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The Neuroimmune Basis of Acupuncture: Correlation of Cutaneous Mast Cell Distribution with Acupuncture Systems in Human

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Abstract: The hypothesis that cutaneous mast cells (MCs) are responsible for skin phenomena in acupuncture was proposed 40 years ago, but very little is known about the correlation of MC distribution with acupuncture systems in human. The aim of this study is to quantify cutaneous mast cells at different body sites and compare them with the distributions of classical acupuncture points and micro-acupuncture systems. Skin biopsies from dermatological practice were evaluated under microscope with H&E or CD117 stains. Dermal MCs were counted and expressed as MCs per high power field. Densities of classical acupuncture points at different body sites were also calculated and expressed as points per dm^2. MC densities at special sites of the body were compared with micro-acupuncture systems. After examining 285 skin biopsies, MC enriched special sites (MESS) were found at peripheral parts of the body and around orifices of body surfaces. Comparative mapping showed that patterns of MC distribution are highly correlated with the distributions of classic acupuncture points in 14 classic acupuncture meridians, with the exception of the trunk areas. Mapping also revealed that all micro-acupuncture systems were established at MESS, including ear, scalp, hand, foot, eye, face, and umbilicus. The conclusion is that the densities of cutaneous MCs are highly correlated with classical acupuncture points and micro-acupuncture systems. These findings provide tissue evidence of neuroimmune basis of acupuncture and suggest that MC is a tissue target for acupuncture stimulation and may serve as a tissue marker for acupuncture points.

Keywords: Mast Cell; Acupoint; Micro-Acupuncture System; Neuroimmune; Mast Cell Distribution.

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Introduction

Acupuncture is one of the most commonly used complementary and integrative therapies and has been recently recommended for various pain conditions by several national organizations in the US with increasing pervasiveness (Vickers *et al.*, 2012; National Academies of Sciences *et al.*, 2017; Qaseem *et al.*, 2017). Its mechanism of action has been investigated extensively in the past, but the focus was mainly on neural and humoral pathways (Pomeranz *et al.*, 1976; Sjölund *et al.*, 1977; Chung *et al.*, 1980; Han, 2004). Some studies suggested that cutaneous tissue responses to needle stimulation may play an important role (Langevin *et al.*, 2007; Goldman *et al.*, 2010). Exploring specific tissue targets of acupuncture needle may aid in better understanding of the biologic basis of acupuncture and further improvement of the clinical practice.

The hypothesis that cutaneous mast cells (MCs) are responsible for skin phenomena in acupuncture was proposed 40 years ago (Song, 1977) and recent animal experiments support the scientific merits of this theory (Wang *et al.*, 2013; Yin *et al.*, 2017). However, the primary cellular target of acupuncture in skin is not well understood, and the evidence supporting a direct correlation between cutaneous MC distribution and acupuncture systems in human is still missing (Li, 2016).

Since its discovery in 1878 (Ehrlich, 1878), cutaneous MCs are recognized as a part of innate immune, neuroimmune, and neuroendocrine systems responding to stimulations from physical irritations and antigens (Abraham *et al.*, 2010; Aich *et al.*, 2015). In addition to its' specific functions such as IgE mediated reactions, cutaneous MCs constantly generated from bone marrow have long been speculated to perform multiple functions in biological system and its specific pattern of distribution in humans is not well understood (Kalesnikoff *et al.*, 2008).

To interpret skin responses to acupuncture and the "meridian phenomena" found in a large clinical survey in China, Song first proposed a hypothesis in 1977 that dermal MCs might be responsible for the cutaneous signals induced by acupuncture stimulation, and MCs might be enriched at acupuncture points and meridians in normal healthy individuals (Song, 1977). Song and others subsequently reported that abundant MCs were found at acupuncture points in human amputated extremities (Sections of Histology *et al.*, 1980). Further studies suggest that MCs might be the responsible cells for the changes of skin appearance, sensation, and deqi phenomena during acupuncture treatments (Lin *et al.*, 1989). Many studies in animals demonstrated that acupuncture treatment could lead to MC activation, degranulation, and suggest MCs are responsible for certain acupuncture effects including analgesia (Zhu *et al.*, 2017). In addition, some animal experiments showed that inhibition of MC activation or nerve blockers could block pain reduction by acupuncture (Zhang *et al.*, 2008). More recently, Zhu *et al*. proposed that cutaneous MC might undergo crosstalk with both central CNS and periphery stimuli, and MCs could be the key cell at sensitized acupuncture points such as ashi point, trigger point, and classic points (Zhu, 2015).

