Evaluation of Minoxidil 5% Topical Lotion Hydrogel on the Proliferation of Human Hair Dermal Papilla Cells *In Vitro*

Summary: The proliferation of human hair dermal papilla cells upon treatment with minoxidil 5% in PCCA Atrevis (PCCA Formula 14068) was evaluated using the BrdU *in vitro* staining assay. Following 24h cell exposure, the compounded minoxidil showed comparable hair cell proliferation to the minoxidil 5% commercial product of reference. The easy-to-compound, alcohol-free formulation is a promising, new therapeutic alternative.

Introduction:

Androgenetic alopecia (AGA) is the most common type of progressive hair loss and it is estimated to affect 50 million men in the United States. Although it is neither life threatening nor painful, it is a distressful condition that may lead to psychosocial consequences. The treatment of reference for AGA consists of the topical application of minoxidil, which has been widely used since the early 1980s [1]. When compounded, minoxidil may be incorporated in variable strengths to a topical base in order to meet the individual needs of men.

A new formulation including minoxidil 5% in Atrevis Hydrogel (PCCA Formula 14068) was developed to obtain pharmaceutically elegant, easy to compound, alcohol-free minoxidil preparations that do not require pH adjustment. The purpose of this *in vitro* study was to investigate the proliferation of human dermal papilla cells upon treatment with minoxidil 5% in PCCA Atrevis in comparison to the minoxidil 5% commercial product of reference, over a period of 24 hours.

Methodology:

The 5-bromo-2'-deoxyuridine or bromodeoxyuridine (BrdU) staining was the assay used to evaluate *in vitro* the proliferation of human hair dermal papilla cells, which are located at the bottom of hair follicles. The BrdU is an analog of the nucleoside thymidine commonly used to identify proliferating cells. When incorporated into nuclear DNA, it represents a label that can be tracked using antibody probes to detect DNA synthesis [3].

Materials and Methods

The dermal papilla cells (Cat #2400, ScienCell Research Cell Laboratories) were divided into 3 groups: minoxidil 5% in PCCA Atrevis (n=18); minoxidil 5% reference product (n=18); and control/negative group (n=12). Each minoxidil formula was diluted with growth medium to a concentration of 1.5 µM. The cells were treated with diluted formula solutions while simultaneously supplied with BrdU (Cat #11296736001, Sigma-Aldrich). For the control group, cells were left untreated. Following 24h exposure, the cells were fixed and prepared for fluorescent detection of the incorporated BrdU in the proliferating daughter cells. Upon completion of BrdU staining, the fluorescence intensity of the proliferating cells was read using the CLARIOstar - BMG Labtech Plate reader (483/530 nm excitation/emission). Images were taken under fluorescent microscopy (Nikon, Japan) (Figure 2). The quantitation of green fluorescence by the proliferating cells was analyzed using the Stars software. Data is presented as the mean relative fluorescence units (RFU) ± Standard Deviation (SD). Two-tailed Student's t tests were used to perform direct comparisons between the test groups, and a p value of <0.05 was considered statistically significant. All statistical analyses were performed using the Excel software.

