

# The effect of periodical and mechanical stretching on cell morphology and proliferation of chondrocytes: a testing model

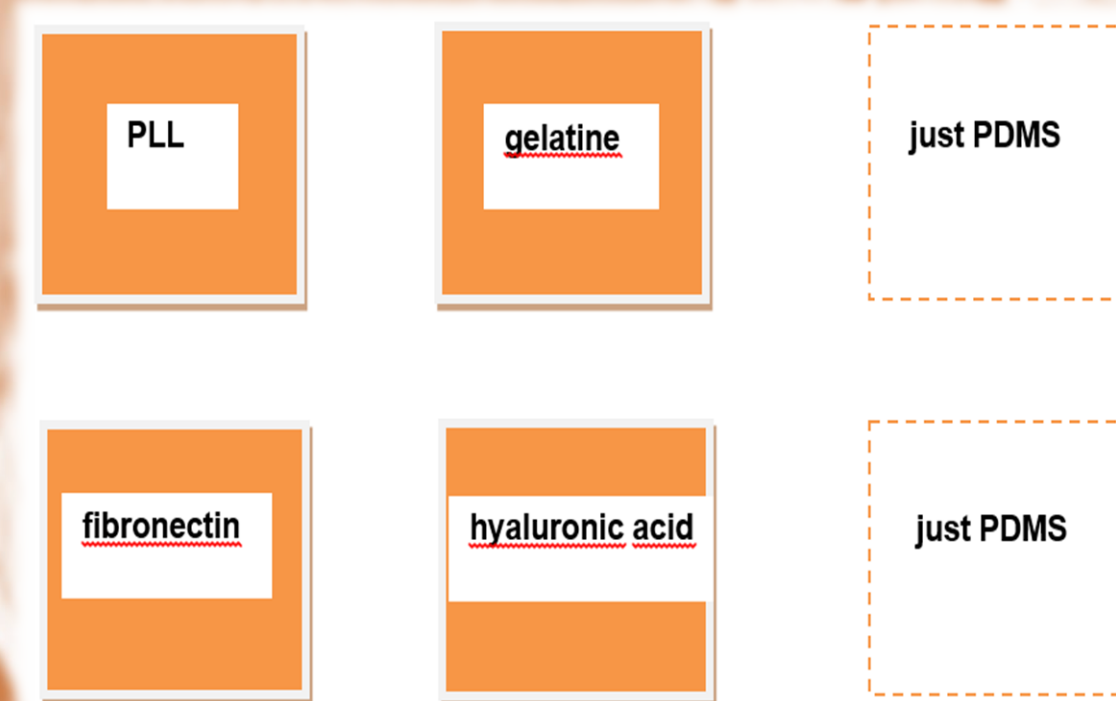
Aylin Yurtseven, Vasiliki Rousidi, Berrin Canbaz, Dipl.-Ing. (FH) Kiriaki Athanasopulu, Prof. Dr. Ralf Kemkemer  
Angewandte Chemie, Hochschule Reutlingen  
Biomedizinische Wissenschaften, Labor Biomaterialien SS 2016

## Background

The cartilage regeneration of knee joints is getting more important in medicine. A usual method to heal damaged cartilage is the application of chondrocyte transplantations with autologous chondrocytes. This method demands a cultivation of chondrocytes *in vitro*. The cells proliferate in cartilage after a transplantation. In the natural environment of the cartilage the chondrocytes are exposed to mechanical forces. The aim of this project is to create a testing model which shows the effect of periodical and mechanical stretching on cell morphology and proliferation.