Currently, very little is known about the relationship between cutaneous MC distribution and acupuncture systems in whole human body (Li, 2016). In this study, 285 human

skin biopsies from different body areas were examined by light microscopy with H&E or specific immunostains for the distribution of cutaneous MCs in dermis. For the first time, the correlation of cutaneous MC distribution and acupuncture systems has been established.

Materials and Methods

Sources of the Skin Biopsies

Histological tissue slides were randomly selected from routine service of dermatopathological diagnosis. Skin biopsies came from diagnostic and cosmetic procedures submitted by dermatologists and general practitioners in clinical practice. The tissue specimens of skin biopsies were processed in diagnostic laboratories (Pathology Solution, NJ, USA) and cut in sections of 4 micros in thickness, mounted on glass slides, and stained with H&E for microscopic evaluation. Only relative normal skin specimens or skin biopsies without conditions affecting MC numbers were included in the study. The inclusion criteria required skin biopsies with full epidermis and dermis, re-excision specimens with adequate normal skin, biopsies without significant inflammatory conditions, and specimens without neoplasm described in exclusion criteria (Patel *et al.*, 2012). The exclusion criteria excluded small and superficial biopsies, specimens lacking full thickness of dermis, marked inflammatory dermatosis, dermal scar, granulation tissue, MC related disorders, and benign neoplasms that might affect MC distributions such as neural tumors and vascular lesions, and specimen bearing malignant tumors such as squamous cell carcinoma, basal cell carcinoma, and malignant melanoma.

A total of 285 skin biopsies were selected for the examination of cutaneous MCs. The specimens include biopsies from ethnic groups representative of the populations in the clinics of the US. The age range of selected skin biopsies was 18 to 85 year-olds, including approximately 58% female and 42% male.

Skin areas for analysis included (1) 13 General Sites: head, foot, hand, abdomen, lower back, neck, forearm, upper arm, chest, upper back, lower leg, buttocks, and thigh; (2) 13 Special Sites: scalp, ear, peri-eye, nose, peri-mouth, face, wrist, ankle, hand, foot, peri-umbilicus, peri-nipple, and perineum/genital/anus/vulva areas. Ten to 50 independent biopsies for each specific area were examined.

Specimen Processing

Skin tissues were processed in pathological laboratories with standard histological protocols for diagnostic pathology. Fresh skin tissues were fixed in 5% formalin immediately following the biopsy procedure and submitted to the laboratory. Fixed tissues were embedded in paraffin and sectioned. The slides were stained with H&E following a standard protocol. Immunochemical stains were done with anti-CD117/C-kit monoclonal antibody (Biocare Medical, Pacheco, CA, USA) with the Leica Biosystems BOND-III system (Buffalo Grove, IL, USA) according to the manufactures recommended protocol. CD117

antibody was chosen as this antibody produced consistent and cleaner results than other MC stains such as anti-MC tryptase antibody, Leder stain, or toluidine blue stain.

MC Counting

According to the inclusion and exclusion criteria, H&E stained slides were first reviewed to select suitable sections under light microscopy (Eclipse 80i, Nikon) with attached CMOS camera and Spot Imaging software (Diagnostic Instrument, Inc.). Selected tissue blocks were cut to have specific immunostains for MCs. Immunostained slides were then examined under microscope and MCs were counted in at least 5 high power fields (HPF x400, 0.15 mm^2) in upper dermis (the most MC abundant area in skin). For each specimen, the average number of MCs from five HPFs was converted into MC number/mm^2, or expressed as the number of MCs/HPFs, representing MC density of one biopsy. At least 10 biopsies from different individuals were analyzed for each specific body site and the average MC density per site was presented as MEAN $\pm$ SD.

MC Enriched Special Site

MC enriched special site (MESS) is defined as the skin site that has twofold or more MCs relative to the average MC density on the trunk (the center of the body).

Areas of Body Surface

Based on the data of a standard total body surface (1.8 m^2 is used) and divisions of the "rule of nines" (Livingston *et al.*, 2000), the general sites of body surface areas are calculated as head 7%, feet 5%, hands 6%, abdomen 9%, lower back 6.5%, neck 2%, forearms 6%, upper arms 8%, chest 9%, upper back 6.5, lower legs 13%, buttocks 5%, and thighs 18%.

