Medical Engineering and Physics 74 (2019) 58–64

Contents lists available at ScienceDirect

Medical Engineering and Physics

journal homepage: www.elsevier.com/locate/medengphy

Chondrocyte colonisation of a tissue-engineered cartilage substitute under a mechanical stimulus

Julia Nachtsheim[a,*], Gözde Dursun[a], Bernd Markert[a], Marcus Stoffel[a]

Institute of General Mechanics, RWTH Aachen University, Germany

ARTICLE INFO

Article history:
Received 25 March 2019
Revised 30 August 2019
Accepted 29 September 2019

Keywords:
Cartilage
Human chondrocyte migration
Bioreactor system
Mechanobiology
Tissue engineering

ABSTRACT

Cell-free collagen scaffolds as cartilage substitute for small focal defects show promising results in first clinical studies. However, chondrocyte migration between scaffolds and the colonisation process of a cell-free implant is yet to be fully understood. We here focus on mechanobiological interdependencies between cell migration and mechanical stimulus in a 3D environment. We develop an *in vitro* model composed of a human chondrocyte-seeded collagen base and adjacent cell-free collagen type I scaffolds of varying collagen concentrations. Constructs are either cultured statically or dynamically under the influence of a physiological compression (0.5Hz, 0.5% initial strain). After 20 days we identify vital chondrocytes inside all collagen implants, proving that chondrocytes migrated from the underlying scaffold into the implants. Chondrocytes have not colonised the entire sample and are predominantly found in the bottom of the implant. In static culture conditions, a nearly equal cell number is found inside of all collagen scaffolds. In dynamic culture, the total amount of cells is increased by 30% to 320%, with the highest population in a commercial implant. Differences in cell population between the materials in dynamic culturing can be referred to differences in mechanical properties of the scaffolds, such as strain-rate insensitivity fostering the colonisation process.

1. Introduction

Articular cartilage is a very sensitive part of the musculoskeletal system redistributing loads and guaranteeing low friction movements. Its mechanical structure and composition are highly complex and vary zonally. Since the tissue is avascular, it exhibits only limited self-regenerative abilities. Nonetheless, healthy cartilage tissue is pivotal for joint function. Untreated tissue injuries can lead to progressive pathological changes of the entire joint with a high risk of an incipient osteoarthritis [1,10,22,28]. Conventional orthopaedic surgeries like microfracturing, autologous cell therapies or mosaic plastics feature major disadvantages and there is a strong need for new therapeutic approaches. In the last years hydrogels have emerged as a promising basis for tissue-engineered cartilage replacement constructs for small focal defects. Hydrogels like alignate, agarose, hyaluronic acid hydrogels, collagen gels, or synthetic hydrogels serve as extracellular matrices and are seeded with chondrocytes which induce a native tissue formation process inside the gel [36]. The hydrogel serves as a partly temporary environment to mend the defect zone and to facilitate cell growth, cell proliferation, and tissue regeneration. *In vitro* studies have extensively analysed tissue formation of cell-laden hydrogels and have identified growth factors and an external mechanical stimulus as critical factors to prevent chondrocyte dedifferentiation and to foster metabolic activity [18,35].

Hence, a variety of bioreactor systems applying direct compressions, shear or hydrostatic pressure to tissue-engineered materials have been developed, mimicking physiological loading regimes and allowing for studying and improving chondrogenesis [7]. In a compression bioreactor, tissue formation of chondrocyte-laden collagen gels under static and dynamic culturing conditions was analysed, identifying a fostered increase in material stiffness in dynamic culturing due to collagen fibre formation [34]. Mauck et al. [14] observed a significant increase of the equilibrium modulus of chondrocyte-seeded agarose gels after 28 days, when applying a dynamic compressive strain of 10% at a frequency of 1 Hz.

Although large progress has been made on cell-laden tissue-engineered cartilage constructs, the *in vitro* cell culturing still has its drawbacks. *In vitro* studies are predominantly performed over a period of up to 4 weeks identifying gene expression towards a native cartilage tissue formation with improving matrix properties. However, cartilage-like tissue formation with zonally varying matrix structure and composition with comparable mechanical properties still remains a challenge for tissue engineering [36]. A

* Corresponding author.
E-mail address: nachtsheim@iam.rwth-aachen.de (J. Nachtsheim).

https://doi.org/10.1016/j.medengphy.2019.09.022
1350-4533/

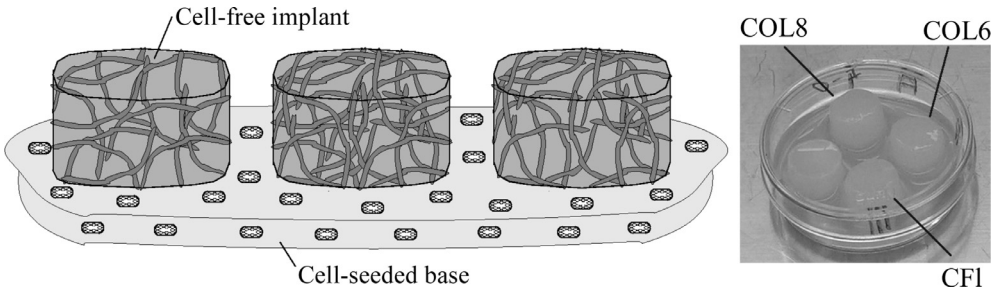

Fig. 1. Schematic figure of the *in vitro* model composed of a subjacent cell-seeded base and four cell-free collagen implants (left), *in vitro* model prepared in a petri dish with four different collagen implants (right).

