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# GROWTH OF HUMAN FIBROBLASTS IN THE PRESENCE OF 6-HYDROXYHEXANOIC ACID

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Biodegradable polymers such as aliphatic polyesters are commonly used in pharmacy and medicine. Because of their biocompatibility, they are utilized to manufacture surgical fibers, devices for bone fracture internal fixation and as the carriers for controlled release of drugs (1, 2). Moreover, they can be easily processed to highly porous scaffolds for use in tissue engineering (2, 3). Biodegradable polymers containing caproyl units belong to the most frequently used biomaterials (2, 4). Poly-ε-caprolactone (PCL) seems to be very attractive because of its excellent thermal properties and permeability to drugs. PCL can be degraded hydrolytically in a humid environment. However, degradation rate of PCL is very slow due to its hydrophobicity and crystallinity. It has been shown that some enzymes can modulate the degradation rate of PCL *in vivo*. The degradation product of poly-ε-caprolactone hydrolysis is 6-hydroxyhexanoic acid. It is converted to adipic acid by ω-oxidation in the endoplasmic reticulum of liver and kidney cells. The adipic acid is then metabolized by β-oxidation and Krebs cycle to carbon dioxide and water (5). 6-Hydroxyhexanoic acid is thought to be biocompatible but there are not any experimental data that demonstrate its impact on human cells *in vitro*.

Recently, in our laboratory, co- and terpolymers obtained from poly-ε-caprolactone have been found to possess a shape memory effect. Shape memory devices are suitable for a wide range of medical applications such as biodegradable stents or surgical clamps (6).

The aim of our study was to determine the impact of different concentrations of 6-hydroxyhexanoic acid on human connective tissue cells. Changes in cell morphology and growth rate were analyzed as well as the concentration of tested compound in cell culture medium was assessed.

## EXPERIMENTAL

HGF-1 cell line (human fibroblasts) was purchased from LGC Promochem (Łomianki, Poland). The cells were grown in minimum essential medium (MEM) supplemented with 10% FBS, 100 U/mL penicillin, 100 ⌠g/mL streptomycin, 1× MEM-Non Essential Amino Acids Solution and 10 mM HEPES. The cell cultures were maintained at 37°C in an atmosphere of 5% $CO_2$/95% air. Human chondrocytes were purchased from Lonza (Basel, Switzerland) and cultured in chondrocyte growth medium (Lonza, Basel, Switzerland).

To study the cell proliferation, cells were plated at an initial density of $10^3$ cells per well in 200 µL of culture medium in 96-well plates. Cells were allowed to attach and grow for 24 h prior to exposure to test reagents. Cells were incubated with 6-hydroxyhexanoic acid for 6 days. Subsequently, they were washed with PBS and fixed in 10% trichloroacetic acid. Proliferation of the cells was quantitated using "In Vitro Toxicology Assay Kit, Sulforhodamine B Based" (Sigma-Aldrich, Poznań, Poland) according to the manufacturer's protocol. The sulforhodamine B is a dye staining cellular proteins. After the liberation of the incorporated dye, absorbance was measured at λ = 570 nm and λ = 690 nm (reference wavelength) using the MRX Revelation plate reader (Dynex Technologies). In

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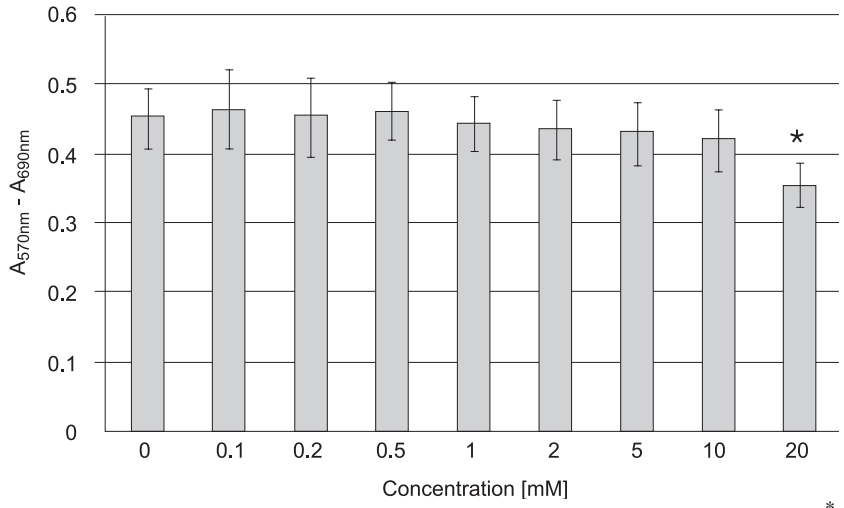