Classic Acupuncture Points

Since there are various versions of classic acupuncture points in ancient Chinese literature, a consensus of acupuncture points in 14 meridians from 93 classic acupuncture books (Liu *et al.*, 2002) and standard acupuncture charts (Jin *et al.*, 2004; Cheng, 1978) were used in this analysis. A total of 654 classic acupuncture points on standard acupuncture charts were allocated to 13 general sites. The density of acupuncture points (expressed as numbers of points/dm^2) was calculated based on the number of acupuncture points and the surface area of each site (Livingston *et al.*, 2000).

Micro-Acupuncture Systems

Micro-acupuncture systems were identified from acupuncture textbooks and major publications (Jin *et al.*, 2004; Cheng, 1978). There are 11 commonly used micro-acupuncture

systems established on the special sites of body surface, including scalp, ear, eye, nose, mouth, face, wrist, ankle, hand, foot, and umbilicus.

Statistical Analysis

The averages of MC densities at different body sites were compared with the average of MC density at the trunk (the average of upper and lower back, chest, and abdomen) by student's *t*-test. The correlation of acupuncture points and MC densities in different body sites were analyzed by best fit logarithmic curve analysis in Excel program.

This is a study of existing pathological specimens in the storage, personal information of the subjects was not identified, therefore IRB review has been exempted.

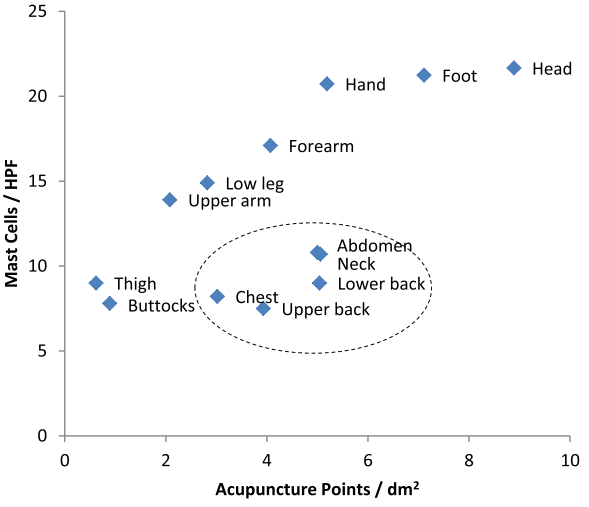
Results

Among 13 areas (general sites) of skin surface, the densities of cutaneous MCs vary greatly with a threefold maximal difference. While the chest, back, and thigh have the lowest density, the foot, hand, and head have the highest density. The distribution of classical acupuncture points also shows an uneven distribution. The foot, hand, and head have the highest densities of acupuncture points in contrast to the buttocks and thighs where lower numbers are seen (Table 1). The densities of acupuncture points in different areas of body surface show direct correlation with the densities of cutaneous MCs (Fig. 1A), except for the trunk areas. When the trunk parts were excluded from the analysis, a linear curve correlation was clearly observed ($R^2 = 0.9548$, $p < 0.01$) (Fig. 1B). It is known that acupuncture points on trunk are often used to stimulate autonomic nerves from spinal cord

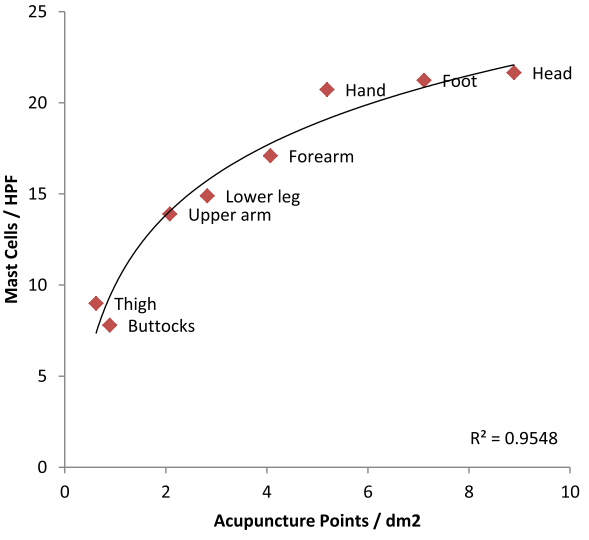
Table 1. The Densities of Acupuncture Points and Cutaneous MCs in Different Human Body Parts

