

Application of Electron Microscope Technique in Anthropological Research: A Review

Anjali Haloi

*Sophisticated Analytical Instrument Facility, North-Eastern Hill University,
Shillong, Meghalaya, India*

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ABSTRACT The purpose of this paper is to review the use of Electron Microscope (EM) techniques in the field of anthropological research. This technique has not been extensively used by anthropology researchers in India, although it can provide a wide range of information about the unseen world in anthropological research. Anthropologists have traditionally made significant contribution in fields like growth and nutrition, reproductive health, genetic disorders, excavated human remains among others by traditional anthropometry methods. In many an instance, one can also utilize or add up the EM facility for such studies. The author outlines the basic sample preparation methods to be selected for the Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM) systems. Examples of application of EM in anthropology and the contributions of researchers in India and elsewhere are discussed.

INTRODUCTION

Anthropology is no longer a single discipline, but rather a range of different practices carried out in a variety of social settings. It is in this context that the nature and purpose of social knowledge, and in particular anthropological knowledge, comes into particular focus (Moore 1996). Important new questions in anthropology now calls for sustained research with traditional methods complemented with modern analytical techniques from the life and physical sciences including molecular biology and x-ray crystallography among others.

One such technique which has been in usage in anthropological research is the Electron Microscope (EM). Although the invention of EM was done in 1930s, its use in practical field especially in anthropology is very few. Here, in this paper author would like to mention about few applications of EM and review some of the work done by researcher across the world in the field of anthropology.

In the 17th century Anthony Leeuwenhock invented the first light microscope that produced magnified images of very small apparently invisible objects perceivable to human eye. Advanced models of the microscope were later developed

which produced clearer images with higher magnification and better resolution.

In a light microscope, a beam of light is directed through a thin object and a combination of glass lenses provide an image which can be viewed by our eyes through an eyepiece. The image formed is realistic because it uses multi colour light. Visible light has wave like nature with a wave length of 400 – 800nm. Since the resolution cannot be less than half the wavelength, the ultimate resolution attainable by using the light microscope is 200 nm. This corresponds to a magnification of 1000 times as compared to an eye. In the 1920's it was discovered that accelerating electrons behave in vacuum just like light. They travel in straight lines and have a wavelength which is about 100,000 times smaller than that of light. Furthermore, it was found that electric and magnetic field have the same effect on electrons as glass lenses and mirrors have on visible light. The discovery formed the basis of electron microscopy.

An electron microscope (EM) uses an electron beam to illuminate a specimen and produce a magnified image. Electron microscopes are used to investigate the ultrastructure of a wide range of biological and inorganic specimens.

Because of the unique capabilities of EM in terms of resolution and magnification, they have been extensively used by physical and life science researchers. Where does the anthropologist stand in the current scheme of things? This is the question which prompted the author to work on this paper.

Address for correspondence:

Dr. Anjali Haloi
Sophisticated Analytical Instrument Facility,
North-Eastern Hill University, Shillong 793022
Meghalaya, India
E-mail: anjali.ghy@gmail.com

According to Jennie (2005), SEM is an increasingly useful tool for anthropologists. Laboratory analyses are central to bio-cultural anthropology and archaeology, and access to state-of-the-art equipment will help researchers in anthropology, engage with the leading edge of research and keep current with new developments. A SEM makes high-magnification, non-destructive analyses possible, opening many new research opportunities. Archaeology researchers would be able to study the surface of sand grains to understand how archaeological sites formed, analyse the composition of thin sections of sediment, pottery and stone artefacts to understand prehistoric environments, technology, artifact composition and function, identify microscopic plant remains such as pollen and phytoliths, and examine details of bone damage and growth. In brief, it is an opportune time for anthropologists to explore their field of interest with the aid of this versatile instrument.

The high cost of an electron microscope and the necessary infrastructure as also trained personnel for its operation makes it out of bounds for a large majority of researchers. Fortunately in India, the Department of Science and Technology (DST) has established central instrument facilities in a number of universities and institutes across the country, wherein EM analyses can be

done at nominal expenses. An indicative list of institutes where EM analysis can be availed is provided for the benefit of interested researchers (Table 1).

While most EM facilities would process and prepare samples to be viewed under an electron microscope on their own, it is nonetheless worthwhile for intending researchers to have an overview of the process of sample preparation which itself is critical in the outcome of the final image.

Standard Procedure of Sample/Specimen Preparation for TEM

Step 1. Sample should be cut into small cube of 1-2 mm size, it is advisable for live specimens to be washed in fixative during dissection.

Step 2. *Fixation* in Karnovsky's fixative or in 3% Glutaraldehyde for 2 to 4 hours at 4°C.

Step 3. *Washing* with 0.1M Buffer (used to prepare the fixative, usually sodium cacodylate or phosphate buffer). 3 changes of 15 minutes each at 4°C.