second disadvantage is the supply of autologous patient's cells. In autologous cell-therapy, a small amount of chondrocytes of about 2000 - 4000 cells/mg is harvested in a first surgery and are subsequently cultivated to increase the cell number [6]. In gel based repair, chondrocyte densities of more than 10 x 10^6 cells/mL are required with the total number of cells depending on the defect size [9]. During this process, there is a high risk of phenotype changes due to cell passage or monolayer culturing as reported by Darling et al. [8]. To overcome the restriction in cell supply, stem cells or co-cultures of chondrocytes and stem cells are used with the purpose of chondrogenic differentiation [5,16,27,29,31]. Overcoming all these limitations of *in vitro* cell culturing and donor site morbidity, the implantation of cell-free hydrogels is a new therapeutic approach for small focal cartilage defects. For the therapeutic success, chondrocyte migration from the adjacent healthy cartilage tissue into the implant is pivotal to enable the above discussed chondrogenic tissue remodelling. The hydrogel can be implanted in a minimally invasive one-step surgery and fills in the defect zone instantaneously. First clinical studies have retrospectively analysed cell-free collagen scaffolds in knee and ankle joints using the subjective clinical function score [24]. A significant improvement of the afflictions one year after surgery and a very good integration to the surrounding cartilage tissue was observed [3,21]. In a clinical case report the presence of vital chondrocytes inside the collagen implant was proven, although it could not be identified if cells have migrated from the underlying intact osseous tissue, the surrounding cartilage or from the synovial fluid [26].

The migration of articular chondrocytes is a highly complex process, which is influenced by various factors, such as the composition and structure of the extracellular matrix or chemoattractants and is yet to be fully understood [17]. Various studies have observed chondrocyte motility *in vitro* with the vast majority focusing on two-dimensional chondrocyte migration [4,17]. Hamilton et al. [12] analysed the influence of surface topography on chondrocyte motility, finding an accelerated movement on grooved surfaces in comparison to flat surfaces. In a second study they assessed the influence of passage number, identifying minor differences between chondrocytes of passage two and passage three in grooved surface, but a decrease in the percentage of moving cells on flat surfaces at increasing passage [11]. Chondrocyte migration inside a three-dimensional 4 mg/mL collagen matrix was studied by Shimizu et al. [30] quantifying a migration speed of 1 µm/h. Parbbruwe et al. [20] identified migration of nasal chondrocytes between two tissues, native cartilage tissue and a cell-seeded or a cell-free collagen-based scaffold. To the knowledge of the author, no studies have assessed the migration of chondrocytes between two collagen based scaffolds under the influence of a physiological mechanical stimulus.

In this study, we analyse the migration of human articular chondrocytes into and inside different collagen scaffolds and assess the effect of a physiological and dynamical compressive stimulus. Therefore, we introduce an *in vitro* model composed of a cell-seeded scaffold and cell-free collagen implants of varying collagen concentrations. We compare the cell-colonisation process of the implants in static and dynamic culturing conditions. An inhouse bioreactor system is used to apply a physiological dynamic compression to the constructs. We hypothesize that chondrocytes migrate from the underlying tissue into all adjacent cell-free collagen implants and initiate the colonisation process. Due to the limitation in culturing time, we do not expect the implants to be entirely and homogeneously seeded by chondrocytes. Furthermore, we expect the colonisation process to be accelerated in dynamic culturing conditions.

2. Materials and methods

2.1. In vitro model

The *in vitro* model is composed of three cell-free implants and a subjacent cell-seeded collagen base as presented in Fig. 1.

For preparation of the cell-seeded base, human articular chondrocyte, purchased from PromoCell GmbH (Heidelberg, Germany) were mixed with 2 mL bovine collagen type I (PureCol EZ gel, Advanced BioMatrix Inc., San Diego, USA) and cast into a petri dish (35x10mm, SARSTEDT AG & Co. KG, Nürmbrecht, Germany). Two experimental series were performed with cells at passage 2 and passage 3. Using chondrocytes of passage two (HCH-P2), a base with a concentration of 100,000 cells/mL was prepared. A second test series was performed with cells of passage three (HCH-P3) and a concentration of 80,000 cells/mL. The used cell concentrations are significantly higher than in native cartilage [32] and are comparable to chondrocyte concentrations used in *in vitro* experiments [11,12,15].