Body Parts	Acupuncture Point Density (Points/dm^2)	MC Density (Cells/HPF, MEAN + SD)
1. Head	8.89	21.66 ± 3.13
2. Foot	7.11	21.24 ± 3.21
3. Hand	5.19	20.73 ± 3.35
4. Abdomen	5.06	10.7 ± 2.36
5. Lower back	5.04	9.0 ± 2.14
6. Neck	5.00	10.8 ± 1.87
7. Forearm	4.07	17.10 ± 1.2
8. Upper back	3.93	7.5 ± 1.72
9. Chest	3.02	8.21 ± 2.01
10 Lower leg	2.82	14.9 ± 1.37
11 Upper arm	2.08	13.9 ± 1.79
12 Buttocks	0.89	7.8 ± 2.3
13 Thigh	0.62	9.0 ± 2.91

Notes: Dermal MCs on CD117 antibody stained tissue slides were counted at high power fields (HPF) of light microscope and expressed as MCs/HPF. The densities of classic acupuncture points at different sites were determined by point number and skin surface areas and expressed as points/dm^2.



(A)



(B)

Figure 1. The correlation of dermal MC densities with classic acupuncture point densities in different parts of skin (A) and a linear correlation of dermal MC densities with classic acupuncture point densities except for trunk areas. (B) The correlation of MCs/HPF in dermis and points/dm^2 were analyzed by logarithmic best fit in Excel program. Acupuncture points in trunk sites were excluded in the analysis.

and have segmented effects on the trunk areas, which might be different from other acupuncture points in term of the mechanism of action (Mori *et al.*, 2002). These data suggest that acupuncture points on the trunk might be different from other areas of the body.

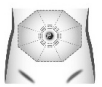
Special sites Acupuncture charts	Special sites Micro-Acupuncture systems	Mast Cell Densities (MCD) Ratio to Trunk Density (RTD) MCD RTD
	**Scalp** **Scalp acupuncture system**	**23.58±2.87 2.72**
	**Ear** **Auricular acupuncture system**	**20.56±3.5 2.34**
	**Eye** **Ocular acupuncture system**	**22.9±3.45 2.64**
	**Nose** **Nasal acupuncture system**	**22.0±2.65 2.53**
	**Mouth** **Oral acupuncture system**	**21.27±3.32 2.45**
	**Face** **Facial acupuncture system**	**20.79±3.24 2.40**
	**Wrist** **Wrist acupuncture system**	**19.3±2.41 2.22**
	**Ankle** **Ankle acupuncture system**	**21.9±3.11 2.52**
	**Hand** **Hand acupuncture system**	**20.73±3.65 2.39**
	**Foot** **Foot acupuncture system**	**21.73±3.03 2.50**
	**Umbilicus** **Umbilical acupuncture system**	**17.29±2.14 1.99**
	**Breast/ nipple** **No acupuncture system**	**17.7±2.0 2.04**
	**Perineum/ anus/ vulva/ genital** **No acupuncture system**	**19.79±3.47 2.28**

Figure 2. MC densities and micro-acupuncture systems at special sites. Mast Cell Densities (MCD) are present as MC/HPF (MEAN $\pm$ SD) and the ratio to trunk density (RTD) is calculated by MCs/trunk MCs (8.7). MC enriched special sites (MESS) have 2 times or more MCs ($p < 0.01$) than trunk areas (8.7 MC/HPF) and are located mainly at peripheral parts of the body and around orifices in normal human skin. All established micro-acupuncture systems are found at MESS. The only two MESS that do not have micro-acupuncture system yet are perineum and peri-nipple areas.

In addition to the peripheral distribution of MCs, histological examinations reveal that MCs tend to accumulate around the body openings (orifices), including ear, eye, nose, mouth, nipple, umbilicus, vulva, anus, and hair follicles. A survey of skin biopsies found that there are multiple MC enriched special sites (MESS), defined as a skin site that has twofold or more numbers of MCs relative to the average MC density on the trunk (8.7 MC/HPF). All identified MESS are located at peripheral parts of the body or around orifices of body openings (Fig. 2).

