

Effect of EnduBerry™ Nu on Telomere Length in Human Fibroblasts



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INTRODUCTION

The present study evaluated the effect of EnduBerry™ Nu on telomere length in primary human fibroblasts and whether the extract demonstrates protective effects on telomere shortening. Telomere length is considered a marker of cellular aging: once telomeres become critically short, cells cease dividing and enter senescence. Such telomere shortening is further associated with age-related diseases and can be induced by oxidative stress and inflammation. For this experiment, cells were treated with EnduBerry™ Nu or vehicle control before being passaged multiple times for 6 weeks (representing replicative senescence). The measured parameters were population doubling and telomere length. Telomere length was measured with a high-throughput (HT) quantitative fluorescence in situ hybridization (Q-FISH) technology.

STUDY DESIGN

Test samples

- 43.3 µg/mL EnduBerry™ Nu

Cells

- Primary human fibroblasts

Test parameter

- Population doubling
- Telomere length analysis (TAT®)

Materials and Methods

- Cell growth: Countess™ cell counter (Invitrogen)
- Fluorescent peptide nucleic acid probe: Alexa488-OO-CCCTAACCCCTAACCCCTAA (Panagene)
- High Content Screening Opera Phenix System (Perkin Elmer)
- Columbus software Version 2.9 (Perkin Elmer)

Cell cultivation and treatment

Cells were grown in fibroblast medium (HEPES and bicarbonate based, pH 7.4) supplemented with 2 % FBS, growth factors and essential nutrients at 5 % CO₂ / 95 % ambient air. Fibroblasts were seeded at 5x10³ cells/cm² prior to experimental start. At the onset of the experiment, cells were treated with EnduBerry™ Nu or left untreated (control). The media and treatment was renewed every 2-3 days and cells passaged at sub-confluence (70-80 %) every 7 days for a total of 6 weeks.

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Measurement of Population Doubling

At each passage, cell growth is monitored by counting the cell numbers in an automated cell counter. Population doubling (PD) was calculated with the formula $PD = 3.322(\text{Log}(C_f) - \text{Log}(C_i)) + x$. C_f represents the final cell number, C_i the initial cell number and x the PD of the previous passage. One PD is equivalent to one round of cell replication.

Telomere Length Detection and Analysis

Telomere length analysis (TAT[®]) was performed with High-throughput (HT) Q-FISH. This method is based on a quantitative fluorescence *in-situ* hybridization method. Briefly, telomeres are hybridized with a fluorescent (Alexa-488) peptide nucleic acid probe that recognizes three telomere repeats. The images of the nuclei and telomeres are captured by a high-content screen system and the intensity of the fluorescent signal detected from the probes can be translated to telomere length. Cells from each weekly passage were seeded to black-walled 384-well plates, with 5 replicates of each treated sample and 8 replicates of untreated each control. After fixation with methanol/acetic acid the cells were treated with pepsin to digest the cytoplasm and the nuclei processed for *in situ* hybridization with the PNA probe. After washing the cell nuclei were further stained with DAPI before continuing to image acquisition. For each well, 15 independent images were captured and the telomere fluorescence intensity measured at the 488 nm wavelength. The results of fluorescence intensity were analysed and calculated with Life Length's proprietary program. Statistical analysis was performed with Student's T-test.

RESULTS

Population Doubling

Treatment with EnduBerry[™] Nu at 43.3 µg/mL stimulated fibroblast proliferation and the cells presented an increase in proliferative capacity after the first week of expansion.

Telomere length analysis (TAT[®])

Normalizing the median telomere length (initial – final) to the population doubling was used to determine the telomere shortening rate. Treatment with EnduBerry[™] Nu unfortunately showed no effect on telomere shortening rate.

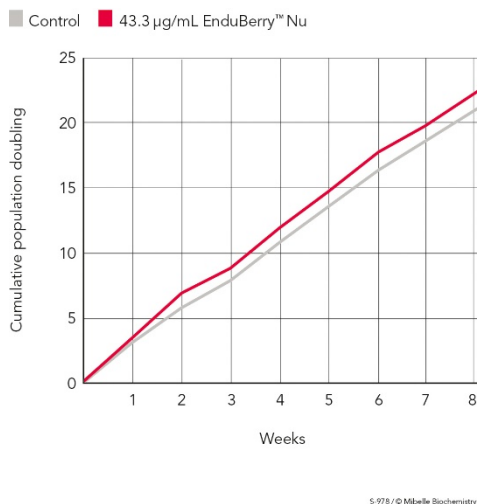


Figure 1: Increase in population doubling after 8 weeks of EnduBerry[™] Nu

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