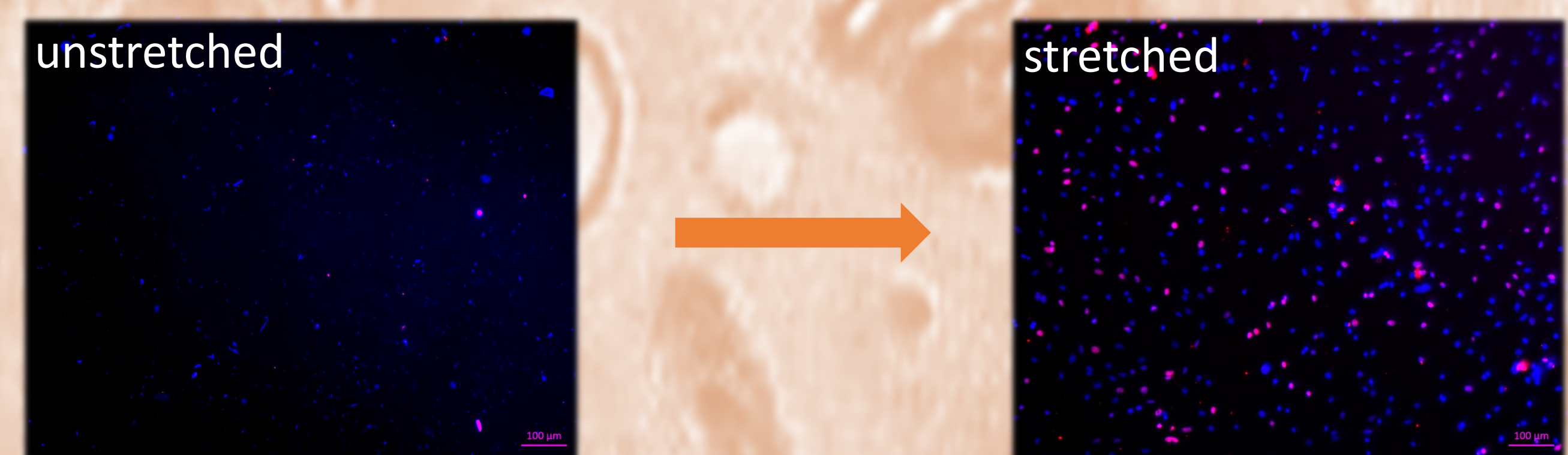
## Material and Methods

- Production of PDMS substrate
- Coating of the substrates with poly-L-lysine, gelatin, fibronectin, hyaluronic acid
- Measurement of the contact angle
- Cultivation of chondrocytes
- Seeding of the chondrocytes on the pretreated substrates
- Incubation
- Starting of the stretching device
- Fixation of the overgrown substrate
- Beginning of the 24-hour experiment
- Fluorescence-staining
- Detection with fluorescence-microscopy
- Cell counting

