

# Isolation and Identification of Melanosomes from Human Hair

— A New Approach to Morphologic Evaluation —

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Melanosomes were isolated from the human hair by graded centrifugation and identified by transmission and scanning electron microscopic examination. Melanosomes were separated from the keratinous structures by treating with strong NaOH solution for 15 hours. The keratinous structures were removed by centrifugation at 2,500xg and 3,500xg for 10 minutes respectively at 0°C. The isolated melanosomes were collected by centrifugation at 7,800xg at 0°C. Scanning electron microscopic examination made it possible to evaluate the global structure of purified melanosomes. (*Ann Dermatol* 3:(1) 12–14, 1991)

*Key Words:* Isolation of melanosomes, Scanning electron microscopy

Within melanocytes, melanin is formed after the tyrosine-tyrosinase reactions on the melanosomal matrix. Fully melanized melanosomes are transferred to the epidermal keratinocytes and hair matrix cells; thus, they are found in the epidermis and hair.

Isolation of purified and nondegraded melanosomes is necessary for morphologic, biochemical and photochemical analysis. Although many isolation methods have been developed<sup>1-7</sup>, they are inadequate due to their inability to breakdown the keratins without the destruction of melanosomal proteins. Borovansky and Hach<sup>8</sup>, examined all the recently published methods and suggested several principles which minimize the undesirable effects of the disintegrating agents.

In the present study, melanosomes were separated from the human hair by modification of Borovansky's method<sup>1</sup>, and the morphologic evaluation of the isolated melanosomes was per-

formed by transmission and scanning electron microscopy.

## MATERIALS AND METHODS

### Materials

Black hairs from Korean males in their twenties were used.

### Methods

#### *Isolation of melanosomes*

Hairs were washed with cold acetone for 10 minutes and diethyl ether for 10 minutes and air dried. These were treated with 1 N NaOH for 15 hours at 37°C in a shaking incubator. The suspension was centrifuged at 2,500xg for 10 minutes. The first supernatant was centrifuged at 3,500xg for 10 minutes. The second supernatant was centrifuged at 7,800xg for 10 minutes. The precipitate was suspended with distilled water, and this suspension was centrifuged at 7,800xg for 10 minutes. The precipitate was melanosomes. All centrifugation procedures were performed at 0°C.

#### **Electron microscopic examinations**

##### *Transmission electron microscopy*

For transmission electron microscopy, the

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diluted pellet of melanosomes was prefixed in a mixed solution of 2.5% glutaraldehyde and 1% paraformaldehyde and postfixed with osmium tetroxide. After dehydration with graded ethanols, the sample was embedded in Epon, cut into several ultrathin sections, and stained with uranyl acetate followed by lead citrate. The sections were examined in a Hitachi H-500 electron microscope.

#### *Scanning electron microscopy*

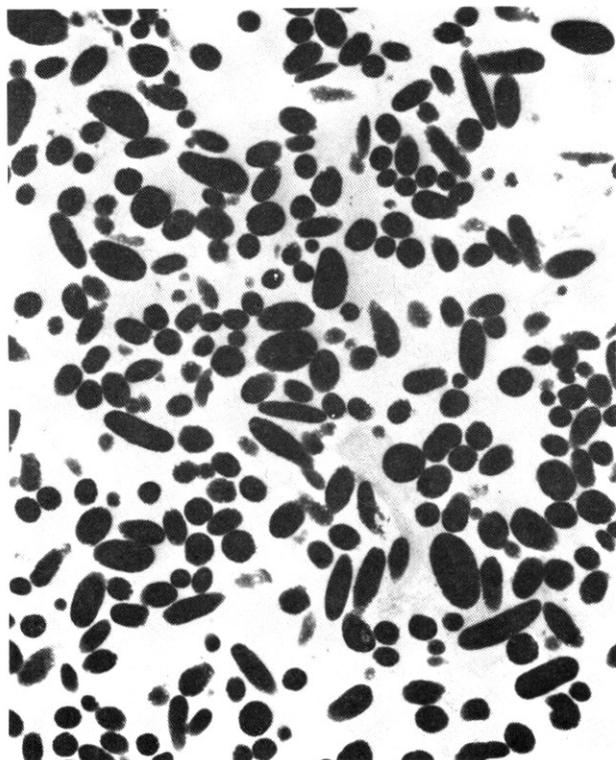
For scanning electron microscopy, the diluted pellet of melanosomes was prefixed in a mixed solution of 1% glutaraldehyde and 1% paraformaldehyde. The prefixed melanosomes were filtered through a 0.4  $\mu\text{m}$  nucleopore membrane filter. The filtered melanosomes were arrayed in a single layer and washed with phosphate buffer (pH 7.4), followed by 1% osmium tetroxide. Following osmitation, the sample was dehydrated

through graded ethanols, transferred to isoamyl acetate, dried with a critical point drier (Hitachi ICP2), mounted on stubs, coated with gold in a sputter coater, and examined through a Hitachi H-450 scanning electron microscope operated at 20 kV.

## RESULTS

On the transmission electron microscopic examination, the isolated melanosomes which were irregularly crosssected showed a lower degree of degradation and contamination of keratins (Fig. 1).

On the scanning electron microscopic examination, the crosssectional features of melanosomes were not observed, but the global morphology of melanosomes which showed varying size and oval to ellipsoidal shape could be observed (Fig. 2).



**Fig. 1.** Transmission electron microscopic findings of isolated melanosomes. No degradation of melanosomes or contamination of keratins are noted ( $\times 12,200$ ).



**Fig. 2.** Scanning electron microscopic findings of isolated melanosomes. The global structures of melanosomes are observed ( $\times 14,000$ ).

## DISCUSSION

Melanosomes are the major determinant of skin color among individuals and prevent the skin damage by nonionizing ultraviolet radiation.

The investigation of melanin and melanosomes was not possible until 1961, when the various subcellular components of melanocytes were separated by sucrose gradient ultracentrifugation<sup>9</sup>. Recently, several reports have been published on melanosomal structure in pigmentary skin disorders<sup>10, 11</sup> and in malignant melanoma<sup>12, 13</sup>.

The isolation of melanosomes from the keratinous structures of hair is difficult because of the resistance of keratin to chemical agents. Finding an agent with a hydrolytic effect sufficiently powerful to liberate melanosomes without causing severe structural damage to the melanosomal proteins has been difficult to achieve<sup>8</sup>. It is generally believed that the denaturation of protein-subcellular particles is dependent primarily on the length of time of the acidic or alkaline treatment and on the concentration of these reagents<sup>1</sup>. Strong acid treatment results in the degradation of melanosomal structures<sup>2</sup>. Attempts to break human hair structure by a mild keratinolytic procedure have not been satisfactory<sup>3</sup>. Alkaline hydrolysis of the keratinous structures is believed to be the method of choice.

Two consecutive centrifugations at 2,500xg and 3,500xg precipitated more keratins and prevented the contamination of keratins. All centrifugation procedures were performed at 0°C to prevent the degradation of melanosomal proteins.

Electron microscopy is the best means for the evaluation of the isolated melanosomes. Transmission electron microscopic examination is useful for the observation of melanosomal internal structure. However, it is not adequate to evaluate the global morphology of melanosomes because of the inconstancy of features due to the direction of the cross-section. In this study, using scanning electron microscopy, the overall morphology of

melanosomes could be observed.

Using this purification and identification method, the morphologic characterization of melanosomes according to age, sex and further biochemical and photobiologic investigations, will be studied.

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