Why Human Prepuce is a Valuable Resource in Science?
Histological, Ultrastructural, Immunohistochemical and Statistical Analysis

¿Por qué el Prepuce Humano es un Recurso Valioso en la Ciencia?
Análisis Histológicos, Ultraestructurales, Inmunohistoquímicos y Estadísticos

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SUMMARY: This research was to examine the histological and ultrastructural characteristics of prepuce samples, as well as vimentin and S100 protein localization and statistical analysis. Urologists have long struggled with the prepuce, which is used to treat a variety of urethral problems. Skin biopsies were collected from the prepuce at the moment of circumcision and processed for light microscopy, electron microscope examination, immunohistochemical techniques, and statistical analysis in a total of six boys. Histologically, the prepuce epidermis displayed focal spiky ridges, which are saw-toothed interspersed with sulci, slight hyperpigmentation, looser connective tissue and plentiful vascular components. Immunohistochemically, the existence of melanocytes and Langerhans cells in the epidermis, as well as smooth muscles in the dermis, was stained positively for vimentin. Also, there was a positive reactivity of the Langerhans cells in the epidermis and around Meissner's corpuscles in the dermis for S100 protein staining. Ultrastructurally, the prepuce's intercellular gaps were widened, melanocytes rested on a folded basement membrane, and desmosomal content was reduced, with a prominent active euchromatic nucleus. Cytoplasmic projections were distended and elongated, and the interstitial blood vessels were surrounded by endothelial cells and rested on a basement membrane. There were also minimal collagen fibers in the interstitium. The prepuce's histological and ultrastructural features, as well as immunohistochemical studies using vimentin and S100 protein as intermediate filaments and statistical analysis, all demonstrated that it is a useful scientific resource.

KEY WORDS: Prepuce; Light Microscopy; Vimentin; S100 Protein; Electron Microscopy; Statistical Analysis.

INTRODUCTION

The foreskin is a unique mucocutaneous tissue that covers the glans penis and serves to shield them from the irritating effects of urine. It is extensively vascularized and innervated (Cold & Taylor, 1999). It has been used to treat difficult wounds and to test novel drugs. In the future, they could be employed to aid in the repair of organs (Glick, 2021). Collagen and elastic fibers are fibrotic components of the cellular matrix that have been linked to pathological changes in various tissues, including the foreskin (Ushiki, 2002; Favorito et al., 2012). Also, it contains free nerve endings, Meissner's corpuscles, and Pacinian corpuscles, all of which have distinct staining behaviors (Martín-Alguacil et al., 2015).

Male urethral reconfiguration is one of the oldest reconstructive surgery problems and has always presented urologists with a significant challenge (Olajide et al., 2010). Surgical repair of severe hypospadias (one of the most
common genital anomalies in male newborns) may require multiple procedures and mucosal grafting of the foreskin (Perovic & Basting, 1999).

Intermediate filaments make up one or more members of a large extended family of cytoskeletal proteins (IFs). In tasks ranging from cell motility to signal transmission, their relevance in regulating cells’ physiological features is becoming well acknowledged (Lowery et al., 2015). All IF proteins are synthesized under strict developmental regulation. Vimentin (VIM) is a type III, IF protein that is the primary cytoskeletal component of mesenchymal cells. Assume that all animal cells contain proteins, as well as tubulin-based microtubules and actin-based microfilaments, which constitute the cytoskeleton. VIM is frequently used as a marker for cells derived from the mesenchyme (Cabeen & Jacobs-Wagner, 2010).

S100 proteins are a family of proteins with a common basic structure that allows them to carry out a variety of intracellular functions related to the regulation of proliferation, energy metabolism, calcium homeostasis, enzyme activities, cell growth and differentiation, and extracellular tasks including such participation in innate and adaptive immune responses, tissue regeneration, and remodeling (Halawi et al., 2014). They additionally act as chemotactic agents and may have a role in the etiology of epidermal disorders like psoriasis. They’re overexpressed in wound healing, skin cancer, inflammation, and cellular stress, among other aspects (Eckert et al., 2004).

Transmission electron microscopy (TEM) is a remarkable technology in cell biology that permits viewing of cells from the ultrastructure to the molecular level (Koster & Klumperman, 2003), and it’s a good opportunity to know more about how they behave. TEM is being used to characterize every cellular component, including organelles, membrane systems, and specialized structures. The loose aspect of the dermal structure adds to high elastin content, which likely contributes to contractility, according to a TEM investigation comparing human foreskin (Tuncali et al., 2005).