Three different collagen type I based cylindrically shaped implants with varying collagen concentration with a diameter of 10 mm and a height of 6 mm were tested. Scaffolds with concentrations of 6 mg/mL (COL6) and 8 mg/mL (COL8) collagen type I were compared to a commercially available product, see Fig. 2. To produce COL8 specimens, an acidic collagen solution of 10 mg/mL was mixed with a gel neutralisation solution (Meidrix Biomaterials GmbH, Esslingen, Germany), which neutralises the pH value of the collagen, at a ratio of 4:1 and cast into cylindrical shape. To prepare COL6 scaffolds, the mixture was further diluted with Dulbecco's Phosphate Buffered Saline (DPBS, Gibco by Fisher Scientific GmbH, Schwerte, Germany) at a ratio of 3:1. ChondroFiller liquid (CFl) (Meidrix Biomaterials GmbH, Esslingen, Germany) was prepared according to the manufacturer's instructions and cylindrical samples were cast.

For each experimental series, a total of six *in vitro* models were prepared, composed of cell-seeded base and three implants, three for dynamic cultivation and three for static cultivation.

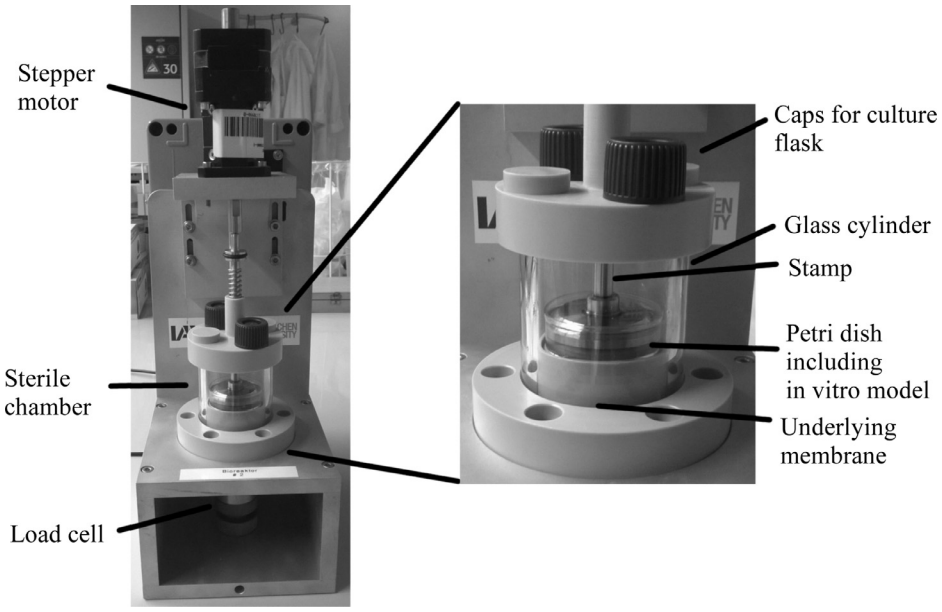

Fig. 2. Bioreactor system composed of a sterile chamber enclosing the *in vitro* model. Deformation controlled compression is applied by a stepper motor at the top of the bioreactor system. Reaction force is recorded by a load cell which is located underneath the sample.

2.2. Bioreactor system

An inhouse bioreactor system adapted from a previous study [33] was used to culture the constructs under dynamic compression and in physiological conditions. The petri dish including the *in vitro* model is encapsulated in a glass chamber and gas exchange is allowed via two caps for cell culture flasks (VWR International GmbH, Darmstadt, Germany) as shown in Fig. 2. A stepper motor is positioned above the sterile chamber driving a solid and flat-ended punch inside the chamber which applies a displacement controlled compression to the collagen implants. At the bottom, the chamber is restricted by a malleable membrane (SILPURAN Film 200 µm, Wacker Chemie AG, Munich, Germany) transferring the reaction force to a load cell.

2.3. Static and dynamic culturing protocols

A total of six *in vitro* models were prepared 24 h after cell-seeded and cell-free collagen preparation, and consecutively cultured either statically or dynamically for a total of 20 days in an incubator with humidified atmosphere with 5% CO_2 at 37 °C. During the experiment, specimens were fully immersed in Chondrocyte Growth Medium (PromoCell GmbH, Heidelberg, Germany), which was exchanged at least every second day. Dynamically cultured *in vitro* models were compressed unconfined and displacement controlled in a triangular manner at a frequency of 0.5 Hz at an initial strain of 5% in vertical direction. Mechanical stimulus was applied in 4 out of 6 days: 2 days static culture - 4 days dynamic culture - 2 days static culture - 4 days dynamic culture - 2 days static culture - 4 days dynamic culture - 2 days static culture. During each day of dynamic culture, stimulus was applied 4 times for 30 minutes with pauses of 60 minutes. At the end of each stimulation day, samples were taken out of the bioreactor and culturing medium was replenished. Due to a significant decrease in sample height, compression was decreased by 50% after the first 4 days of dynamic cultivation.