Step 4. *Post Fixation* is carried out with 2% OsO₄ in 0.2M Sodium cacodylate buffer or 0.1M Phosphate buffer (used to prepare the fixative) for 2 hr at 4°C.

Step 5. *Washing* with 0.1M Buffer (used to prepare the fixative). 3 changes of 15 minutes each at 4°C.

Table 1. A list of institutes with EM facility

<i>Institute</i>	<i>Address</i>
IIT Bombay	Sophisticated Analytical Instrument Facility (SAIF), IIT-Bombay, Powai, Mumbai-400 076
IIT Rorkee	Institute Instrumentation Centre, Indian Institute Of Technology, Roorkee, Roorkee-247 667
IIT Chennai	Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Chennai-600 036
Bose Institute	Sophisticated Analytical Instrument facility Bose Institute Kolkata-700 009
Central Drug Research Institute	Sophisticated Analytical Instrument Facility, Central Drug Research Institute, Lucknow-226031
North Eastern Hill University	Sophisticated Analytical Instrument Facility, North Eastern Hill University, Shillong- 793022
Panjab University	Sophisticated Analytical Instrumentation Facility (SAIF), Panjab University, Chandigarh- 160 014
AIIMS	Sophisticated Analytical Instrument Facility (For Electron Microscopy), All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110 029.
Kochi University	Sophisticated Analytical Instrument Facility, Sophisticated Test and Instrumentation Centre, Kochi University, Kochi-682 022
The National Electron Microscope Facility (NELMIF)	Department of Metallurgical Engineering, Institute of Technology, Banaras Hindu University, Varanasi - 221005
Central Facility for transmission and scanning electron microscopy	Institute of Medical Sciences, Banaras Hindu University, Varanasi-221 005

Step 6. Dehydration

o 30 % Acetone	- 15 minutes	x	- At 4° C
	2 changes		
o 50 % Acetone	- 15 minutes	x	- At 4° C
	2 changes		
o 70 % Acetone	- 15 minutes	x	- At 4° C
	2 changes		
	(can be left over night)		
o 80 % Acetone	- 15 minutes	x	- At 4° C
	2 changes		
o 90 % Acetone	- 15 minutes	x	- At 4° C
	2 changes		
o 95 % Acetone	- 15 minutes	x	- At 4° C
	2 changes		
o 100 % Acetone	- 15 minutes	x	- At 4° C
	2 changes		
(Dry Acetone)			
Dry Acetone	- 2 changes after every 30 min. each		- At room temperature

Step 7. Clearing:

Propylene Oxide - 2 changes after every 30 min. each - At room temperature

Step 8. Infiltration:

1 part of Embedding Medium and 3 parts of Propylene Oxide

(can be left over night)

2 parts of Embedding Medium and 2 parts of Propylene Oxide - 1 hour

3 parts of Embedding Medium and 1 part of Propylene Oxide - 1 hour

(Under vacuum)

Pure Embedding Medium - 1 hour (Under vacuum)

Step 9. Embedding:

Embedding the sample in pure Embedding Medium using Beem capsules or flat embedding mould (if orientation of the specimen is required).

Step 10. Polymerization:

The embedded blocks are kept in the Embedding oven at 50°C for 24 hours. Then raise the temperature to 60°C and keep for 24- 48 hours.

Step 11. Ultramicrotomy. The ultrathin sections are collected on to grids.

Step 12. Staining: Sections are then stained by double staining method with lead citrate and uranyl acetate to get good contrast while viewing in the TEM.

Standard Procedure of Sample/Specimen Preparation for SEM

Step 1. Primary fixation: 2-3mm size samples should be fixed in 2.5 -3% Glutaraldehyde or Karnovsky's Fixative

Step 2. Washing: Washed in 0.1M Sodium Cacodylate Buffer 3 changes of fifteen minutes each at 4 °C

Step 3. Post-Fixation (Optional): 1% Osmium Tetroxide in 0.1M Sodium Cacodylate Buffer at 4 °C.

Step 4. Washing : Washed in 0.1M Sodium Cacodylate Buffer 3 changes of fifteen minutes each at 4 °C

Step 5. Dehydration: All steps to be carried at 4 °C

o 30 % Acetone	-15 minutes	x	- At 4° C
	2 changes		
o 50 % Acetone	-15 minutes	x	At 4° C
	2 changes -		
o 70 % Acetone	-15 minutes	x	- At 4° C
	2 changes		
	(can be left over night)		
o 80 % Acetone	-15 minutes	x	- At 4° C
	2 changes		
o 90 % Acetone	-15 minutes	x	- At 4° C
	2 changes		
o 95 % Acetone	-15 minutes	x	- At 4° C
	2 changes		
o 100 % Acetone	-15 minutes	x	- At 4° C
	2 changes		
	(Dry Acetone)		

Step 6. Drying :

o Critical Point drying: Samples are to be dried with Liquid Carbon dioxide at its Critical Point that is, 31.5 °C at 1100p.s.i.

o TMS method: the specimens are immersed in Tetra Methyl Silane for 5-10 minutes for two changes at 4° C. Then they are brought to room temperature (25-26 °C) to dry.

