Microwave irradiation for rapid epidermis-dermis separation and improved epidermal cell immunodetection

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Abstract

We explored the effects of microwave irradiation on epidermal-dermal separation and subsequent immunostaining of epidermal cells. Epidermal sheets were obtained after incubation in 0.02 M EDTA in PBS and microwave irradiation with 4 pulses of 420 watts for 5 sec, with a total incubation period of 4 min. The control epidermal sheets were immunostained for Langerhans cells and dendritic epidermal T cells using a conventional immunoperoxidase method. The experimental immunodetection of these cells was assisted by incubating the primary antibodies for 10 min at 70 watts. We showed a simple and rapid method for separation of the epidermal-dermal junction and immunostaining of epidermal cells with optimal morphological preservation.

Key words: dendritic epidermal cells, epidermal/dermal separation, immunohistology, Langerhans cells, microwave

The use of epidermal sheets in immunohistochemical procedures has allowed the characterization of epidermal cells, particularly in skin diseases where the epidermis plays an important role. To this end, epidermal-dermal separation is required for immunostaining procedures in large tissue samples. The epidermal-dermal separation procedures described in the literature are time-consuming, and on some occasions may cause significant tissue damage, thus complicating cell characterization.

Previously, we evaluated the efficacy of different epidermal-dermal separation procedures and concluded that the best separating agent was a solution of 0.02 M EDTA in phosphate-buffered saline (PBS), pH 7.3, compared to treatment with trypsin and thermolysin (Sánchez et al. 1990). The enzymatic treatments resulted in faster separation, but difficulties in removing the enzymes caused great damage to tissue integrity. In addition, although EDTA solution allows good separation, the long incubation period, 150 min at 37° C, may contribute to normal degenerative processes.

Microwave irradiation has been widely used in tissue fixation (Chiu and Chan 1987, Azumi et al. 1990), and its success in immunocytochemistry may be due to the brief exposure of the tissue to the fixative, allowing better preservation of antigens.

Leong and Milios (1986) were the first to use microwave irradiation for immunocytochemistry to shorten the incubation period with primary antibodies recognizing T and B cell molecules in blood samples. Also, Chiu and Chan (1987) accelerated the three steps of immunoperoxidase staining in a microwave oven. In both cases, the results of subsequent experiments were inconsistent (Leong and Milios 1990).

The potential of immunostaining with microwave irradiation has been shown with a limited number of tissues and antigens. Takes et al. (1989) showed a substantial reduction in the total incubation time required for an immunoperoxidase procedure to detect lymphocyte antigens, neural proteins, intermediate filaments, muscle proteins and tissue enzymes in frozen and paraffin sections. Van Vlijmen and Van Erp (1993) enhanced the

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immunocytochemical signals to different cutaneous antigens in psoriasis lesions. These investigators and others proposed the use of different microwave incubation protocols for a distinct series of antibodies based on source and affinity of each particular antigen (Login and Dvorak 1992, Boon and Kok 1994, Marani 1998, Noyan et al. 2000).

In the present study, we used microwave irradiation to improve immunodetection of dendritic cells in separated epidermal sheets. The epidermal-dermal separation and immunoperoxidase staining incubation periods were substantially reduced, and gave excellent and consistent results.

Materials and methods

Epidermal separation

Skin biopsies from the hind footpads of BALB/c mice were cut into 1 mm^2 pieces. Each piece was immersed in a solution of 0.02 M EDTA in PBS, pH 7.3, in 15 × 45 mm (2 cm^3) glass vials. Some samples were incubated under different conditions of microwave irradiation and others following a conventional incubation procedure at 37° C for 150 min. The irradiation protocol is summarized in Table 1. Briefly, pulses of microwave irradiation at different wattage (power percentages) were carried out using a microwave oven (EM-A 500T, Sanyo Electric Co., Osaka, Japan) with a maximum power of 700 watts. Between each pulse of irradiation, samples were incubated on ice.

After each schedule, the skin samples were washed in cold PBS, and the dermis was removed from the epidermis under a stereomicroscope using wooden toothpicks. The criterion for evaluating the epidermal separation was based on the ability to achieve a simple separation without tissue damage.

Monoclonal antibodies

Rat monoclonal antibody NLDC-145, which recognized the scavenger receptor DEC-205 on murine dendritic cells including epidermal Langerhans cells (Kraal et al. 1986), was diluted 1:30 (culture supernatant); a rat monoclonal antibody against Thy-1.2 (30H12), was diluted 1:50 (culture supernatant). Dilutions and immunostaining were carried out using PBS, pH 7.2. Both antibodies were kindly provided by George Kraal, Free University of Amsterdam, The Netherlands.

