Toxicology Analysis of Tissue-Mimicking Phantom Made From Gelatin

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Abstract. Skin phantom mimics the biological skin tissues as it have the ability to respond to changes in its environment. The development of tissue-mimicking phantom could contributes towards the reduce usage of animal in cosmetics and pharmacokinetics. In this study, the skin phantoms made from gelatin were tested with four different commonly available cosmetic products to determine the toxicity of each substance. The four substances used were; mercury-based whitening face cream, carcinogenic liquid make-up foundation, paraben-based acne cleanser, and organic lip balm. Toxicity test were performed on all of the phantoms. For toxicity testing, topographical and electrophysiological changes of the phantoms were evaluated. The ability of each respective phantom to react with mild toxic substances and its electrical resistance were analysed in to determine the toxicity of all the phantom models. Four-electrode method along with custom made electrical impedance analyser was used to differentiate electrical resistance between intoxicated phantom and non-intoxicated phantom in this study. Electrical resistance values obtained from the phantom models were significantly higher than the control group. The result obtained suggests the phantom as a promising candidate to be used as alternative for toxicology testing in the future.

1. Introduction

Tissue-mimicking (TM) phantoms or well known as tissue-mimicking materials are objects that mimic or resemble human tissue such as skin [1]. The low cost phantom development were already studied extensively for spectroscopy and imaging field. A TM phantom that accurately represents human tissue, are important in safety testing and other tissue testing application [2]. Phantom has been developed recently in such a various ways in order to get the most accurate results for its similarity in mimicking the human tissue. These materials may be classified into several categories based on few ingredients [3]. Basically phantoms are made for imaging modalities related with tissue frequency and electrical properties in field such as ultrasound, magnetic resonance imaging (MRI), and computed tomography (CT). The most reliable organic material developed as phantom is gelatin because of its desirable mechanical properties [2], [4]–[7].

At certain level, all harmful chemical can cause harm to the cells and tissues. However, the chemical is considered to be non-toxic when it only causes damage with a very large amount. When one speaks about toxicity test in food or domestic product, issues that will arise is necessarily the animal testing [8]. A report has stated that at least one million of animal testing are done all over the world annually.
One of the toxic substances, mercury is the most common chemicals found in lightening product. Liang and colleagues have reported that mercury is presence in most common skincare product such as eye drops, nasal drops, antibiotics and cosmetic products [10]. Women who used mercury-lightening product often have elevated mercury levels in the hair, blood and urine [11]. Parabens are also the most commonly used additives in many cosmetic products and they act as preservatives in cosmetic [12]. Methylparaben, propylparaben, and butylparaben are the compounds that can easily be found in cosmetic product especially face and body cleanser.

Electrical resistance analysis is low cost yet non-invasive commonly used in body composition approach and clinical condition assessment [13]. Electrical resistance analysis is not limited to only body composition and single sample measurement; it has also been used in clinical condition measurement. One such example is the use of electrical impedance characterization to compare normal and cancerous hepatic liver tissue [14].

In this study, phantom made from gelatin was tested for its toxicity by looking at its microscopic examination, weight reduction and electrical resistance. The topographical condition of the phantom, its percentage of weight loss, and electrical resistance are important criteria in order to distinguish between normal and intoxicated phantom.

2. Material and methods

2.1. Materials

Gelatin, ovalette, and sunflower oil were purchased from a bakery supplier store (Bake With Yen Sdn Bhd). Propylene glycol and formalin (37% formaldehyde) were obtained from Sigma-Aldrich (St.Louis,MO,USA). Whitening night cream, UV foundation make up base, acne cleanser, and organic lip balm were purchased from a cosmetic store in Kuala Lumpur, Malaysia.

2.2 Fabrication of the phantom

The phantom was fabricated according to the method described by Hahn and Noghanian with slight modification to obtain the composition intended for this study [5]. The phantom was made based on this composition; 7 g of calf-skin gelatin (200 bloom), 60 ml of distilled water, 7 ml of propylene glycol, 5 ml of sunflower oil, 0.30 g of ovalette (surfactant), and 20 drops of formalin (37% formaldehyde).

60 ml of distilled water was heated until it reached 50°C. 7ml of propylene glycol was then added as a stabilizer followed by the addition of 7 g of gelatin. The temperature was allowed to increase until it reached 70°C. In a separate container, another solution of 0.30 g ovalette and 5 ml of sunflower oil was prepared. These two solutions were then mixed together. The ovalette acts as a surfactant that will assist in the bonding between oil and water parts in the solution.

The mixture was then stirred until a compound mixture formed. Formaldehyde was then added to the mixture. The mixture was then poured in petri dishes and stored in the refrigerator for 10 hours to ensure complete solidification.