Notably, the most commonly used micro-acupuncture systems are established at MESS areas, either around orifices or at the peripheral sites of the body (Fig. 2). There are only two exceptions, the nipples and perineal areas (anus, vulva, and penis). What is known today about these areas is limited. The reason for these two areas have not been used often for acupuncture treatment is probably due to inconvenience in clinical practice rather than their usefulness or ineffectiveness. MC densities and ratios to trunk MCs of these special sites are also shown in Fig. 2.

The unique pattern of cutaneous MC distribution is shown in Fig. 3 (left). When cross referenced to a traditional acupuncture chart, it is clear that all areas with dense classic

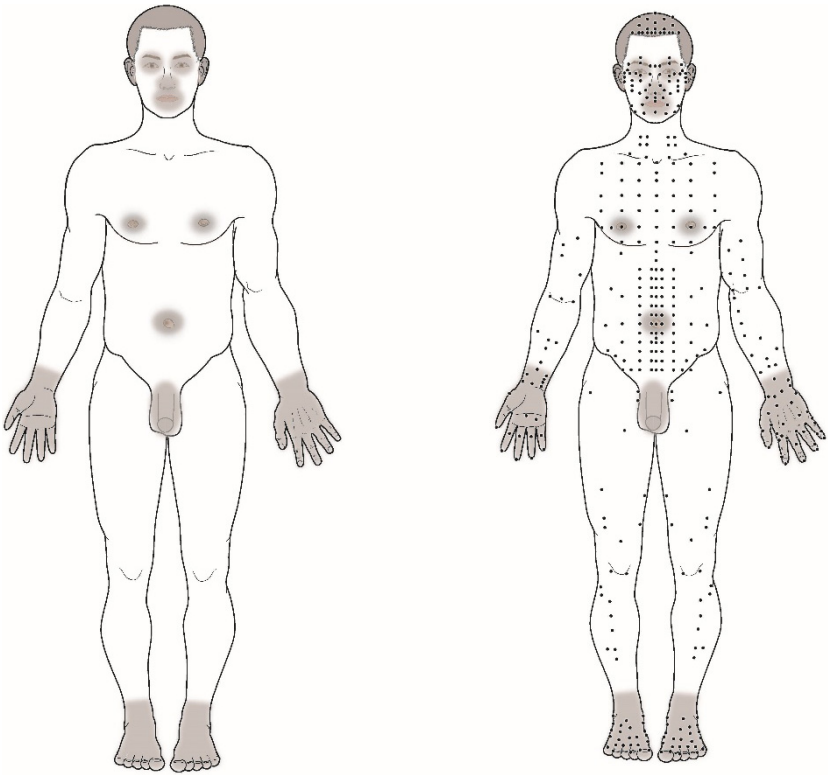


Figure 3. MESS distribution overlaps with the areas of high density acupuncture points. MESS are found around orifices and at peripheral skin (left) and that overlaps with acupuncture points in classic chart (right).