Step 7. Mounting: The specimen are mounted on Brass or Aluminum Stubs

Step 8. Sputter coating: Coating is carried out using silver or gold, the coating should be of about 35 nm thick.

The sample preparation procedures listed above may look daunting to the uninitiated researcher. In any case, samples for EM analysis need only be prepared till step 2 (fixation) before they are accepted by most centres for further processing.

The requirement of fixation for electron microscopy is very crucial since the fixative should preserve the organization of the cell down to the macro molecular pattern. In order to protect this pattern from the solvents used in embedding, from the mechanical forces involved in the sectioning, and from the action of the electron beam, the fixative should replace the weak intermolecular associations with more stable bonds to pre-

vent the collapse of the molecular structure. After pre and post fixation, specimen should be rinsed thoroughly to wash off the excess fixative with buffer which is the best vehicle and allows the least amount of extraction of cellular materials and also maintain the pH. The water is removed by passing the samples through a series of solution of ascending concentration of the dehydrating agent. After dehydration, for SEM, the sample needs to dry by critical drying method and then mounted to facilitate viewing in the instrument. However for TEM, though acetone is easily miscible with resin, it is advantageous to clear it off with a clearing agent to facilitate penetration of resin. Very few tissues are rigid enough to be cut into thin sections without an additional support. Therefore tissue must be attached to a block which is sufficiently strong to be handled safely during sectioning. This can be accomplished by infiltrating resin into the tissues and further hardening it, so that it will take the form of a block embedded with tissue. Infiltration is done by gradually decreasing the concentration of clearing agent and proportionately increasing the concentration of embedding medium. Embedding is done in the embedding medium using flat embedding mould or beam capsule. Polymerization is the process by which liquid resin is hardened. This is usually done by heat. After polymerization, the blocks are removed from the capsules or flat embedding moulds and are trimmed to produce a pyramid form at the tip of the block where the tissue is located. For cutting ultrathin sections use fresh glass knives or a diamond knife. The sections are then picked onto grids. To obtain a good contrast, a double staining method using uranyl acetate and lead citrate is routinely followed.

APPLICATION OF ELECTRON MICROSCOPE IN ANTHROPOLOGY

Because electron microscopes use a beam of highly energetic electrons to examine objects on a very fine scale, the examination can provide unique information. That includes, topography—the surface features of an object, its texture, direct relation between these features and materials properties and morphology—the shape and size of the particles making up the object that related between these structures and material properties which character specially in the biological anthropological fields. Electron micros-

copy has been widely applied in life science and material science studies, anthropologist world over have also successfully used EM in various fields. Some important areas of anthropology research where EM has been applied and the techniques of sample preparations shows the wide applicability of this technique for the ingenious researcher.

Indicative Research Fields

a) Studies on Bone and Fossil remains

In order to explain the diversity within and between human populations, physical anthropologists must study past populations of fossil hominins as well as the non-human primates. The microscopic examination of fossilized bone tissue is a sophisticated and increasingly important analytical tool for understanding the life history of ancient organisms. Bone must be considered not only as a structural member but also as a mineral bank that can be drawn upon in times of need. It is important therefore to recognize that bone tissue appears morphologically as though it is under the control of bone cells. Bone, being a mixture of hard mineralized matrix and soft cells can be a difficult tissue to prepare for electron microscopy but the methods that are used are basically the same as for other tissues. To process the undecalcified bone for EM study one has to obtain a biopsy of human iliac crest bone by using an electric drilling machine. Slices about 1 mm thick are cut from the biopsy bone cylinder with a razor blade (Schulz 1977) and then followed by standard EM protocol for sample preparation.

Conventional ultramicrotoming of fully mineralized cortical bone results in significant distortions of its internal structure. Ion milling and the related technique of focused ion beam (FIB) milling produce essentially no distortion because no stress is applied to the bone during milling. In an attempt to improve understanding of the ultrastructure of bone, McNally et al (2012) have used a relatively untried method of sample preparation, cryo-ion milling, to prepare sections for TEM analysis. They showed that analogous structures to those seen in human cortical bone can be observed in other human bones (including trabecular bone) and in bones of other vertebrate species.

b) Study of Conjunctiva

Relatively few morphological studies on human cornea have been performed due to the difficulty in obtaining material. The specimens may be obtained either by superficial scraping-off, limited to the epithelial layer, or from corneal buttons obtained at keratoplasty. Conjunctiva, on the other hand, may be obtained by painless biopsy. Impression cytology usually removes only 1–3 cell layers and does not yield the same information as a flat mount or cross section preparation of the ocular surface (Singh et al. 2005).