The purpose of this study was to look into the unique histologic structure of the human foreskin, with a focus on the histologic structures, immunohistological studies using vimentin and S100 protein as intermediate filaments, transmission electron microscopy to evaluate the fine structures and statistical analysis was performed.

MATERIAL AND METHOD

Collection of human skin tissue. Skin slices measuring 1–2 cm² were inserted in a sterile tube filled with phosphate-buffered saline (PBS) without Ca²⁺ Mg²⁺ containing 1 % penicillin/streptomycin (Invitrogen, USA) and transferred to the lab on ice for rapid processing and storage at 4 °C.

Light microscope procedure. For light microscopy, the specimens were maintained in 10 % neutral buffered formalin for 24–48 hours before being dehydrated in a graded alcohol series, cleaned in xylene, and embedded in paraffin wax. Hematoxylin and eosin (H&E) were used to stain paraffinized specimens, which were subsequently viewed under a light microscope (Olympus CX31, Japan) and photographed with a digital camera (Olympus, Camedia-5060, Japan) (Bancroft & Gamble, 2008).

Immunohistochemistry procedure (IHC). The ready-to-use IHC kit was used on paraffin-embedded, formalin-fixed skin slices (4 µm). Using a streptavidin-biotin peroxidase system and 1:100 rabbit anti-(rat S100) serum (Biogenex, San Ramon, Calif., USA) and anti-Vimentin antibody, Dako Clone V9, the sections were immune-stained. Diaminobenzidine (DAB) was used as the peroxide-sensitive chromogen, which was counterstained with Mayer’s hematoxylin, dehydrated in escalating degrees of alcohol, cleaned in xylol, and mounted with Canada balsam (Nadji & Morales, 1986).

Transmission electron microscope procedure (TEM). Foreskin samples were preserved in 2.5 percent glutaraldehyde for two hours, then trimmed and diced into one cubic millimeter pieces, fixed in glutaraldehyde solution in 0.1 M sodium cacodylate buffer, pH 7.2, and stored in a thermal box cooled to 4°C. They were post-fixed in a sodium cacodylate buffer containing 1 % osmium tetroxide, then dehydrated in an escalating series of ethyl alcohol before being embedded in Spur’s resin. The Electron Microscopy Unit, Pathology Department, College of Medicine, King Khalid University evaluated ultrathin sections stained with uranyl acetate and lead citrate using a TEM (JEM-1011, Jeol Co., Japan) operating at 80 kV (Eid et al., 2021).

Statistical Analysis. The arithmetic mean, median and standard deviation were used to present data. Mann-Whitney non-parametric test of significance was used to detect data of foreskin. The chosen level of significance was 5 %.

RESULTS

Histological findings: The epidermis of the human foreskin and the connective tissue beneath it were shown. The succession of epidermal layers with a higher content of keratinocyte layers and ridges associated with sulci was visible in histological
cross-sections of foreskin specimens (Fig. 1A). Meissner’s corpuscles were more abundant at the crests of ridges, typically in little bunches extending the terminals of papillae that appeared as elongated bodies linked to the basement membranes. With looser connective tissue, the substantial vascular component may be appreciated (Fig. 1B).

**Immunohistochemistry**

**Vimentin Figures:** In foreskin samples, VIM labeling demonstrated strong positivity in epidermal (melanocytes and Langerhans cells) and mesenchymal (such as smooth muscle cells) cells throughout the dermis (Fig. 2A).

**S100 protein Figures:** Langerhans cells were discovered in all strata of the epidermis but are particularly prominent in the stratum spinosum, as demonstrated by S100 protein staining of foreskin specimens. The dermal papillae, which are near to Meissner’s corpuscles, also showed a strong reactivity (Fig. 2B).

**Transmission electron microscope (TEM):** Different layers of the epidermal skin with keratinocytes dispersed among them, melanocytes were resting on a folded basement membrane with its lamina densa of the dermo-epidermal junction. Minimal collagen fibrils in its dermis were also seen (Fig. 3A).
Keratinocytes with an active euchromatic nucleus and a pronounced nucleolus terminated at the desmosomes that joined the cells and formed the vast cellular extensions (Fig. 3B). Melanocytes had melanin granules filled the majority of its cytoplasm, a prominent euchromatic nucleus, nucleolus, abundant mitochondria, widened intercellular gaps and fewer desmosomes (Fig. 3C). A lot of interstitial blood vessels was surrounded by endothelial cells and a basement membrane were also seen (Fig. 3D).