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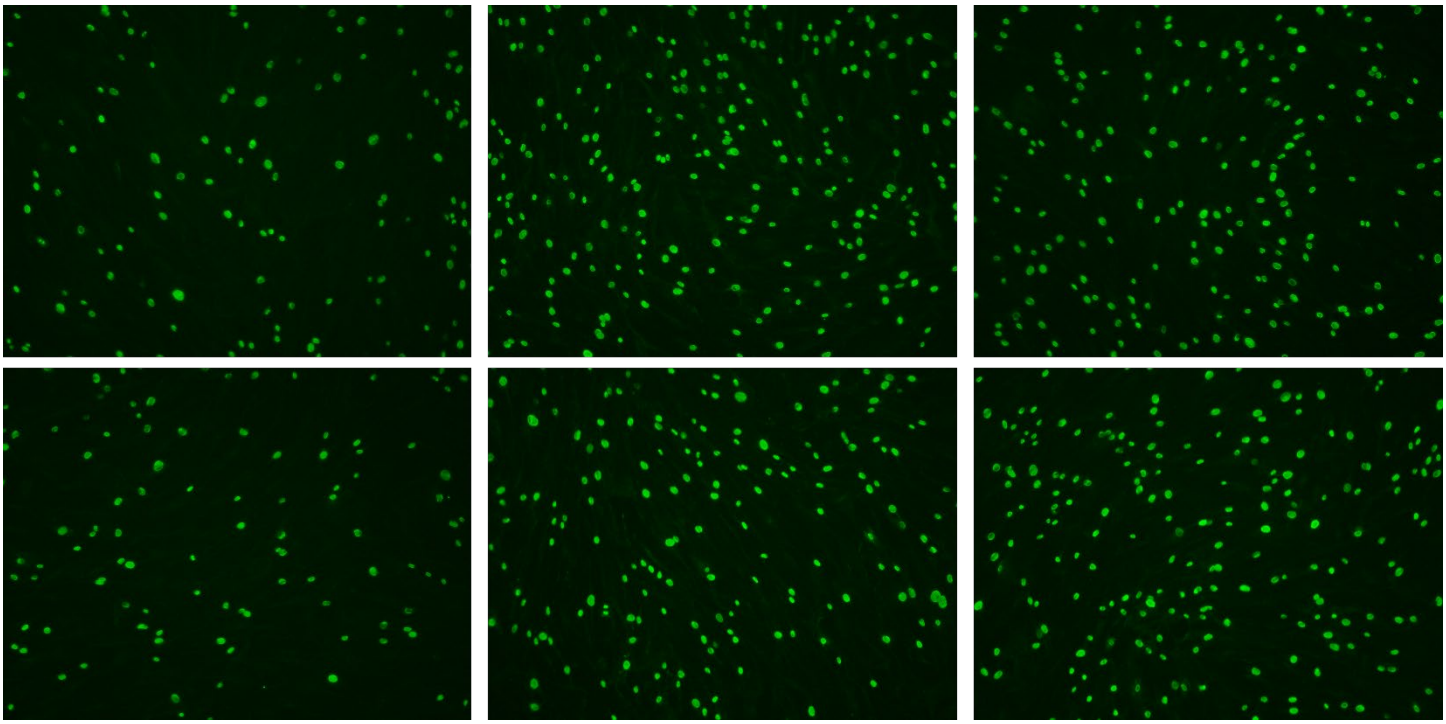

Figure 2. *Proliferation of human dermal papilla cells upon treatment with minoxidil 5% reference product, in comparison with minoxidil 5% topical lotion hydrogel (PCCA Atrevis, PCCA Formula 14068) over a period of 24 hours.*

Results and Discussion:

The proliferation effect of minoxidil on the human dermal papilla cells was significant in both groups when compared to the negative control (untreated cells). For the cells exposed to the minoxidil 5% in Atrevis Hydrogel (PCCA Formula 14068), the average RFU was 183,109±24,416 (p=0.006), and for the minoxidil 5% commercial product of reference it was 181,446 ±38,171 (p=0.021). As shown in Figure 2, the corresponding percentages of change were 22% (minoxidil 5% in PCCA Atrevis) versus 20% (minoxidil 5% reference product). As a result, the *in vitro* efficacy of the compounded minoxidil is comparable to the commercial minoxidil of reference. In addition, the compounded minoxidil is an alcohol-free formulation that may reduce the skin irritation commonly associated with the minoxidil topical products. It is also easy to compound and does not require pH adjustment.

Conclusion:

The minoxidil 5% in PCCA Atrevis has shown *in vitro* comparable hair cell proliferation to the reference product. The easy-to-compound, alcohol-free formulation is a promising, new therapeutic alternative.

References:

1. Cash TF. The psychology of hair loss and its implications for patient care. *Clin Dermatol.* 2001;19(2):161-166.

2. Marotta JC, Patel G, Carvalho M, Blakeney S. Clinical Efficacy of a Topical Compounded Formulation in Male Androgenetic Alopecia: Minoxidil 10%, Finasteride 0.1%, Biotin 0.2%, and Caffeine Citrate 0.05% Hydroalcoholic Solution. *Int J Pharm Compd*. 2020 Jan-Feb;24(1):69-76. PMID: 32023218.

3. abcam (2021) *BrdU staining and BrdU assay protocol.* Available at: https://www.abcam.com/protocols/brdu-staining-protocol (Accessed: September 6th, 2021)