2.4. Microscopic evaluation

Cell distribution inside the collagen implants was analysed with a fluorescence microscope (BZ-9000, Keyence Deutschland GmbH, Neu-Isenburg, Germany) in combination with a Live/Dead cell staining. For Live/Dead cell staining, implants were washed with DPBS (Gibco by Fisher Scientific GmbH, Schwerte, Germany), then immersed for one hour in 10 µM calcein solution (Calcein, AM, cell-permeant dye, Thermo Fisher Scientific Inc., Waltham, USA), and subsequently for 10 minutes in propidium iodide (Thermo Fisher Scientific Inc., Waltham, USA). After staining, specimens were positioned on a petri dish and scanned with the fluorescence microscope with a two-fold magnification. To cover the entire sample diameter, five adjoining Z stacks were acquired. Subsequent image processing was performed with Fiji [23]. The five Z stacks were merged to one single Z and the position of each cell, vital or dead, was analysed as following: 1. the background was subtracted, 2. the threshold was adjusted for each Z stack individually, 3. the holes were filled in, 4. the image was converted, 5. touching objects were separated by watershed algorithm, 5. 3D image counter algorithm [2] was used to determine the position of the centroid of volume of each cell. Statistical analysis of the depth dependent cell distribution was subsequently performed with MATLAB (R2017b, MathWorks Inc., Natick, USA).

2.5. Unconfined compression tests

Unconfined compression tests of the three implants were performed to identify differences in material properties. Collagen samples were prepared as described previously and immersed in DPBS until testing to avoid dehydration of the hydrogel. Unconfined compression relaxation tests were performed on an uniaxial testing machine (zwickiLine Z2.5, Zwick GmbH & Co. KG, Ulm, Germany) equipped with force sensor Xforce HP with a nominal force of 50 N (Zwick GmbH & Co. KG, Ulm, Germany). Collagen gels are positioned on a solid plate and compressed strain controlled with a solid and flat-ended punch, see Fig. 3. Samples were approached at a velocity of 25 µm/s until a pre-load of 0.02 N was reached and held constant for 60 s. Subsequently, samples were compressed with a velocity of 5 µm/s or 10 µm/s until a compression of 15% was reached and relaxation was measured for 120 s. During the entire experiment, the sample diameter was recorded with a video extensometer (ME46-155, Messphysik Materials Testing GmbH, Fürstenfeld, Austria) and used to calculate the true stress. As shown in Fig. 3, the contrast was increased with contre-jour. A minimum of

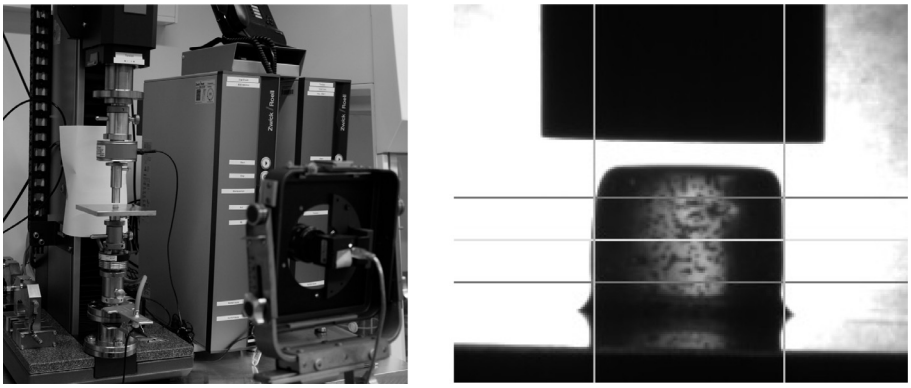

Fig. 3. Unconfined compression relaxation setup including the test machine and the video extensometer (left). Video extensometer recording the lateral sample expansion during compression (right).

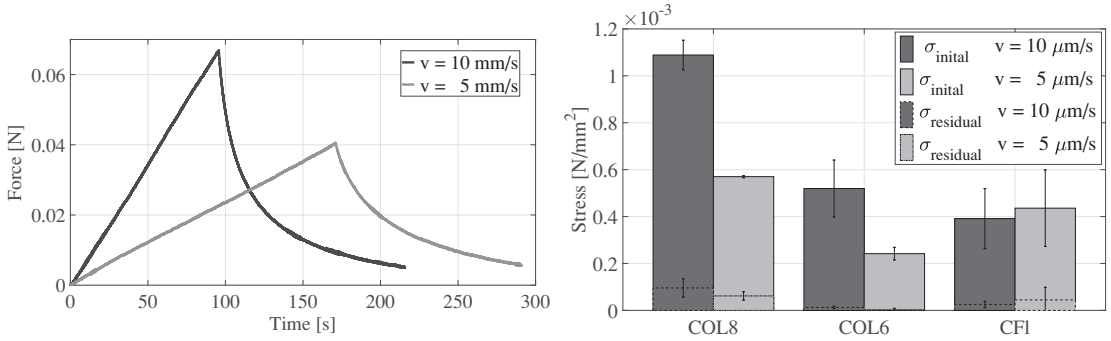

Fig. 4. Reaction force during unconfined compression experiment of a COL8 specimen (left). Mean initial and residual stress during relaxation period, error bars describing the standard deviation (right).

three specimens were tested for each contraction velocity. Stress-strain behaviour was analysed in MATLAB.