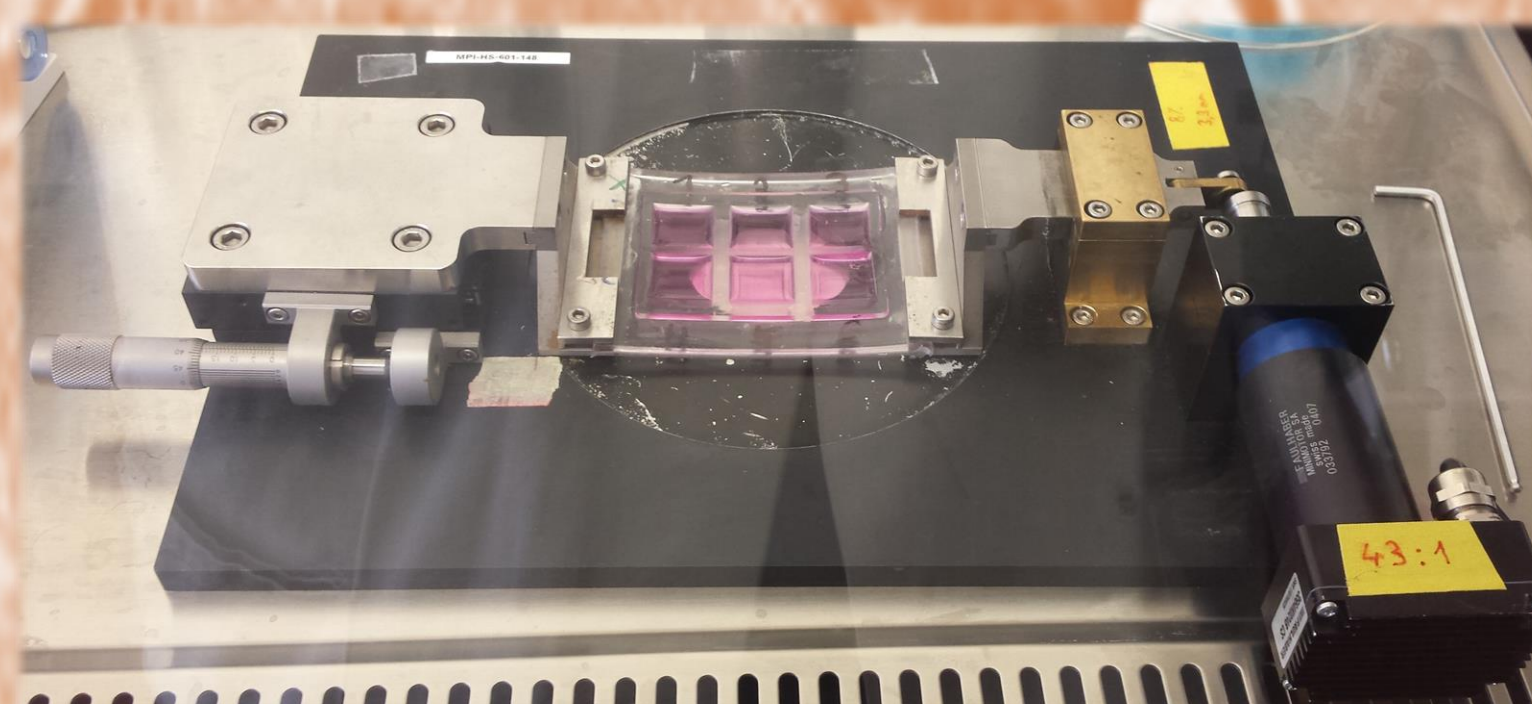


## Fluorescence staining

- Integration of 5'-Ethynyl-2'-Desoxyuridin (EdU) in the synthesized DNA before stretching
- Fixation of the cells and preparation of the click-iT® reaction cocktail containing Alexa Fluor® azide for staining of the proliferating cells
- 4',6-Diamidin-2-Phenylindol (DAPI) staining of total nuclei



