a link between lithic analysis and hominid palaeoethology.

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