Immunoperoxidase staining

Immunoperoxidase staining was carried out as described previously (Sánchez et al. 1993) with some modifications for immunocytochemical characterization in epidermal sheets. Briefly, after fixation in fresh acetone for 5 min, the epidermal pieces were transferred to round-bottom microplates, hydrated in PBS, and incubated with primary rat monoclonal antibodies at room temperature for 90 min in the conventional protocol. In the microwave protocol, the microplates were placed in a humid chamber and tested at different incubation times (5, 10, 15, 20 and 30 min) in a microwave with 10% power (70 watts) and very short incubations (2 and 5 min) at medium level 50% power (350 watts). Two glass bottles filled with 100 ml ice-cold PBS were positioned in opposite corners of the microwave chamber as water loads. After primary antibody incubation, the rest of the procedure was carried out at room temperature using biotinylated rabbit anti-rat IgG (Vector Labs, CA) diluted at 1:30 (17 μg/ml) for 45 min, and avidin-biotin-horseradish peroxidase conjugate (Vectastain Elite, Vector Labs, CA) at 1:10 dilution

Table 1. Microwave irradiation schedules for dermal-epidermal separation

Power (%)	*Watts*	*Pulses (no.)*	*Time/pulse (sec)*	*Total time (min)*	*Result**	*Morphological preservation***
	700		2	3	2	1
	700		5	2	4	1
	20		10	10	4	2
	420		5	4	3	4
	350		5	4	2	3
	280		5	4		2
	280		5	8	2	2
	210		5	4	1	2
	210		5	8	2	2
	70		5	8	1	1

*Score for dermal-epidermal separation: 1, none; 2, difficult; 3, easy; 4, spontaneous.
**Score for morphological preservation: 1, poor; 2, moderate; 3, good; 4, optimal.

for 15 min. Five-minute washes with cold PBS were carried out between incubations. The reaction was developed for 5 min with 90 µM H_2O_2 and 3-amino-9-ethyl-carbazole (AEC), final concentration 0.88 mM, which was dissolved in 50 mM N,N-dimethylformamide in 0.1 M acetate buffer, pH 5.2. The epidermal sheets then were washed and mounted on glass slides with glycerin-gelatin. Controls consisted of omission of the primary antibody or use of an antibody of irrelevant specificity at the same concentration.

Results

The results of the epidermal separation are summarized in Table 1. Rapid separations were obtained with microwave pulses at high power (420–700 watts), but the heat generated caused significant tissue damage. No separation was obtained at low power irradiation (70–320 watts). The best result was found with 420 watts (60% power) using four pulses of 5 sec each and intervals of 60 sec at 4° C with a total incubation period of 4 min instead of the 150 min used in the conventional procedure. With this schedule, the epidermal separation was easier and the morphology better preserved than in the conventional method.

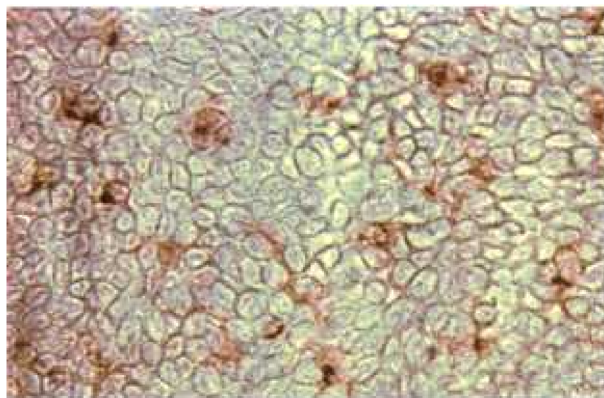
Once we tested different incubation times at low power microwave irradiation to avoid hot spots and their effect on the yield of antigen-antibody complexes, best immunohistologic results were obtained with incubations of 10 min for the primary antibodies, thus reducing the immunostaining period by 89%.

Epidermal sheets immunostained with microwave-assisted primary antibody incubation showed similar cell proportions, but the morphology of immunoreactive cells was preserved better than in conventionally stained tissue (Fig. 1).

Ten-minute microwave irradiations reduced the incubation time without affecting the formation of antigen-antibody complexes, whereas tissues subjected to higher incubation times (15, 20 and 30 min) showed strong and diffuse background and weakly immunostained epidermal cells (Fig. 2). Sheets incubated for a short time at 350 watts (50% power) showed weak immunostaining compared to non-microwaved control tissue (data not shown).