## Experimental set-up:

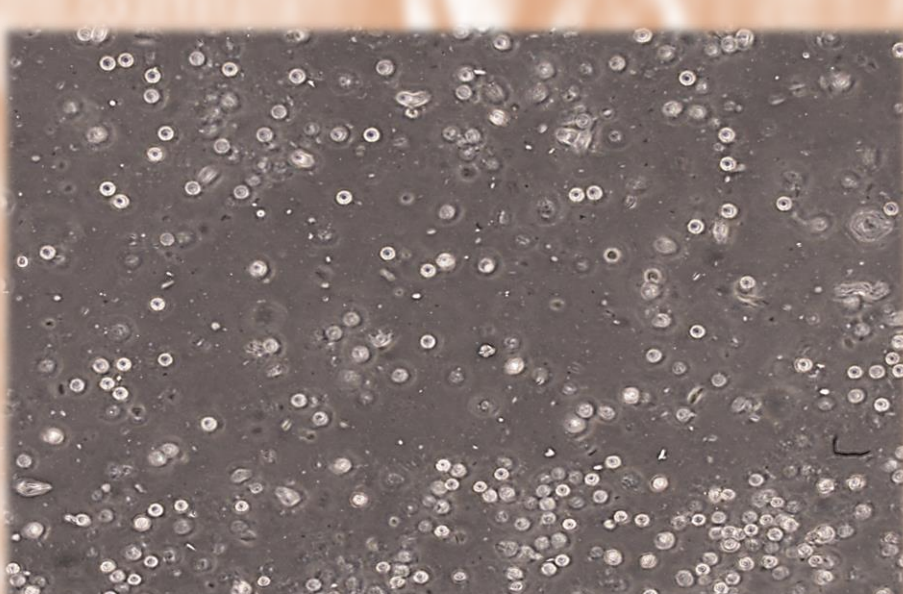


- stretching grade: 8 %
- gear motor: 43:1
- stretching in mm: 7 mm

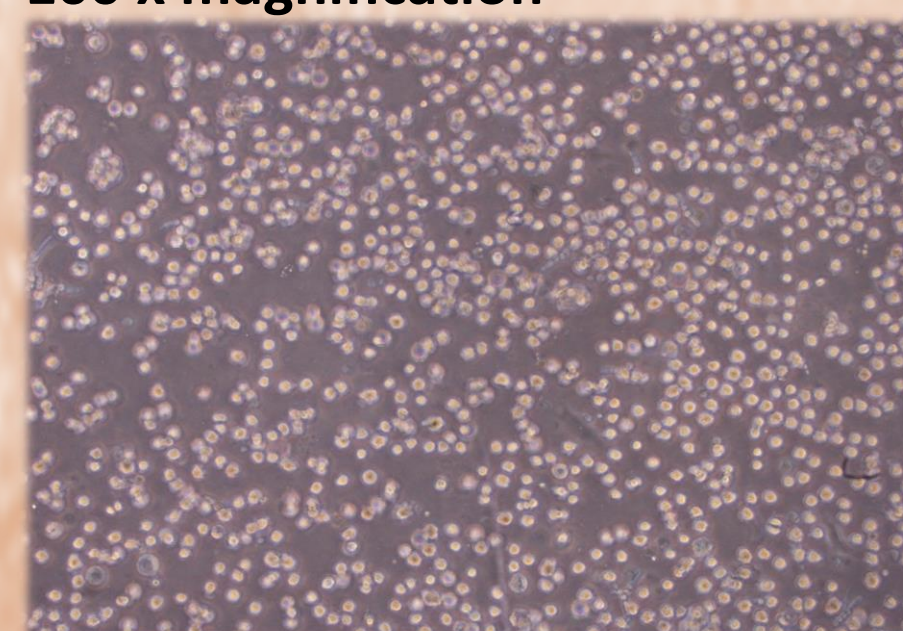
## Calculation of the proliferation rate in %

Way of stretching	unstretched cells			
Coating	Fibronectin	Gelatin	Hyaluronic acid	Poly - L - Lysin
Amount of cells with DAPI	61	541	31	37
Amount of cells with EdU	22	472	16	15
Proliferationsrate in %	36	87,2	52,6	40,5
Way of stretching	stretched cells with V500			
Coating	Fibronectin	Gelatin	Hyaluronic acid	Poly - L - Lysin
Amount of cells with DAPI	111	665	171	964
Amount of cells with EdU	3	38	21	205
Proliferationsrate in %	2,7	5,7	12,3	21,3

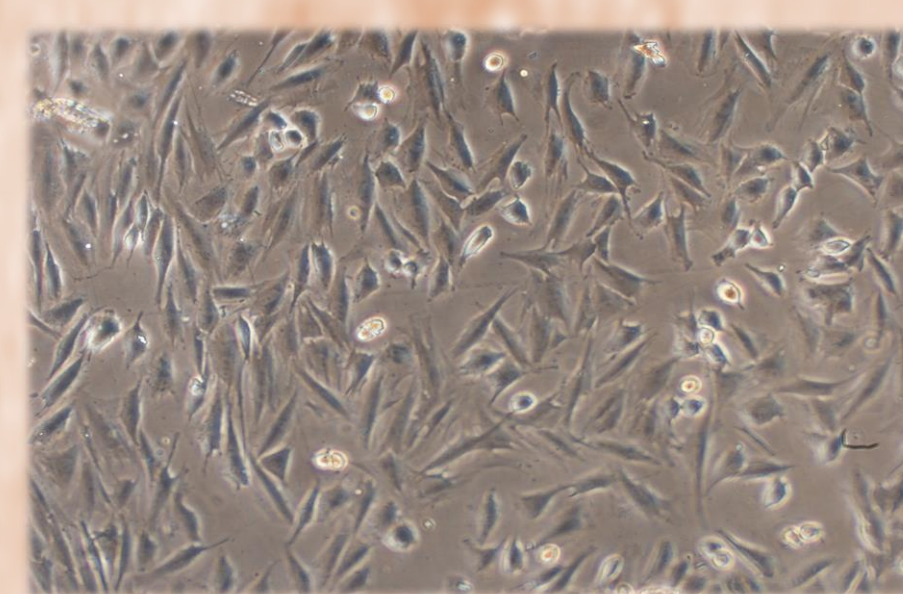
## Morphology using the example of PLL and hyaluronic acid (h.a.)