3. Results and discussion

3.1. Mechanical tests

In unconfined compression tests, all three collagen gels possess a viscoelastic material behaviour with distinct relaxation characteristics. A representative force-time characteristic of a COL8 specimen is shown in Fig. 4. The stress-strain correlation reveals the linear-elastic characteristic with COL6 and COL8 materials being strain-rate dependent, see Fig. 5. CFl in contrast does not show a strain-rate dependent behaviour. Higher material stiffness of COL8 in comparison to COL6 and CFl can be referred to more densely packed collagen fibres due to higher collagen concentration. During relaxation, the strain decreases fast in all collagen gels, approaching zero after 120 s. Fig. 4 compares the initial strain at the beginning of the relaxation period to the residual strain after 120 seconds. During compression, we observe fluid expulsion which has been extensively analysed by Neel et al. [19]. Fluid content prior and after testing have not been further analysed.

3.2. Cell migration

Cell migration in different collagen type I based implants is studied in static and dynamic culture conditions. Three specimens of COL6, COL8, and CFl are cultured dynamically and statically. Two

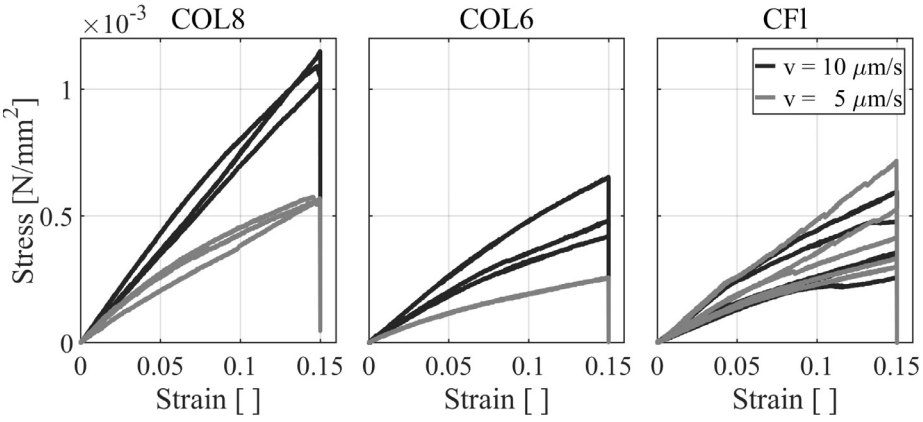

Fig. 5. Stress-strain curve of unconfined compression experiments of the four collagen scaffolds.

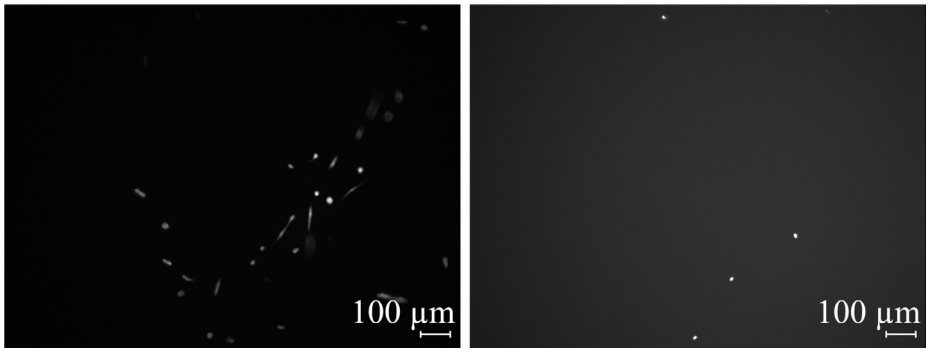

Fig. 6. Fluorescence microscopic imaging of a Live/Dead stained HCH-P2 inside a dynamically cultured CFl specimen at a 10-fold magnitude. Vital calcein stained chondrocytes on the left, dead propidium iodide stained chondrocyte on the right.