c) Palaeo-anthropology

The investigation of human skeletons gathered from archeological and anthropological excavations is of capital importance for the enlightenment of human history. Every piece of human skeleton is an important data source in this process. Teeth, the most durable pieces of the body against all factors, can be protected without deterioration and is found intensively in excavations. The relationship between tooth of living species and nutrition is so important and a constant research topic for anthropologic researches that gives information about diet, environmental relationships and cultural structure. Several recent studies have demonstrated the efficacy of dental microwear analysis (DMA) for dietary reconstruction among nonhuman primates, early hominids, and prehistoric humans. It has been proved that the food stuffs, which living species consumed, have a fundamental role on tooth wear. The micro marks have several characteristics about the ingredients, hardness, size and chewing force of the nutrients (Serpil et al. 2013).

Researchers have recognized for more than three decades that patterns of microscopic use wear on teeth hold the potential to provide information about the diets of early hominids. Dental microwear, usually analyzed using scanning electron microscopy (SEM) techniques, is a good indicator of the abrasive potential of past human population diets. Dental casting is a very common procedure for making high-quality replicas of paleo-anthropological remains. Several commercial products can be used in molds. High-resolution casts are studied rather than original teeth because they are more convenient, often more durable, and relatively easy to curate. Casts were made of a single, second mandibular molar

from each individual. The casting process began with the rinsing of the original teeth with ethyl alcohol (95% solution) and then allowing them to air-dry. Molds of the original teeth were taken with a polyvinyl siloxane impression material, Coltene's President Jet (light body). After setting up for 6–10 min, the molds are carefully removed and allowed to de-gas overnight. The molds were rinsed with alcohol, allowed to air-dry, and filled with super hard epoxy resin at a hardener to base ratio of 4 to 1. The hardener and base are thoroughly, manually mixed before it being placed into the molds with a plastic pipette. Care has to be taken to avoid trapping air bubbles between the mold and resin. Each filled mold are placed in a manual centrifuge for 15–30 sec. The centrifuge forces the resin deep into the relief of the mold and help remove air bubbles from the interface of the molding and casting materials. The casts are allowed to harden overnight. Preparation for electron microscopy included mounting the casts on 10-mm aluminum stubs with copper tape and conductive silver paint, and then sputter-coating them with 200 Å of gold-palladium (Christopher 2001).

d) Forensic Anthropology

Scanning electron microscopy is often used in investigating forensic evidence. The areas of forensic medicine where the above methods have already been used, as well as the results obtained, are with hairs, bones, muscle and skin.

The advantages of using the Scanning Electron Microscope (SEM) for human hair studies far surpass the technique of light microscopic studies. Determinations of surface characteristics such as scale count, hair diameter, surface debris, hair shape, scale structure and surface damage, whether physical or chemical may be significant in investigative crime studies. Use of an optical light microscope gives very poor topographic resolution of hair features. While the Transmission Electron Microscope (TEM) gives better resolution, there is still the matter of replication of the surface of the sample to be investigated, which gives another possibility for error in investigation. The SEM is not only a better tool for studies, since the depth of field and resolution of topographical work is better, but preparation is rapid and easy. Hair samples are mounted on metal stubs with either double-stick cellophane tape or conductive paint on the ends; the

hairs are then metal vaporized with a thin layer of aluminum, coating the sample with a featureless metal at a thickness of less than 200 Å, which is under the resolution of the SEM. This enables the sample to be observed without disturbing it; observations are made of the sample directly without replication. Other forensic samples like bone study and its preparation are mentioned above whereas samples like muscle, skin etc. are to be processed following standard protocol for EM.

e) Human Genetics

DNA carries all our genetic information across the generations. The 1953 breakthrough describing the double-helix was confirmed using x-ray crystallography. Direct imaging becomes important when the knowledge at few/single molecule level is requested and where the diffraction does not allow to get structural and functional information. Gentile et al. (2012) recently reported on the direct imaging of double stranded β -DNA in the conformation, obtained by combining a novel sample preparation method based on super hydrophobic DNA molecules self-aggregation process with TEM. The experimental breakthrough is the production of robust and highly ordered paired DNA nano fibers that allowed its direct TEM imaging and the double helix structure revealing. The new research is different because it visualizes DNA directly with an electron microscope. The new research created a water repellent surface that caused liquid in samples to dry out very quickly and deposit suspended strands of DNA. The surface is made up of tiny micro pillars that catch the DNA, holding it up where images can be taken. The results are pretty amazing - it can actually show the repeating spiral pattern of DNA. The work could eventually allow scientists to closely study the way proteins and chemical agents interact with DNA. A variety of preparation methods are available for TEM and SEM including ultra-thin sections of conventionally fixed samples, immunogold labeling, plunge-freezing cryo-EM and tomography. EM of DNA is commonly done by the kleinschmidt method, in which the nucleic acid is first absorbed to a positively charged protein monolayer formed at an air-water interface. The spread DNA is then picked up on a film (for example, nitro cellulose) supported on an EM grid. In an earlier study on kinetoplast DNA, the mitochon-

drial DNA of trypanosomatids, Perez-Morga and Englund (1993) had developed a technique of DNA sample preparation for EM studies. Using a common modification of the kleinschmidt method, this technique requires much smaller amount of DNA for each spreading.