2.3 Toxicity testing

Two groups of four phantoms were prepared, with four belongs to the test group and another four belong to the control group. All of the test phantoms were test simultaneously with the control
phantoms. The four cosmetic products (whitening night cream, UV foundation make up base, acne cleanser, and organic lip balm) were heated with a solvent to ensure better diffusion when the substances were introduced into the phantom. Distilled water was used as the solvent, with 1 g of each substance was added to 1 ml of distilled water. The phantom with the added cosmetic substance was then left in the drying oven at 37°C for 10 hours to allow proper reaction to take place. Equal amount of the liquefied cosmetic substance was added to the phantom every day during the 7-day course of testing.

2.3.1 Microscopic observation

The surface of each phantom was observed through light microscope. A 5X magnification was used to obtain the image.

2.3.2 Weight reduction analysis

The initial weights of all the phantoms (±73 g) were measured and the weight loss during 7 days of experiment was recorded. The readings obtained then analysed using one-way ANOVA calculations (SPSS software).

2.3.3 Electrical resistance measurement

The variation of the electrical resistance values in a continuous range of frequency is termed as galvanic profile. The general morphology obtained from the galvanic profile of the test phantoms was used and compared with the galvanic profile of the control group.

3. Results

3.1 Microscopic observation

Toxic substances in cosmetic products have particular effect to the surface of the phantom. To observe the skin phantom feasibility on toxicology analysis, the image of phantom’s surface on control phantom and every test substance were obtained and compared. The images were obtained from Light Microscope with 5X magnification.

Figure 1. Surface of skin phantom models from control group (a) and after toxicity test of; organic lip balm (b), paraben-based acne cleanser (c), carcinogenic liquid foundation (d) and mercury-based face cream (e).

Figure 1(a) shows a normal microscopic image of agar/gelatin gels. The image presented was from one of the control phantoms. The light area represents the gelatin region and the dark spot indicated air bubbles and open pores. Air bubbles are trapped on the surface and in the structure itself during the mixing process of the phantom.
Figure 1(b) showed almost similar morphology as in control group, apart from hardened composition of the phantom due to the presence of beeswax in the organic lip balm. Figure 1(c-e) showed the presence bigger air bubbles and also surface lesion on the surface of the phantoms.

3.2 Weight reduction analysis

The initial weights of all phantoms were measured respectively and a mean weight of ±73g was obtained for a single phantom. Weight reduction percentage demonstrates the percentage of weight loss throughout the whole test duration for a maximum of 7 days.

![Mean Percentage Reduction of Phantom’s Total Weight](image)

**Figure 2.** Phantom's weight reduction percentage (with a respective p<0.05 to test subjects)

A one-way measure ANOVA was ran on five set of 15 values of weight reduction percentage of skin phantom sample to determine if there was difference in phantom’s total weight loss among test and control group. There were statistically significant in means among intoxicated phantom group and normal phantom group with p<0.05.

3.3 Electrical resistance measurement

The general morphology of the galvanic profile, as measured in both groups, resembles an increase in the electrical resistance, corresponding with the increase of the current frequency, at a constant voltage of 7V of alternating current.
Figure 3. The electrical resistance of the skin phantom in the test group and the control group at the lower end of frequency range. Each point represents the average of measurement for one specific frequency.

When the galvanic profiles between control group and test group were compared, the electrical resistance was significantly higher in the mercury based face cream-intoxicated group, especially on high frequency measurements.

4. Discussion

After toxicity test was conducted, the topographical evaluation towards intoxicated skin phantom by acne cleanser, carcinogenic foundation and mercury-based face cream revealed a minor destruction on the surface of the phantom which can be clearly seen on Figure 1(c-e). Each test substance (Figure 1(c-e)) contained toxic substances which could leads to breast cancer and other health side effects. [15]. Paraben-based cleanser is capable of storing extra fat in human skin tissue and that can be related to the presence of bigger air bubbles beneath the surface area of the phantom (Figure 1(c)). The bigger air bubbles and surface lesions were inferred to be caused by the reaction of the highly active toxic ingredients in the test substances itself. On figure 1(e), mercury-based face cream affect the surface of the phantom by causing minor erosions and making the phantom loses more weight than other test substances. This phenomenon also happened in carcinogenic liquid foundation (Figure 1(d)), in which the whole phantom turns into yellowish-brown color due to synthetic dye used to make the substances. Organic lip balm shows a less than 5% difference when compared with the control group; no presence of potential toxic substances was made as a conclusion to this finding. Organic lip balm, however, harden the composition of the skin phantom due to the presence of beeswax in the ingredients organic lip balm.