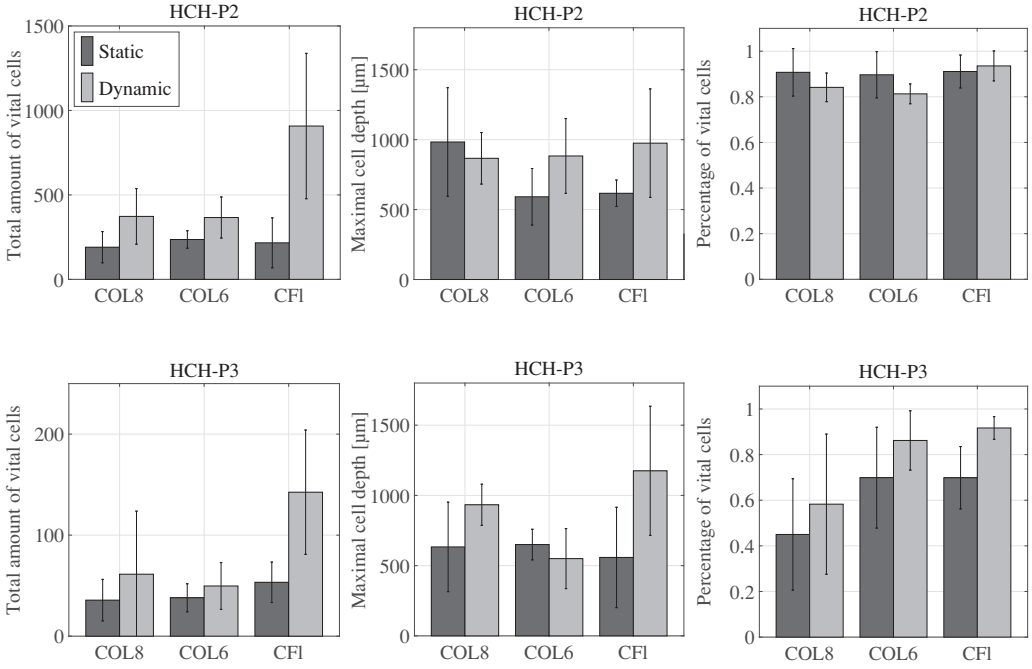

Fig. 7. Experimental results of cell migration experiment with HCH-P2 (top) and HCH-P2 (bottom). Total number of vital cells inside the implants (left), maximal cell migration depth describing the distance between tissue boundary and vital cell (centre), and percentage of vital cells (right). Bars graphing the mean values and error bars describing the standard deviation.

experimental series are performed with chondrocytes of different passages: passage two (HCH-P2) and passage three (HCH-P3). During dynamic cultivation, one CFl implant has been destroyed in HCH-P3 culture. For this reason, the evaluation of HCH-P3 migration includes only two CFl samples.

After 20 days of dynamic or static culturing, we have identified vital chondrocytes inside all three collagen gels. Human articular chondrocytes have migrated from the underlying tissue into the collagen implants. Fig. 6 portrays vital and dead chondrocytes of passage 2 inside a dynamically cultured CFl implant. The fluorescence microscopic image reveals a higher amount of vital chondrocytes than dead chondrocytes. Moreover, the cell shape of vital chondrocytes and blurry cells outside the focal plane show that chondrocytes reside in the three-dimensional environment of the implant. Our finding confirms the hypothesis by Schneider et al. [25] that some of the *in vivo* observed cells inside the collagen implants have migrated from the surrounding healthy cartilage tissue and do not only derive from the bone marrow in case of subchondral lesion.

In Fig. 7 on the left, the total amount of vital chondrocytes colonising the implants are displayed. We observe no differences in HCH-P2 population between the three implants in static culture. The same applies for HCH-P3 cells. Due to dynamic compression, population of HCH-P2 and HCH-P3 is increased in all three collagen implants. The amount of HCH-P2 colonising COL6 is raised by 55% and the amount of HCH-P2 inside COL8 is doubled in comparison to static culturing. An even larger effect of dynamic stimulation is revealed for CFl with HCH-P2 population being more than tripled. The interdependency between biological processes and mechanical stimulation, or in other words, the mechanobiological activity, has already been reported in previous studies for cell-laden scaffolds [14,34]. In the present study, this mechanobiological impact on chondrocytes can also be observed fostering the colonisation process.

We identify a similar increase of the cell population due to dynamic cultivation in COL6 and COL8 scaffolds, in comparison to a significantly larger increase in CFl scaffolds. In unconfined compression test we have identified differences in the material

stiffness between COL6 and COL8 materials. At an equal level of compressive strain, the state of stress inside COL8 scaffolds is larger than in COL6 scaffolds. With the number of cells inside both scaffolds being similar, we conclude that the differences in the state of stress (which cells are exposed to inside both scaffolds) do not influence the cell growth significantly. During dynamic stimulation, the state of stress changes within one compressive cycle to a larger extent in a strain-rate dependent material like COL6 or COL8 than in a strain-rate insensitive material like CFl. This implies that smaller changes in the state of stress in oscillating compressive stimulus support the colonisation process.

The quantitative differences in the total amount of HCH-P2 and HCH-P3 inhabiting the implants can be explained by a decreased cell proliferation rate due to higher passage number as reported by Kang et al. [13] and by a lower HCH-P2 concentration inside the collagen base with the growth rate following an exponential function.

A second measure for evaluating cell migration is the cell depth which is graphed in the middle in Fig. 7 and displays the distance between the tissue boundary and a stained vital cell. In dynamic HCH-P2 experiments, an increase in maximal cell depth has been observed in COL6 and CFl. We notice that chondrocytes are only inhabiting the bottom part of the implant (approximately 1 µm) and are not found throughout the entire sample height which would be possible if compared to a previously reported cellular velocity of 1 µm/h in less dense collagen gels [30] and assuming migration taking place in random directions. During each stimulation period, the collagen implants are compressed plastically, its total height is reduced and its diameter is increased. Hence, upwards migrated cells are pushed back towards the tissue boundary and the measure of cell depth is reduced. Furthermore, in unconfined compression tests, we observed water squishing out of the tissue. The collagen gel is compressed and the local concentration of collagen fibres is increased and a lateral alignment of the fibres may result, which has been reported in a previous study [34]. In clinical application, the lateral expansion of the implant is restricted by the surrounding tissue.