Availability of ancient DNA is important for studies in forensic anthropology, migration and lineage studies and identification of diseases etc. Several factors like well preserved micromorphology, with small areas of localized demineralization, lamellae integrity, compact appearance of bone in scanning electron micrographs and high collagen content has been correlated with preservation of DNA in archaeological bones (Coulson-Thomas et al, 2015). The authors analyzed medieval human bone samples by transmission electron microscopy, and observed intact osteons and an apparently well-organized bone matrix, containing molecules such as collagen and osteocalcin. They were able to isolate DNA from such samples and perform real time PCR amplification of ancient DNA with fragments up to 250 kb.

f) Study of Human Blood

Studying human blood is important in forensic anthropology as well as in human genetics and human health and nutrition and various blood related human diseases etc. Also, blood is the source of DNA extraction from particular individuals and used for comparative analysis. These above mentioned areas can be studied by using EM for detailed information and the method of preparation of blood sample for EM studies includes, immediately after collecting the blood tissue from the subject is fixed with primary fixative and then followed by standard TEM and SEM protocol for sample preparation to study/view in both TEM and SEM. Interesting recent applications of EM in the study of blood stains on weapons and stones have been described by Hortola (2012) where it was suggested that a variable pressure SEM working in low-vacuum mode can be used fruitfully to detect blood remains in medium-sized reed and bone antique aboriginal artifacts. This procedure can prospectively help ethnographic museum curators and aboriginal-art surveyors as an easy guiding test in the valuation of antique traditional weapons.

Contribution of International Anthropologists

The contribution of anthropologist from across the world in the above mentioned areas of research by the sophisticated technique in different peer-reviewed journals is considerable. Apart from the use of traditional anthropometric method along with a few biochemical test in the field of anthropological research, anthropologist today are also relying on EM technique for such research and some of the significant studies in these field are described.

Bartelink et al. (2001) studied the statistical variation in cut mark width between control and test samples on bone using a scalpel blade, paring knife, and kitchen utility knife with the use of SEM. Alunni-Perret et al. (2005) reported on their macro and microscopy study of bone lesions made by a sharp force instrument (a single blade knife), and a sharp-blunt instrument classified as a chopping weapon (a hatchet). Each weapon was used on human bones where emphasis has been placed on the value of SEM as an anthropologist's tool in bone lesion injuries because the microscope facilitates analysis unachievable with macroscopic methods. Some three-dimensional characteristics are also not visible to the naked eye were clearly defined with its use.

The use of TEM for structural and chemical analysis of historical materials was described by Fredrickx et al. (2003). In 17th century Kunckel glass they reported colouring grains of iron oxide (Fe_2O_3) next to the expected gold (Au) particles. In parchments, the authors could elucidate details of the fabrication process

Ubelaker (2009) mentioned that in recent years, research and case experience have greatly augmented knowledge regarding the effects of extreme heat on skeletal remains. As a result of this effort, enhanced interpretation is now possible on such issues as the extent of recovery, reconstruction, trauma, individual identification, size reduction, thermal effects on histological structures, color variation, the determination if remains were burned with or without soft tissue, DNA recovery and residual weight. The rapidly growing literature in this area of forensic science includes experimental research that elucidates the dynamics of the thermal impact on skeletal structure and morphology.

Long-lasting inflammation is a major problem in treatment after severe eye burns and may find expression in an altered elemental composi-

tion of the conjunctiva. Particulate contamination of biological tissue induces such inflammatory processes. In the anterior eye segment, trauma or subsequent therapy may give rise to such contamination. Scanning electron microscopy analysis is able to detect traumatic residues of submicron size and changes of the elemental composition (Schirner et al. 1995). Koufakis et al. (2006) had studied the ultrastructural appearance of the conjunctival surface epithelium in patients with Sjogren's syndrome compared with normal subjects by using TEM and found that the ultra structural morphology of the apical conjunctival epithelium is altered in patients with Sjogren's syndrome. The findings suggest that an intact ocular surface glycocalyx may play a key role in the maintenance of a healthy ocular surface, possibly by preventing abrasive influences on the apical epithelial cells.

Weyrich et al. (2015) have reviewed archaeological dental calculus research. The authors state that scanning electron microscopy (SEM) of archaeological human and animal dental calculus samples was used to further identify food particles and to record the first observation and description of in situ calcified oral microbes by Dobney and Brothwell in 1988. Further studies revealed the extensive and ubiquitous presence of well-preserved calcified microorganisms in human calculus, and formed the first investigations into the ancient oral microbiome.

