Developing New Graft Holding Solutions: *In Vitro* Evaluation of Bioactive Molecules Identifies Vitamin B12 as Micrograft Stabilizing Substance

Walter Krugluger, MD, Katharina Laciak, Karl Moser, and Claudia Moser *Vienna, Austria*

Introduction

Recent progress in studying the biology of isolated micrografts during hair restoration procedures identified key factors for high viability micrografts. This has led to the development of new storage solutions that have been shown to support hair growth after transplantation in various preclinical and clinical trials. Factors that negatively affect micrograft viability and that can be eliminated by storage solutions are dehydration, pH changes, poor nutrient supply, and apoptotic cell death in hair follicle cells. Storage solutions that meet these requirements are substantially modifications of tissue culture media and have been used in clinical trials under names such as HypoThermasol™, Custodiol™, and Moser's solution. However, the beneficial effects of these solutions have some disadvantages that limit their clinical use. Among these, high cost of the solutions is a major consideration. In addition, the capacity of these solutions to bind H_2O molecules limits dehydration of micrografts but also makes the graft more slippery, which results in technical disadvantages during planting and prolonged hair transplant sessions.

These disadvantages of storage solutions were the basis for our efforts to investigate a number of different bioactive substances known to positively influence the metabolic pathways in hair follicle cells and for their ability to improve the metabolic state of the isolated micrograft.

Methods

For this study, micrografts were stored for 5 hours in phosphate buffered saline (PBS) and simultaneously stimulated with different molecules. Micrografts stored in PBS served as a control. Thereafter, micrografts were transferred into hair follicle organ culture for an additional 3 days. Hair shaft elongation was measured in cultured micrografts during the 3 days in culture. After the culture period, micrografts were harvested and total mRNA was prepared by standard procedures. To quantify the amounts of specific mRNA, mRNA was reverse transcribed and subjected to real-time PCR (polymerase chain reaction). Real-time PCR allows the determination of mRNA transcription of genes of interest by amplification of the target genes with specific primers and measurement of PCR products after each PCR cycle with a DNA-binding fluorescent dye (SYBR-green). Depending on the amount of specific mRNA in the sample, logarithmic amplification occurs earlier or later during the PCR reaction, and the shift of the logarithmic phase of the PCR reaction can be used to quantify changes in the amount of mRNA relative to control samples. To exclude quantitative differences in starting mRNA levels, amplification of target genes was normalized to amplification of the housekeeping gene GADPH in each sample.

Results

In vitro hair shaft elongation (HSE) reveals increased elongation in micrografts stored in PBS containing vitamin B12 as compared to PBS alone. In PBS alone we found 1.2% HSE, in DMEM, which served as positive control, HSE was 17.6%. Supplementation of PBS with 2.5 ug/ml and 25 ug/ml vitamin B12 led to a dose-dependent increase in percent HSE (22.0% and 27.4%, respectively).

Analysis of mRNA transcription of intracellular hair follicle growth promoting molecules by real-time PCR revealed a dose-dependent induction of ß-catenin transcription (a key molecule of the *wnt* pathway) by vitamin B12 (see Figure 1). Transcription of glycogen-synthase-kinase 3 (GSK-3), which serves as an intracellular inhibitor of the *wnt* pathway, remained unchanged after stimulation with different doses of vitamin B12.

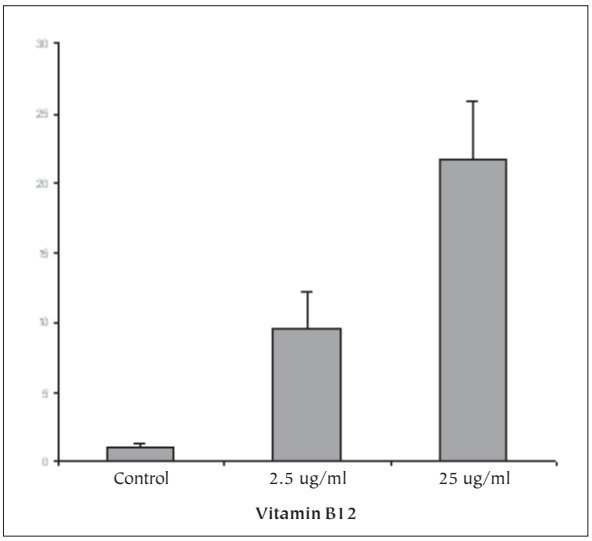

Figure 1. Fold increase of ß–catenin mRNA in stored micrografts

Discussion

Over the past 5 years, progress in understanding the molecular events during the storage period of micrografts has been made. This has led to the development of storage solutions that take the needs of isolated hair follicle cells into account. However, none of the developed solutions has made its way into routine hair restoration surgery. One reason for the reserved use in clinical practice is the influence on surgical routine. The micrografts stored in the developed solutions turned out to be well hydrated, making planting more difficult and time consuming. On the other hand, routinely used saline leads to satisfactory results and reasonable growth rates and percentages of growing micrografts. The benefits of defined storage solution, which are earlier regrowth and higher post-transplantational hair

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growth, might not justify the high costs of the formulated storage solutions.

In this first *in vitro* study, we evaluated the effect of PBS-based storage solutions, which are supplemented with selected molecules. Although these solutions did not cover some of the previously uncovered key factors for optimal micrograft preservation (i.e., nutrient supply and apoptotic cell death), an externally induced molecular switch ("priming") of the micrograft to a "proliferating" growth state might positively influence the post-transplantational behaviour of the micrograft.

In our study, it turned out that one substance that might meet this criteria is vitamin B12. We found a clear induction of hair shaft growth rate and, more important, the induction of a cellular signal transduction pathway, the *wnt* pathway, which is highly correlated to cell proliferation in the hair follicle. These *in vitro* findings strongly argue for an increased growth potential of the treated micrografts, and this might support micrograft engraftment and hair growth *in vivo*. However, more studies have to be done to identify molecules or combinations of molecules that support micrograft engraftment, and clinical trials have to confirm the *in vitro* results. However, using sophisticated molecular methods in the identification of substances supporting micrograft engraftment and growth might lead to the development of inexpensive solutions that meet the criteria necessary for routine use.✧

References

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