Inside the CFl sample the most promising results are achieved concerning cell migration and cell depth. Maximal depth of HCH-P2 cells is increased by 50% due to dynamic cultivation and the maximal depth of HCH-P3 cells is doubled. For COL6 and COL8 materials, no major differences in the maximal cell depth are revealed for HCH-P3, concluding that cell motility of chondrocytes of passage two and of passage three is similar in the here given three-dimensional environment. This relates to the findings by Hamilton et al. [11] that chondrocyte migration in a two-dimensional environment depends not only on the passage number, but also on the surface topography.

The percentage of vital cells is used as a control instance for the experiment, as insufficient quality in biocompatibility and sterility or a mechanical stimulus can result in cell death. In HCH-P2 tests, we find a high percentage of vital cells at a minimum of 80%. In HCH-P3, the average fraction of vital cells is lower and we observe an increase in the percentage of vital cells in dynamic culture.

In the frame of the study, we did not analyse the differences in microscopic material composition and structure such as permeability, fibre orientation or pore size. That is why we cannot relate differences in the colonisation process to microscopic material differences. In combination with numerical methods, relaxation characteristics can reveal diffusion properties of the hydrated collagen gels which can be associated to the potential of cells moving inside the tissue.

It must be noted that the number of cells inside the implants is not only dependent on the process of cell migration but also to cell proliferation. We observe two superimposed cellular processes which cannot be distinguished in the frame of this experimental setup. Additionally, the experimental setup does not allow for live-monitoring of the migration process, nor can we analyse the cell distribution at multiple times to assess which influence the pauses in dynamic cultivation have on migration and proliferation. Nonetheless, chondrocytes can only be found inside the implants if cells are motile and have migrated into the tissue and a distinction between the processes of proliferation and migration is not of importance for the therapeutic success of the cell-free implant.

4. Conclusions

Here, we have introduced an *in vitro* model to analyse chondrocyte migration from a three-dimensional donor tissue towards cell-free tissue-engineered cartilage substitutes of varying collagen concentrations. We have confirmed that chondrocytes are motile and alive inside a cartilage based tissue and that, under satisfactory contact conditions, chondrocytes are capable to migrate between adjacent tissues towards a cell-free collagen based implant. In dynamic culture conditions, with the application of a physiologically mechanical compression, cell population inside all three tested collagen scaffolds is increased. Most promising results concerning colonisation and cell depth are achieved for CFl implants. A mechanobiological process seems to play an important role in strain-rate insensitive materials, because the cell population and cell depth in dynamic cultivation is several times higher than in static culture conditions. We could not identify differences in the mechanical properties of strain-rate sensitive implants as a driving factor for differences in cell-population inside the implants.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Approval

Not required.

Declaration of Competing Interest

There are no competing interests to declare, no external fundings, and no ethical approval required.

References

[1] Alford JW, Cole BJ. Cartilage restoration, part 1: basic science, historical perspective, patient evaluation, and treatment options. Am J Sports Med 2005;33(2):295–306.
[2] Bolte S, Cordelieres FP. A guided tour into subcellular colocalization analysis in light microscopy. J Microsc 2006;224(3):213–32.
[3] Breil-Wirth A, von Engelhardt L, Lobner S, Jerosch J. Retrospektive untersuchung einer zellfreien matrix zur knorpeltherapie. OUP 2016;9:515–20.
[4] Chang C, Lauffenburger DA, Morales TI. Motile chondrocytes from newborn calf: migration properties and synthesis of collagen II. Osteoarthr Cartil 2003;11(8):603–12.
[5] Cheng HW, Luk KD, Cheung KM, Chan BP. In vitro generation of an osteochondral interface from mesenchymal stem cell-collagen microspheres. Biomaterials 2011;32(6):1526–35.
[6] Davies RL, Kuiper NJ. Regenerative medicine: a review of the evolution of autologous chondrocyte implantation (ACI) therapy. Bioengineering 2019;6(1):22.
[7] Darling EM, Athanasiou KA. Articular cartilage bioreactors and bioprocesses. Tissue Eng 2003;9(1):9–26.
[8] Darling EM, Athanasiou KA. Rapid phenotypic changes in passaged articular chondrocyte subpopulations. J Orthop Res 2005;23(2):425–32.
[9] Foldager CB, Gomoll AH, Lind M, Spector M. Cell seeding densities in autologous chondrocyte implantation techniques for cartilage repair. Cartilage 2012;3(2):108–17.
[10] Gaissmaier C, Fritz J, Mollenhauer J, Schneider U, Marlovits S, Anders J, et al. Verlauf klinisch symptomatischer knorpelschden des kniegelenks: ergebnisse ohne und mit biologischer rekonstruktion. Deutsches Rzteblatt 2003;100:2448–53.