Christopher (2001) studied the microwear evidence for a paleobotanically suggested change in the types of foods that were consumed by two temporally distinct populations of the North American eastern woodlands. High-resolution casts of adult mandibular second molar protoconid phase II wear facets were viewed via a scanning electron microscope. A dietary transition is evidenced by a statistically significant increase in the mean number of pits and concomitant decreases in scratch width and scratch length from the Late Archaic to the Early/Middle Woodland. Overall, the diet became harder and less abrasive. In palaeo-anthropological research, the relationship between diet, dental morphology, and tooth use is biologically very significant. Specifically, recent changes in our diets imply adaptability to a new lifestyle, and direct consequences of many contemporary health problems can be found. In this context, scanning electron microscopy (SEM) technologies in tooth enamel exploration are an important tool for document-

ing dietary habits in extant and extinct primate species (Romero and De Juan 2012).

Jordi et al. (2006) analyzed SEM image resolution and enamel surface feature definition of tooth molds at various magnification levels. Results, through comparison with the original teeth, show that both the negative molds and the positive casts are highly reliable in replicating enamel surfaces. However, positive cast quality is optimal for SEM observation only till the fourth consecutive replica from the original mold, especially at high SEM magnification levels.

Ancient DNA recovered from 16 Jomon skeletons excavated from Funadomari site, Hokkaido, Japan was analyzed by Noboru et al. (2008) to elucidate the genealogy of the early settlers of the Japanese archipelago, providing direct evidence for the genetic relationships between these populations. It appears that the genetic study of ancient populations in northern part of Japan brings important information to the understanding of human migration in northeast Asia and America. Morris et al. (2011) presented the first structural analysis of the effects of autophosphorylation on the trimeric DNA-PK enzyme, performed by electron microscopy and single particle analysis and observed a considerable degree of heterogeneity in the autophosphorylated material, which is resolved into subpopulations of intact complex, and separate DNA-PKcs and Ku, by using multivariate statistical analysis and multi-reference alignment on a partitioned particle image data set. Bell et al. (2012) directly visualized the sequence of DNA molecules using electron microscopy. This report represents the first identification of DNA base pairs within intact DNA molecules by electron microscopy. Image contrast is further enhanced by using annular dark-field scanning transmission electron microscopy.

Nikfarjam et al. (2003) provided ultrastructural details of the blood supply of colorectal liver metastases and their association with the portal vein and hepatic artery. This study concluded that SEM provides useful additional information on the morphological features of tumour vasculature. Depault et al. (2006) studied the genotoxic effects of potassium chromate (K_2CrO_4) and cadmium chloride ($CdCl_2$) in human blood lymphocytes in vitro as measured by the electron microscopy in situ end-labeling (EM-ISEL). This study brings new information on the utility of EM-ISEL for the evaluation of genotox-

icity and confirms the genotoxic effects induced by chromium and cadmium. Fibrinogen is the key to the maintenance of hemostasis and is an acute phase protein that is part of the coagulation cascade of proteins. It plays a fundamental role in inflammation, particularly as indicator for a pro inflammatory state and is a prominent marker for developing vascular inflammatory diseases. Current research therefore investigates the establishment of a laboratory fibrinogen model to study that might mimic fibrin fiber generation that is achieved using plasma from healthy and diseased individuals. The ultrastructure of fibrin nets can be studied using scanning electron microscopy (SEM) with the addition of thrombin to plasma (Pretorius et al. 2013). In inflammatory conditions such as thromboembolic ischemic stroke and diabetes, the fibrin networks are changed to from dense matted fibrin deposits (DMDs) instead of typical netlike appearance. Author concludes that SEM is a very effective tool for the visualization of circulatory consequences of the interaction of iron-induced hydroxyl radicals with human fibrinogen. Studies of human bloodstains on non biological materials have been previously carried out using a high-vacuum scanning electron microscope (HV-SEM) in secondary-electron mode without any sample treatment by Hortola (2013) to assess whether biological substrates can affect the morphology of human erythrocytes in bloodstains, three fragments of different biological material (bone, shell, and wood) were smeared with peripheral human blood. The obtained results suggest that HV-SEM is suitable for examining untreated bloodstains on biological substrate.

Research in India

The use of EM techniques in anthropological research in India is not very extensive, although researcher from other various disciplines like biological and physical science has made notable contribution of research using this technique.

