**STUDY PROTOCOL**

**A randomised, controlled comparative study of the wrinkle reduction benefits of a No-Tox®, a plukenetia/niacinamide/plankton-based topical cosmeceutical formulation *vs.* 0.5 mg (0.05% w/w) tretinoin, a prescription-strength topical retinoic acid formulation**

***(Abbreviated Name: No-tox Vs. Retinoid Study NOVRET Study)***

**Background**

Wrinkles can be due to photo-aging and chronological aging. Fair-skinned individuals in sunny or high-UV climates are prone to photo-aging that results in wrinkles, *deep furrows*, hypo-or-hyper pigmentation, rough skin, loss of the skin tone, solar elastosis, and even skin cancer [1]. Chronological aging, on the other hand results in cutaneous laxity and *fine wrinkles*, as well as development of benign growths such as seborrheic keratoses and angiomas [2]. UV-induced reactive oxygen species (ROS) in human skin become responsible for stimulation of numerous signal transduction pathways matrix metalloproteinases (MMPs) like collagenase-1 (MMP-1), stromelysin-1 (MMP-3), and gelatinase A (MMP-2), that specifically degrade connective tissue such as collagen and elastin [3]. Therefore, the demand for wrinkle-reducing agents, and non-invasive facial rejuvenation therapies continues to grow [4]. Topical retinoids, such as Tretinoin have been considered a benchmark for topical applications to reduce wrinkles, especially when used over a period of a few months [5]. No-Tox®, which is a proprietary plukenetia/niacinamide/plankton-based cosmeceutical formulation has been shown to reduce wrinkles over 3-12 weeks in a study [6]. Given the skin irritation potential of prescription-grade topical retinoids, this study aims to compare the efficacy of No-Tox® a proprietary cosmetic formulation *vs*. a prescription-grade formulation with 0·05% Tretinoin in improving the appearance of facial wrinkles.

**Methods/Design**

A randomised, open label, single-blind, trial will be conducted at the Skin Surgery Clinic, Auckland, New Zealand in association with the Auckland University of Technology. Participants fulfilling the eligibility criteria will be selected. Enrolled participants will be randomly allocated to one of two parallel groups: the No-Tox® group or the Tretinoin group. There will be no cost to the participants, and all study products will be provided to the subjects at no cost, sponsored by the Skin Surgery Clinic. The Skin Surgery Clinic does not have any direct financial relationship with the manufacturers of either of the study products which are both commercially available. No researcher will receive any specific study-related funding from manufacturers of the study products or the sponsoring clinic.

**Recruitment:**

We will recruit participants from existing clients of the Skin Surgery Clinic. The Clinic has been in operation for 25 years and has a large database of clients that will be contacted via email or telephone text messaging. Respondents will be contacted by clinical trial coordinators to determine eligibility via a telephone pre-screen. If an applicant meets the study criteria, he or she will be invited to the clinical research centres to be further clinically examined for eligibility.

**Eligibility Criteria:**

Healthy volunteers, aged between 25 and 75 without acute or chronic skin diseases will be enrolled. Participants will be instructed regarding their participation during the run-in, treatment, and follow-up periods. Written informed consent will be obtained from each participant. Participants will be randomised into two groups of 30. Participants will be assigned random numbers using a random number generator program. These random numbers will be sent to the clinic, and the randomisation table will be kept blind by the organisation from the investigators during the research period. Ultimately, 60 participants will be randomised into two groups of 30 (minimum 20) each. Researchers into randomisation of clinical studies note that having 40 subjects in two groups, with 30 subjects allocated to each group, set the statistical power at a minimum of 80% [7] which is a desired minimum benchmark.

The flow chart (Fig 1) indicates the design of this study. Each participant will be examined for any skin condition or irritation before and during the study. Written informed consent for participation in this study will be obtained from all subjects in accordance with the Helsinki II declaration, and the protocol approved by an Institutional Review Board (HDEC, NZ). The trial will be registered by the Australia New Zealand Clinical Trial Registry (ANZCTR).

No-Tox® (manufactured under license by Smoothe Inc, DE, USA) will be applied twice daily over the face, as recommended by the manufacturers; Tretinoin 0.5 mg/g (ReTrieve® Cream, iNova Pharmaceuticals (New Zealand) Ltd) will be used once daily at night as per manufacturer recommendations. Measurements and clinical reviews will be undertaken at 1-month and 3-month periods.

Measurements of wrinkles (wrinkle depth) and percentage changes in wrinkles over the duration of this study will be measured using the Pear 3D (DermaQuip LLC, Marietta, GA 30062, USA) scanner, a 3-dimensional skin scanner that uses fluorescence spectroscopy and high-resolution photography as its modalities. The Pear 3D system uses three light sources: normal, polarized, and Ultraviolet (UV). Autofocus is achieved at a resolution of 15 million auto pixels. Normal light is used for spot, wrinkle, texture, and pore analysis. UV light is for assessment of sun damage below the surface, as well as bacterial counts on skin. The polarized light is used for the surface sun damage as well as to analyse blood vessels on the skin.

A follow-up to evaluate the long-term safety and side effects will also be conducted at the end of the trial via participant questionnaires. All statistical evaluation and analyses will be undertaken independently by the Department of Mathematical Sciences of the Auckland University of Technology.

**Discussion**

Given the potential of causing skin irritation is a known side effect of prescription retinoids that have been the benchmark for topical wrinkle-reduction, this study aims to evaluate the potential of a cosmeceutical that has shown promising results in a previous trial. It is generally assumed that such cosmeceutical products do not have clinical efficacy comparable with that of prescription topical therapies and hence this study to test this hypothesis.

**References**

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**Fig 1. Flow chart of the No-tox Vs. Retinoid Study**