**Statistical Results:** These table show the foreskin melanocytes (median=17.00), collagen bundles (median=2.00), keratinocytes (median=8.00), Langerhans cells (median=23.00), vimentin antibodies (median=4.00), Meissner's corpuscles (median=6.00), papillary ridges number (median=6.00) and vascularity (median=48.00) (Fig. 4 and Table I).

**DISCUSSION**

The foreskin serves as a donor source for childhood autologous keratinocyte grafting (Rosado *et al.*, 2012) and as mucosal grafting for surgical reconstruction of serious hypospadias (Stein, 2012). This research simulated the...
microstructural study of human foreskin that is clinically important. The foreskin is densely innervated with sensory receptors, including Meissner's corpuscles, which is remarkable (Cold & Taylor, 1999).

The current foreskin specimens indicated a succession of epidermal layers with a greater content of keratinocyte layers and localized, ridge crests. Skin resistance, cornified envelope formation, and desmosome/tight junction integrity are all aided by the suprabasal skin layers (Simpson et al., 2011; Wallace et al., 2012).

Meissner's corpuscles were more frequent in this study, sometimes clumped together to extend the terminals of papillae that looked as elongated entities linked to the basement membranes. Some researchers assume that the ridged band, with its distinct structure, tactile corpuscles, and other nerves, is primarily sensory tissue that collaborates with other foreskin components (Lakshmanan & Prakash, 1980). The limited proportion of melanocytes in this foreskin sample resulted in little darkening, which can be explained by the fact that melanin synthesis continues, as evidenced by ultrastructural criteria in this and other DOPA incorporation assays (Wilkins et al., 1985).

The connective tissue in the currently reported foreskin structure had a lower content of collagen fibrils and a higher proportion of vascular parts. There is no dense collagenous region, both in density and thickness, indicating that the profuse vascular component might be appreciated with looser connective tissue (Halata & Munger, 1986).

VIM antibody positivity was observed in epidermal Langerhans cells (ELCs) and melanocytes in the foreskin tissues, as well as smooth muscle cells in the dermis, according to immunohistochemistry. VIM is found in all animal cells, including the cytoskeleton, and is expressed in mesenchymal cells (Eriksson et al., 2009).

In the current study, the ELCs of the epidermal skin's stratum spinosum and dermal papillae, particularly those were surrounding the Meissner's corpuscles, reacted strongly to S100 protein staining. ELCs secrete cytokines, which are low-molecular-weight hormone-like molecules that control the strength and duration of immune responses (Weiss et al., 1993).

The stratum spinosum keratinocytes were bound to each other by cell junctions, or desmosomes, according to TEM analysis of the foreskin samples from the current investigation. To provide structural support for tight junctions, involucrum is generated in the stratum spinosum as a terminal indication of keratinocyte maturation (Murthy et al., 1993). Proteins like claudins and occludin generate homotypic interactions between nearby cells and regulate water, ions, and huge molecules transit across paracellular channels (Furuse et al., 2002; Shen et al., 2011).

The intercellular gaps between the keratinocytes were also broadened using TEM, and the cytoplasmic projections of the foreskin in this investigation were distended and elongated. Desmosome internalization in cytoplasmic vesicles has been observed in fresh skin specimens (Iwatsuki et al., 1989). Endocytosis of split desmosomes was identified, leading to the conclusion that antigen-antibody complexes are internalized through this method, according to these scientists.
The TEM images of the foreskin samples in this study revealed different layers of epidermal skin with keratinocytes dispersed among them. Melanocytes are supported by a folded basement membrane with a dermo-epidermal junction lamina densa. Epithelial and mesenchymal components work together to generate organized basement membranes and control normalized epithelial proliferation and tissue architecture in human skin (Andriani et al., 2003). When human neonatal keratinocytes were plated on top of a human dermal fibroblast, the lamina densa was disrupted and there were no anchoring fibrils.

The histological and ultrastructural characteristics of the foreskin, as well as immunohistological experiments employing vimentin and S100 protein as intermediate filaments and statistical analysis, all revealed that it could be a useful scientific resource in the future for a variety of difficulties.

ETHICAL APPROVED AND INFORMED CONSENT.
The Department of Plastic Surgery, College of Medicine, King Khalid University of Abha, Saudi Arabia, generously contributed tissue samples from the foreskin (from six boys ages 1, 2, and 3 years, respectively). Human tissue research was carried out in accordance with Saudi Arabia's Medical University's regulations. Surgical procedures were performed by a urological surgeon who was trained and licensed. Before surgery, all patients signed a written informed consent form.

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