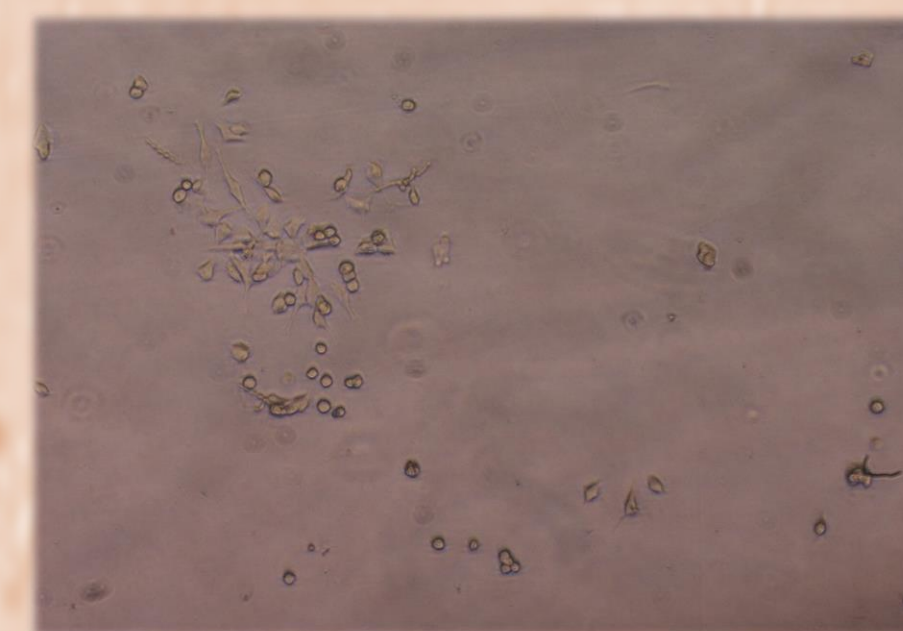
PLL coating before stretching  
100 x magnification



h.a. before stretching  
100 x magnification



PLL coating after stretching  
100 x magnification



h.a. after stretching  
100 x magnification

The images on the left show chondrocytes on a PLL and hyaluronic acid coating before stretching.

The images on the right show the chondrocytes after stretching with velocity 500 (V500) equates ca.12 rotations/min.

The images were taken by phase contrast microscope.

## Results

The example of PLL shows rounded and bright cells after 3 days of cultivation before stretching. The morphology changes during the 24-hour experiment. Considering the results of different coatings on the unstretched substrate the proliferation rate of the chondrocytes on the gelatin surface is with 87,2% the highest and on the fibronectin surface with 36 % the lowest. The chondrocyte population on the stretched substrate is in total higher than the population on the unstretched substrate. The cells on the PLL surface proliferate with a rate of 21,3 % the strongest and on the fibronectin surface only 2,7 %. But at the unstretched substrate the proliferation rates are significant higher.

## Conclusion

The calculated proliferation rates of the different coatings predicate that as well as the coating and the stretching can influence the morphology and proliferation of chondrocytes. The stretching of the cells have a negative effect on the cell proliferation rate, but a positive effect on the cell morphology. Besides the cells prefer certain coatings like gelatin and PLL. The stretching of the substrate improve the adhesion of the cells on the coatings. In addition there is no relation between the cell count and the proliferation rate. In conclusion it is important to determine the optimal coating and to degrade the stress level.