The phantom weight loss is due to several factors. Firstly, fluid exodus happened right after the phantom is kept under 37°C. A normal phantom lost about 10% or less of its total weight through the process of evaporation with maximum of 6 days after solidification. Secondly, water exit from the phantom was due to the reaction of chemical substances contained in the test materials with the internal structure of the phantom. The test group shows increased values in weight percentage reduction especially on mercury-based face cream followed by carcinogenic liquid foundation which may be due to the presence of salicylic acid and retinol found in the test substances [16].
The purpose of measuring the resistance of skin phantom analogues for this experiment is as an attempt to conclude if the intoxicated phantom has a measurable difference of electrical properties compared to normal skin phantom. Relating to biologic tissue, another interesting attribute of the electrical properties is that the resistance of the tissue increase with tissue necrosis. It is because when the cells die, the cell membrane loses its ability to maintain its insulating properties and ionic gradients [17]. The differences in the electrical properties of normal phantom and intoxicated phantom will play a large role in determining the viability of the phantom to act as an alternative medium in toxicology analysis. Since toxicology analysis is a wide range of study, focusing in developing a skin phantom that mimics human biological tissue will be a good step towards starting a phantom-based toxicology analysis in the future. The data gathered for this thesis established the fact that intoxicated skin phantom tissue has higher electrical resistance than normal phantom. The electrical resistance was higher in the intoxicated group, especially on high frequencies range of the electrical current. Mercury-based face cream demonstrated significant effects on skin phantom particularly in the erosion of the phantom surface and high electrical resistance. This can be related to a research on mercury effect in most skin-whitening cream which demonstrated a permanent damage of rat liver, kidney and brain when treated with mercury for three times a week [18]. The main cause for the increased of electrical resistance of the intoxicated group could be the destruction of cross-linked polymeric structures. In general, fat has lower electrical conductivity than structures containing water. The measurement of high frequency is relevant for living tissue because they have the capacity to pass through membranes.

Acknowledgement

This research is supported by University of Malaya Research Grant (UMRG: RP022C-14AFR).

References

[1] N. Ortega-Quijano, F. Fanjul-Velez, I. Salas-Garcia, and J. L. Arce-Diego 2011 Proceedings of 21st International Conference Radioelektronika 2011 1–4
[2] A. I. Farrer, H. Odéen, J. de Bever, B. Coats, D. L. Parker, A. Payne, and D. A. Christensen 2015 J. Ther. Ultrasound 3 9
[3] M. Lazebnik, E. L. Madsen, G. R. Frank, and S. C. Hagness 2005 Phys. Med. Biol. 50 4245–4258
[4] P. G. Anderson, N. C. Rouze, and M. L. Palmeri 2011 Ultrasound. Imaging 33 134–142
[5] C. Hahn and S. Noghanian 2012 Int. J. Biomed. Imaging 2012 1–12
[6] M. M. Nguyen, S. Zhou, J. Robert, V. Shamdasani, and H. Xie 2014 Ultrasound Med. Biol. 40 168–176
[7] A. M. R. Pinto, P. Bertemes-Filho, and A. S. Paterno 2015 Gelatin as a Skin Phantom for Bioimpedance Spectroscopy178–182
[8] J. Tannenbaum 1999 ILAR J. 40 97–110
[9] S. K. Doke and S. C. Dhwale 2015 Saudi Pharm. J. 23 223–229
[10] L. Liang, J. Gilkeson, E. Swain, E. Bennett, M. Li, M. Deng, and P. Pang 2013 J. Cosmet. Dermatological Sci. Appl 3 256–262
[11] P. del Giudice and P. Yves 2002 Int. J. Dermatol. 41 69–72
[12] R. Winter 2009 A consumer’s dictionary of cosmetic ingredients: complete information about the harmful and desirable ingredients found in cosmetics and cosmeceuticals Three Rivers Press
[13] S. Khalil, M. Mohktar, and F. Ibrahim 2014 Sensors 14 10895–10928
[14] S. Laufer, A. Ivorra, V. E. Reuter, B. Rubinsky, and S. B. Solomon 2010 Physiol. Meas. 31 995–1009
[15] P. D. Darbre, A. Aljarrah, W. R. Miller, N. G. Coldham, M. J. Sauer, and G. S. Pope 2004 J. Appl. Toxicol. 24 5–13
[16] E. C. Davis and V. D. Callender 2010 J. Clin. Aesthet. Dermatol. 3 20–31
[17] X. Chen, W. Li, J. Ren, D. Huang, W. He, Y. Song, C. Yang, W. Li, X. Zheng, P. Chen, and J. Han 2014 Cell Res. 24 105–121
[18] I. Al-Saleh, N. Shinwari, I. El-Doush, G. Billedo, M. Al-Amodi, and F. Khogali 2004 BioMetals 17 167–175