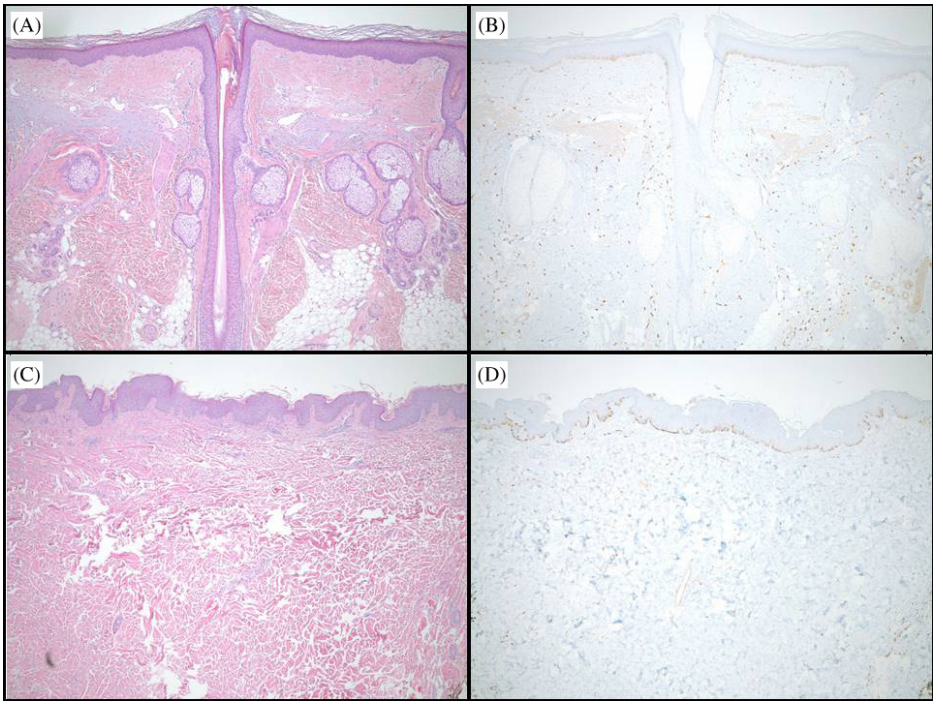


Figure 4. Dermal MC densities vary at different sites. Scalp skin stained with H&E (A) and CD117 (B) and back skin stained with H&E (C) and CD117 (D), showing increased CD117-stained MCs at scalp comparing with back.

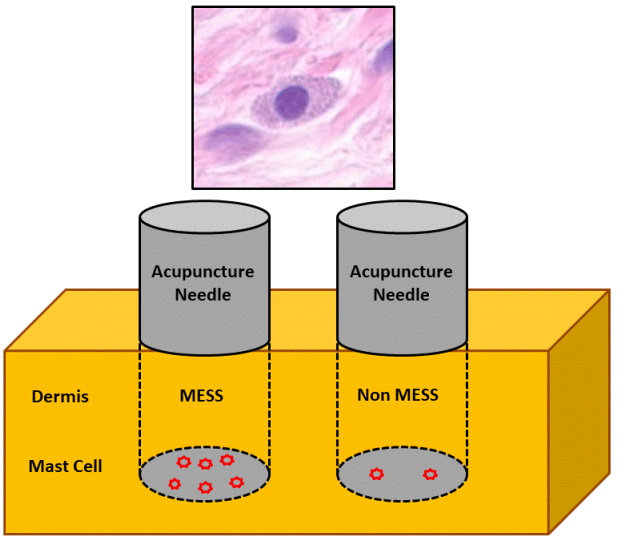


Figure 5. Acupuncture needle hits more MCs at MESS sites. Top: Mast cell with intracellular granules (600x, H&E stain). Bottom: Estimated numbers of needle hitting MCs at MESS and non-MESS points.

acupuncture points, such as head, feet, hands, ankles, and wrists overlap with MESS (Fig. 3, right), except for the trunk. Most micro-acupuncture systems were developed in the last half century after the fine metal needles made available. The fact of these systems all located in MESS areas is probably not by accident but due to underlying tissue selection.

Because of various MC densities in dermis (Fig. 4), an acupuncture needle penetrating through the skin can hit different numbers of MCs depending on location (Fig. 5). For example, an average acupuncture needle (0.25 mm in diameter) will hit approximately 2, 4, 5, and 6 MCs at a cross-section of upper dermis in chest, forearm, hand, and scalp, respectively. Apparently, needling at MESS acupuncture points of the skin physically contacts three folds more MCs than non-MESS sites and could lead to increased MC activation, as MCs are known to respond to physical simulation. In clinical practice, additional needle twisting and manipulation techniques may further amplify MC reaction.

Discussion

Historically, the search for cutaneous points suitable for acupuncture treatment was presumably done by trial and error, built upon several thousand years' effort needling at different areas of the body and evaluating clinical improvement (or lack thereof). Classic acupuncture points and meridians were described about two thousand years ago when primitive forms of large thicker needles were used (Huang, 2003). As the modern stainless steel needles became ubiquitous in last century, acupuncturists gained the opportunity to explore the more delicate areas of human body with fine thin needles. This exploration has led to the establishment of a number of micro-acupuncture systems at special sites based on clinical outcomes (Jin *et al.*, 2004). The question that remains in acupuncture research is whether these acupuncture point selections had any tissue or cellular basis. If so, whether these newly identified special sites of micro-acupuncture systems utilize a congruous MC pathway relative to classic acupuncture points.

Here we found that the distribution patterns of cutaneous MCs are highly correlated with the densities of classic acupuncture points and that all established micro-acupuncture systems are located at MESS. This phenomenon is not likely a coincidence, as cutaneous MCs have been suspected to be responsible for acupuncture induced reactions in addition to the needle stimulation of neural, vascular, and humoral systems (Kalesnikoff *et al.*, 2008; Shiota *et al.*, 2010).