Figure 1. Growth of HGF-1 cell line in the presence of 6-hydroxyhexanoic acid. Each bar represents the mean ± SD; $^{*}p < 0.05$ compared with control

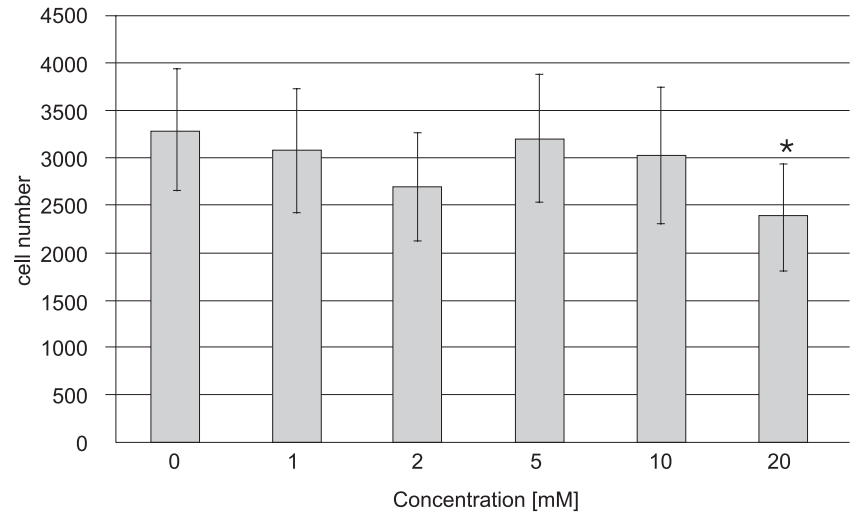


Figure 2. Growth of chondrocytes in the presence of 6-hydroxyhexanoic acid. Each bar represents the mean ± SD; $^{*}$ $p < 0.05$ compared with control

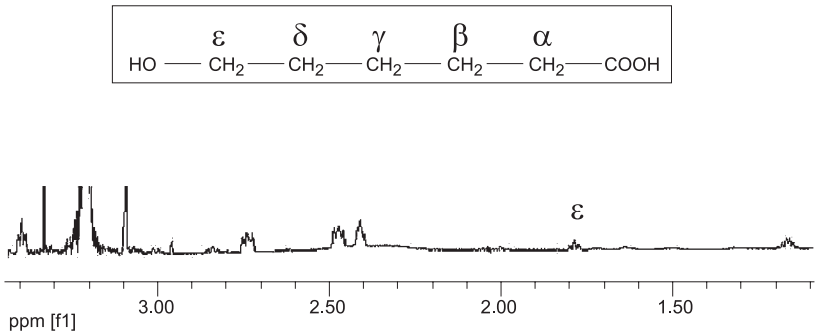


Figure 3. $^{1}H$ NMR spectrum of the culture medium supplemnented with 5 mM 6-hydroxyhexanoic acid after the culture period. Signal corresponding to the ε-$CH_2$ group is indicated

some assays cell number was quantitated by use of CyQUANT® Cell Proliferation Assay Kit according to the manufacturer's (Invitrogen) instructions. The assay utilizes the green fluorescent dye (CyQUANT GR dye) which exhibits strong fluorescence enhancement when bound to cellular nucleic acids. Fluorescence emission was measured at $\lambda = 535$ nm after excitation at $\lambda = 485$ nm using VICTOR$^2{}_{TM}$ multilabel counter (Perkin Elmer).

The presence of 6-hydroxyhexanoic acid in the culture medium was determined by use of the $^1$H NMR technique.

## RESULTS AND DISCUSSION

Cells were cultured in the presence of various concentrations (0.1, 0.2, 1.0, 2.0, 5.0, 10.0 and 20.0 mM) of 6-hydroxyhexanoic acid.

Fibroblasts growing in the presence of 6-hydroxyhexanoic acid at all concentrations displayed normal morphology, remained spread on the substratum and any cell detachment was not observed. Some inhibition of cell growth was seen exclusively at the highest concentration of the tested substance (Fig. 1). Additionally, the impact of the highest concentrations of 6-hydroxyhexanoic acid on the cell growth was tested with the use of human chondrocytes (Fig. 2). Analysis of $^1$H NMR spectra revealed that concentration of 6-hydroxyhexanoic acid in the medium did not change during the culture period (Fig. 3).

Generally, results of our study evidence the biocompatibility of the degradation product of poly-ε-caprolactone. Therefore, some negative cell response to the poly-ε-caprolactone observed by certain authors can not be attributed to the degradation product of the material but rather to possible contaminants or its hydrophobicity (7, 8).

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