There are a number of biological studies like, on human blood, human DNA, health and nutrition, human conjunctiva etc. in India using Electron Microscope. For example, in 1993, a team (Dinda et al. 1993) from department of pathology, All India Institute of Medical Sciences, New Delhi had conducted a study on the tumor microblood vessels (MBVs) of 25 cases of gliomas

of varying grades and compared with those in peritumoral region using both transmission and scanning electron microscopy (TEM and SEM). To determine the morphology and mineralization of Sharpey's fibers in bundle bone of humans with reference to interdental area- Ranjith et al. (2005) has taken human interdental alveolar bone between mandibular 1st and 2nd molar teeth and observed under scanning electron microscope (SEM). Sen (2006) studied conjunctival tissue in ocular leprosy. Emphasis in this study was on ultra structural (Cyto-skeletal) changes of conjunctiva, the most superficial (but vital) ocular structure which have to bear the brunt of many diseases, affecting the eye. The author suggests that studies of the cells and bacteria of the transmission electron microscopic level have fascination not only for the sake of knowledge on this complex syndrome, but also for long term preventive measures. To elucidate morphological changes in red blood cells in patients of skeletal fluorosis living in endemic fluoridated areas, the cross sectional study was conducted at Bathinda region of Punjab, India by Aggarwal and Meenakshi in 2012. The SEM analysis revealed multiple discrete blisters on the surface of red blood cells, and formation of hypochromic red cells, leptocytes, stomatocytes, spherocytes, schistocytes, keratocytes, degmacytes, and dacryocytes in patients afflicted with fluorosis. A paper by Misra et al. (2012) deals with the electron micrographs of the enzyme-DNA complex which shows the enzyme as a blob with DNA looping out of it, but they are unable to determine the configuration of the DNA within the blob.

However, no extensive studies have been performed by the Indian anthropologist so far. The biological anthropologist, evolutionist, archaeologist and palaeo-anthropologist has not attempted studies by using EM in anthropological research like, genetics, health and nutrition, forensic anthropology, palaeo-anthropology, etc. where EM can be used to get information in a broader way.

CONCLUSION

Electron Microscopic study in various anthropological researches is valuable for detailed information. Anthropologists, especially in India, are yet to take advantage of this analytical technique in a significant way. Further, this study would like to bring the attention of the anthro-

pological scholars to conduct researches and explore this new site of applied anthropology where, seminar/workshop in this regard may also provide more information and awareness.

RECOMMENDATIONS

No single analytical technique can solve all the research problems. Each technique has its particular advantage. Method of sample preparation for EM study of anthropologic specimens and samples may be the same as for other biological/physical samples or maybe suitably modified. This itself can be an important research contribution by the anthropologist. The author would like to propose the introduction of analytical techniques like electron microscopy in the university curriculum of anthropology courses in India. Practical portion of such courses may be readily conducted at the regional sophisticated instrument centres.

REFERENCES

- Aggarwal S, Meenakshi G 2012. Scanning electron microscopic study of human red blood cell abnormalities in fluoride toxicity. *Int J of Sc Inn and Disc*, 2: 274-289.
- Alunni-Perret V, Muller-Bolla M, Laugier JP, Lupi-Pegurier L, Bertrand MF, Staccini P, Bolla M, Quatrehomme G 2005. Scanning electron microscopy analysis of experimental bone hacking trauma. *J Forensic Sci*, 50: 796-801.
- Bartelink EJ, Wiersema JM, Demaree RS 2001. Quantitative analysis of sharp-force trauma: An application of scanning electron microscopy in forensic anthropology. *J Forensic Sci*, 46: 1288-1293.
- Bell DC, Thomas WK, Murtagh KM, Dionne CA, Graham AC, Anderson JE, Glover WR 2012. DNA base identification by electron microscopy. *Microsc Microanal*, 18: 1049-1053.
- Christopher W S 2001. Dental microwear evidence for a dietary shift between two non- maize reliant prehistoric human populations from Indiana. *AJPA*, 114: 139-145.
- Coulson-Thomas YM, Norton AL, Coulson-Thomas VJ, Florencio-Silva R, Ali N, Elmrghni S, Gil CD, Sasso GRS, Dixon RA, Nader HB 2015. DNA and bone structure preservation in medieval human skeletons. *Forensic Sci Int*, 251: 186-194.
- Depault F, Cojocaru M, Fortin F, Chakrabarti S, Lemieux N 2006. Genotoxic effects of chromium (VI) and cadmium (II) in human blood lymphocytes using the electron microscopy in situ end-labeling (EM-ISEL) assay. *Toxicol In Vitro*, 20: 513-518.
- Dinda AK, Sarkar C, Roy S, Kharbada K, Mathur M, Khosla AK, Banerji AK 1993. A transmission and scanning electron microscopic study of tumoral and