The MC distribution patterns described here confirms results observed in previous studies. A survey on human skin biopsies found that cutaneous MC has a gradient distribution pattern in healthy individuals with the lowest number at central skin sites such as the abdomen and highest number at peripheral skin sites such as chin and nose (Weber *et al.*, 2003). Another investigation reported MC density is high at distal and low in proximal parts of human body (Janssens *et al.*, 2005). For the first time, this study reveals that MCs are concentrated around orifices of body surface in addition to the gradient distribution toward extremities. The pattern of MC distributions around openings seems to be consistent with MC role as defense cells, a part of innate immunity, and repairing cells for tissue healing (Abraham *et al.*, 2010).

MCs are known as the first line of defense, responding to physical stimulus from environments including temperate, pressure, microorganism, trauma, and other injuries (Shiota *et al.*, 2010). Despite the potential detrimental effects that MCs can have on immune homeostasis, neuroimmune, and neuroendocrine systems, these cells are indispensable to the host, as suggested by evolutionary preservation across many species and that humans without MCs have never been described (Abraham *et al.*, 2010). Acupuncture treatment with the insertion of metal needles into skin can induce focal micro tissue injury and lead to MC activation with a series of subsequent local and system reactions (Kalesnikoff *et al.*, 2008; Abraham *et al.*, 2010). Animal studies demonstrate that acupuncture treatment could induce MC degranulation and migration (Zhu *et al.*, 2017). In addition, the analgesia effect of acupuncture could be significantly reduced by applying blockers to MC activation (Zhang *et al.*, 2008) and mechanical stress receptor of TRPV2 on MCs (Zhang *et al.*, 2012).

Statistical analysis from 93 ancient classic acupuncture books showed that acupuncture points at proximal parts of the body, such as head, forearm, hand, lower leg, and foot are not only at higher densities but also much more frequently used than acupuncture points on the central trunk areas, such as chest, abdomen, and back (Liu *et al.*, 2002). Micro-acupuncture systems have become very popular in last half century and many practitioners use micro-acupuncture only to treat certain conditions. For examples, ear acupuncture is often used for pain and detoxification (Shwartz *et al.*, 1999) and scalp acupuncture is commonly selected for neurological disorders (Wang *et al.*, 2012). Therefore, it is no surprise that the higher densities of acupuncture points at peripheral parts are correlated with MC densities and all micro-acupuncture systems are located in MESS.

The two exceptional MESS areas with no micro-acupuncture systems established so far are perineum and peri-nipples (areolas). This is likely due to the inconvenience to practice acupuncture in these private areas rather than lack of acupuncture effectiveness. Micro-acupuncture systems in these areas may be developed in the future after understanding the MC distribution patterns.

In addition to abundant MCs, MESS areas appear to have more nerves, sensors, and vascular structures, but the densities of these structures are not as uniform as MCs. The relationship of MESS and acupuncture systems with nerves and vessels remains an area requiring further exploration.

A limitation of this study is that the biopsies came from clinic practice labeled with only approximate locations on the human body. It is impossible to specify the close relationship of these biopsy sites to specific acupuncture points or meridians. Theoretically, these biopsies were from abnormal skins samples and might affect the observation, but the non-biased selection, high numbers of biopsies, and significant differences in MC numbers with small standard deviations support that the observed phenomena is highly repeatable.

In conclusion, a consistent pattern of cutaneous MC distribution has been identified in adult skin, which has a close correlation with the distributions of both classic acupuncture points and micro-acupuncture systems. MESS were found at peripheral parts of the body and around orifices of skin surfaces. The densities of both dermal MCs and classic acupuncture points show centrifugal gradient distribution and most micro-acupuncture systems

are established at MESS. This is the first report to describe specific patterns of human dermal MC distribution in reference to acupuncture points and micro-acupuncture systems. These findings provide tissue evidence of the neuroimmune basis of acupuncture and suggest that MC density may be one of the key underlying factors for acupuncture point selection. MCs might be one of the skin targets for acupuncture stimulation and can also serve as a marker for identification of additional acupuncture points. The results may shed light on the mechanism by which acupuncture triggers internal healing.

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