[11] Hamilton DW, Riehle MO, Monaghan W, Curtis ASG. Articular chondrocyte passage number: influence on adhesion, migration, cytoskeletal organisation and phenotype in response to nano-and micro-metric topography. Cell Biol Int 2005;29(6):408–21.
[12] Hamilton DW, Riehle MO, Rappuoli R, Monaghan W, Barbucci R, Curtis ASG. The response of primary articular chondrocytes to micrometric surface topography and sulphated hyaluronic acid-based matrices. Cell Biol Int 2005;29(8):605–15.
[13] Kang SW, Yoo SP, Kim BS. Effect of chondrocyte passage number on histological aspects of tissue-engineered cartilage. Biomed Mater Eng 2007;17(5):269–76.
[14] Mauck RL, Soltz MA, Wang CC, Wong DD, Chao PHG, Valhmu WB, et al. Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. J Biomech Eng 2000;122(3):252–60.
[15] Mauck RL, Wang CB, Oswald ES, Ateshian GA, Hung CT. The role of cell seeding density and nutrient supply for articular cartilage tissue engineering with deformational loading. Osteoarthr Cartil 2003;11(12):879–90.
[16] Mauck RL, Yuan X, Tuan RS. Chondrogenic differentiation and functional maturation of bovine mesenchymal stem cells in long-term agarose culture. Osteoarthr Cartil 2006;14(2):179–89.
[17] Morales TI. Chondrocyte moves: clever strategies? Osteoarthr Cartil 2007;15(8):861–71.
[18] Nebelung S, Gavenis K, Rath B, Tingart M, Ladenburger A, Stoffel. Continuous cyclic compressive loading modulates biological and mechanical properties of collagen hydrogels seeded with human chondrocytes. Biorheology 2011;48(5–6):247–61.
[19] Neel EAA, Cheema U, Knowles JC, Brown RA, Nazhat SN. Use of multiple unconfined compression for control of collagen gel scaffold density and mechanical properties. Soft Matter 2006;2(11):986–92.
[20] Pabbruwe MB, Esfandiari E, Kafienah W, Tarlton JF, Hollander AP. Induction of cartilage integration by a chondrocyte/collagen-scaffold implant. Biomaterials 2009;30(26):4277–86.
[21] Roessler PP, Pfister B, Gesslein M, Figiel J, Heyse TJ, Colcuc C, et al. Short--term follow up after implantation of a cell-free collagen type i matrix for the treatment of large cartilage defects of the knee. Int Orthop 2015;39(12):2473–2479.
[22] Saris DBF, Dhert WJ, Verbout AJ. Joint homeostasis: the discrepancy between old and fresh defects in cartilage repair. J Bone Joint Surg Br Vol 2003;85(7):1067–76.
[23] Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T. Fiji: an open-source platform for biological-image analysis. Nat Methods 2012;9(7):676.
[24] Schneider U. Controlled, randomized multicenter study to compare compatibility and safety of ChondroFiller liquid (cell free 2-component collagen gel) with microfracturing of patients with focal cartilage defects of the knee joint. Vid J Orthop Surg 2016;1:1–8.
[25] Schneider U, Schmidt-Rohlfing B, Gavenis K, Maus U, Mueller-Rath R, Andereya S. A comparative study of 3 different cartilage repair techniques. Knee Surg Sports Traumatol Arthrosc 2011;19(12):2145–52.
[26] Schüettler KF, Struewer J, Rominger MB, Rexin P, Efe T. Repair of a chondral defect using a cell free scaffold in a young patient-a case report of successful scaffold transformation and colonisation. BMC Surg 2013;13(1):11.
[27] Schuurman W, Levett PA, Pot MW, van Weeren PR, Dhert WJ, Hutmacher DW, et al. Gelatin-methacrylamide hydrogels as potential biomaterials for fabrication of tissue-engineered cartilage constructs. Macromol Biosci 2013;13(5):551–61.
[28] Sellards RA, Nho SJ, Cole BJ. Chondral injuries. Curr Opin Rheumatol 2002;14(2):134–41.
[29] Sheehy EJ, Vinardell T, Buckley CT, Kelly DJ. Engineering osteochondral constructs through spatial regulation of endochondral ossification. Acta Biomater 2013;9(3):5484–92.
[30] Shimizu M, Minakuchi K, Kaji S, Koga J. Chondrocyte migration to fibronectin, type i collagen, and type II collagen. Cell Struct Funct 1997;22(3):309–15.
[31] Snyder TN, Madhavan K, Intrator M, Dregalla RC, Park D. A fibrin/hyaluronic acid hydrogel for the delivery of mesenchymal stem cells and potential for articular cartilage repair. J Biol Eng 2014;8(1):10.
[32] Stockwell RA. The interrelationship of cell density and cartilage thickness in mammalian articular cartilage. J Anat 1971;109(Pt 3):411.
[33] Stoffel M, Willenberg W, Azarnoosh M, Fuhrmann-Nelles N, Zhou B, Markert B. Towards bioreactor development with physiological motion control and its applications. Med Eng Phys 2017;39:106–11.
[34] Stoffel M, Yi JH, Weichert D, Zhou B, Nebelung S, Mller-Rath R, Gavenis K. Bioreactor cultivation and remodelling simulation for cartilage replacement material. Med Eng Phys 2012;34(1):56–63.
[35] Urban JPG. The chondrocyte: a cell under pressure. Rheumatology 1994;33(10):901–8.
[36] Yang J, Zhang YS, Yue K, Khademhosseini A. Cell-laden hydrogels for osteochondral and cartilage tissue engineering. Acta Biomater 2017;57:1–25.