- peritumoral microblood vessels in human gliomas. *J Neurooncol*, 16: 149-158.
- Fredrickx P, Wouters J, Schryvers D 2003. The application of transmission electron microscopy (TEM) in the research of inorganic colorants in stained glass windows and parchment illustrations. *Dyes in History and Archeology*, 19: 137-143.
- Gentile F, Moretti M, Limongi T, Falqui A, Bertoni G, Scarpellini A, Santoriello S, Maragliano L, Zaccaria RP, Fabrizio E 2012. Direct imaging of DNA fibers: The visage of double helix. *NanoLetters*, 12: 6453-6458.
- Hortola P 2012. Human bloodstains on antique aboriginal weapons: A guiding low-vacuum SEM study of erythrocytes in experimental samples on ethnographically documented biological raw materials. *Microscopy Research technique*, 75: 1007-1011.
- Hortola P 2013. Human bloodstains on biological materials: high-vacuum scanning electron microscope examination using specimens without previous preparation. *Microscopy and Microanalysis*, 19: 415-419.
- Jennie JR 2005. A replication technique for scanning electron microscopy: Applications for anthropologists. *AJPA*, 62: 255-261.
- Jordi G, Estebarez F, Martinez L, Romero A, Juan JD, Turbon D, Pe Rez-Pe- Rez A 2006. Comparative analysis of dental enamel polyvinylsiloxane impression and polyurethane casting methods for SEM research. *Microscopy Research and Technique*, 69: 246-252.
- Koufakis DI, Costas HK, Lazaros IS, Athanasia A, Anastasios KM, Dimitris ZC 2006. Conjunctival surface changes in patients with Sjogren's syndrome: A transmission electron microscopy study. *Invest Ophthalmol Vis Sci*, 47: 541-544.
- McNally EA, Schwarcz HP, Botton GA, Arsenault AL 2012. A model for the ultrastructure of bone based on electron microscopy of ion-milled sections. *PLoS One*, 7(1): e29258
- Misra JC, Mukherjee S, Das AK 2012. A mathematical model for enzymatic action on DNA knots and links. *Mathematical and Compu Modelling*, 39: 1423-1430.
- Moore HL 1996. *The Future of Anthropological Knowledge*. London: Routledge.
- Morris EP, Rivera-Calzada A, Fonseca PCAD, Llorca O, Laurence HP, Spagnolo L 2011. Evidence for a remodelling of DNA-PK upon autophosphorylation from electron microscopy studies. *Nucleic Acids Research*, 1-11. doi: 10.1093/nar/gkr146.
- Nikfarjam M, Muralidharan V, Malcontenti-Wilson C, Christophi C 2003. Scanning electron microscopy study of the blood supply of human colorectal liver metastases. *Eur J Surg Oncol*, 29: 856-861.
- Noboru A, Shinoda K, Umetsu K, Matsumura H 2008. Mitochondrial DNA analysis of Jomon skeletons from the Funadomari site, Hokkaido, and its implication for the origins of Native American. *AJPA*, 138: 255-265.
- Perez-Morga DL, Englund PT 1993. Microtechnique for electron microscopy of DNA. *Nucleic Acids Research*, 21: 1327-1328.
- Pretorius E, Vermeulen N, Bester J, Lipinski B 2013. Novel use of scanning electron microscopy for detection of iron-induced morphological changes in human blood. *Microsc Res Tech*, 76: 268-71.
- Ranjith S, Madhavan RN, Ramanathan RS, Ramachandran CR 2005. A preliminary study on human interdental alveolar bone using scanning electron microscopy and energy dispersive X-ray spectroscopy. *JOM-FP*, 9: 6-11.
- Romero A, De Juan J 2012. SEM, teeth, and palaeoanthropology: The secret of ancient human diets. In: H Schatten (Ed.): *Scanning Electron Microscopy for the Life Sciences*. New York: Cambridge University Press, pp. 236-256.
- Schirner G, Schrage NF, Salla S, Reim M, Burchard WG 1995. Conjunctival tissue examination in severe eye burns: A study with scanning electron microscopy and energy-dispersive X-ray analysis. *Graefes Arch Clin Exp Ophthalmol*, 233(5): 251-256.
- Schmidt CW 2001. Dental microwear evidence for a dietary shift between two non-maize reliant prehistoric human populations from Indiana. *AJPA*, 114: 139-145.
- Schulz A 1977. A reliable method of preparing undecalcified human bone biopsies for electron microscopic investigation. *Microsc Acta*, 80: 7.
- Sen S 2006. Transmission Electron Microscopic Study of the Ultra-Structural Changes of Conjunctival Tissue in Ocular Leprosy. *Bengal Ophthalmic Journal*. From <<http://www.oswb.org/journal/journal06/>> ((Retrieved on 6 October 2013)).
- Serpil O, Yavuz AY, Erol AS 2013. The relationship between microwear on human teeth and nutrition: Samples from ancient Anatolian societies. *Natural Science*, 5: 449-455.
- Singh R, Joseph A, Umapathy T, Tint NL, Dua HS 2005. Impression cytology of the ocular surface. *B J of Ophthalmol*, 89: 1655-1659.
- Ubelaker DH 2009. The forensic evaluation of burned skeletal remains: A synthesis. *Forensic Sci Int*, 183: 1-5.
- Weyrich LS, Dobney K, Cooper A 2015 Ancient DNA analysis of dental calculus. *J Hum Evol*, 79: 119 - 124.