Discussion

Although microwave irradiation has been widely used for tissue fixation and immunostaining procedures, care must be taken to avoid damage to the tissue integrity. Hjerpe et al. (1988) showed that the



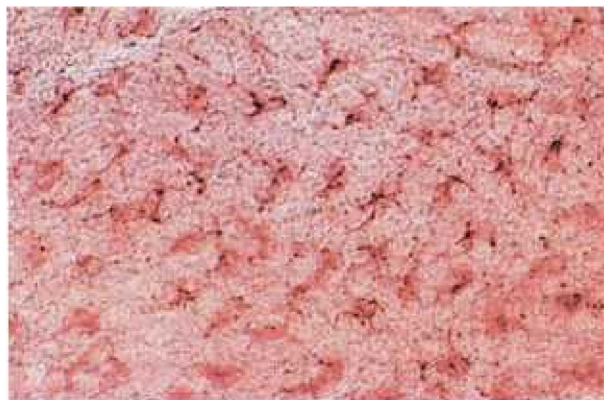


Fig. 1. Detection of DEC-205 positive Langerhans cells on separated epidermis. A) Microwave-assisted epidermal-dermal EDTA separation at 420 watts with 4 pulses of 5 sec during 4 min, and primary antibody incubation at 70 watts for 10 min. × 1000. B) Conventional epidermal-dermal EDTA separation for 150 min, and primary antibody incubation for 60 min. × 1000.

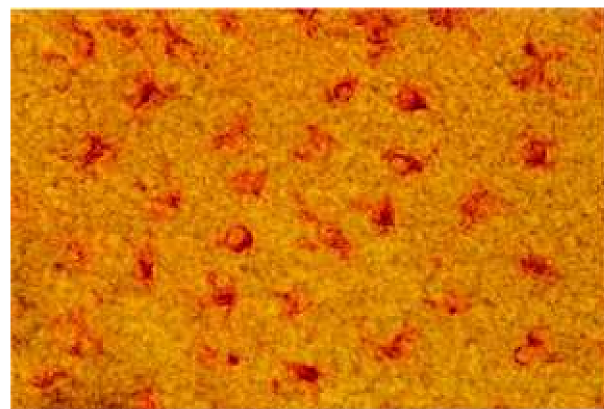


Fig. 2. Detection of DEC-205 positive Langerhans cells on EDTA-separated epidermis. Microwave-assisted epidermal-dermal separation at 420 watts with 4 pulses of 5 sec during 4 min, and primary antibody incubation at 70 watts for 20 min. × 1000.

temperature in the droplets must be controlled to avoid reduction in the formation of antigen-antibody complexes. Moreover, Yasuda et al. (1990) demonstrated that 2 ml PBS water loads increase the temperature by 10° C in 10 sec microwave irradiation at 700 watts (100% power). For that reason, we used irradiation pulses of 5 sec at 420 watts with cooling on ice between each pulse to avoid excessive temperature increase and to favor optimal epidermis separation. This procedure allowed epidermis-dermis separation in only 4 min instead of the 150 min incubation required for the conventional method. The results also showed that 4 min microwave-assisted EDTA rapidly and efficiently disrupted the hemidesmosomal junctions between dermis and epidermis, but that additional incubation time may affect keratinocyte tight junctions, causing severe tissue damage.

The reproducibility of the present microwave-assisted immunocytochemical method for the characterization of epidermal cells was successfully achieved by carrying out the incubations with the secondary biotinylated antibody and the avidin-biotin-peroxidase complex outside the microwave oven to maintain the stability of the reactants.

In the present study, microwave-stimulated immunocytochemistry may favor accelerated diffusion of antibodies through agitation during the incubation time, thus improving the sensitivity of the method. Although the microwave action mode is still unknown, evidence shows that the rapid oscillation of dipolar molecules (i.e., water) and polar molecules (i.e., proteins) causes molecular collisions and acceleration of the chemical reaction rate (Yasuda et al. 1990). In addition, the heat generated by microwave irradiation can contribute to the increase in the reaction rate, but it can also damage biological components such as proteins, antibodies, antigens, and even the general structure of the tissue. For that reason, we used water loads containing the incubation buffer to diminish the heat inside the sample during the extended irradiation step.

We also tested microwave-stimulated secondary fluoresceinated antibody, and obtained appropriate immunostaining of Langerhans cells using 210 watts and irradiation for 10 min with no decrease in the fluorescence stability compared to the conventional indirect immunofluorescence procedure (data not shown).

The microwave device used in the present study is a conventional household oven, not a laboratory microwave with accurate temperature control. Short microwave pulses at specific wattage and appropriate water loads ensure reproducible and successful epidermis-dermis separation and improved epidermal cell immunostaining.

Microwave irradiation is useful for reducing the incubation time for epidermal separation and immunohistology, improving immunostaining detection, and maintaining tissue integrity in separated epidermis.

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