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Towards bioreactor development with physiological motion control and its applications

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ABSTRACT

In biomedical applications bioreactors are used, which are able to apply mechanical loadings under cultivation conditions on biological tissues. However, complex mechanobiological evolutions, such as the dependency between mechanical properties and cell activity, depend strongly on the applied loading conditions. This requires correct physiological movements and loadings in bioreactors. The aim of the present study is to develop bioreactors, in which native and artificial biological tissues can be cultivated under physiological conditions in knee joints and spinal motion segments. However, in such complex systems, where motions with different degrees of freedom are applied to whole body parts, it is necessary to investigate elements of joints and spinal parts separately. Consequently, two further bioreactors for investigating tendons and cartilage specimens are proposed additionally. The study is complemented by experimental and numerical examples with emphasis on medical and engineering applications, such as biomechanical properties of cartilage replacement materials, injured tendons, and intervertebral discs.

1. Introduction

Bioreactors are important parts of any biomechanical and biomedical researches over the last several decades, since bioreactors could accelerate the studies of material properties during remodelling, degeneration and regeneration of biological soft tissues such as cartilage, intervertebral discs, and tendons under cultivation conditions. In studies of Waldman et al. [21] and Vunjak-Novakovic et al. [20] especially changes in material stiffness of cartilage were described after mechanical loadings in bioreactors. Moreover, a bioreactor with two degrees of freedom composed of combined compression and torsion loadings on spine motion segments was proposed by Illien-Jünger et al. [7]. Liu et al. [9] proposed a knee simulator without bioreactor environment for porcine knee joints to stimulate the native porcine cartilage with kinematics of human total knee replacements, scaled to porcine joint limits. In studies of Gao et al. [5], a knee joint bioreactor was proposed, which can subject compression and rolling motions to biological material. Sun et al. [19] described the necessity to apply physiological loadings to articular cartilage and proposed a bioreactor with compression and rolling movements. Combined compression and shear loadings for the cultivation of cartilage replacement specimens were presented by Yusoff et al. [25]. In studies of Grad et al. [6], the high potential of bioreactor systems is described, which lies in the development of bioreactors exhibiting physiological joint motions for optimising tissue replacements. For this purpose, Grad et al. [6] use bioreactors with spheres, which apply compression and rolling on specimens. Especially in [26], the authors note to foresee that bioreactors in future will mimic in vivo mechanical loadings by complex mechanism systems. Consequently, straightforwardly, we discuss bioreactor systems exhibiting more than one degree of freedom. The necessity for combined loadings with different frequencies and repose times in cartilage were studied by Panadero et al. [13] as well.

Numerical simulations as an evaluation method lead to the determination of material parameters and stress and strain distributions during the bioreactor cultivation. A comparison between calculated and measured results can lead to a validated numerical model, which offers the possibility to analyse e.g. stress peaks, damage, or remodelling inside the investigated tissue more precisely. In this way, injuries (Little et al. [8]) or mechanobiological couplings (Soukane et al. [14]) can be modelled. Due to the fact that combined tissue loading with compression and torsion seems to be an important criterion for creating the real physiological conditions, the development of these bioreactors is essential.

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The aim of the present study is the development of four different bioreactors with different functions. By using uniaxial tension and compression bioreactor systems for tendon and cartilage the material parameters such as Young's Modulus and Poisson's Ratio can be determined. Afterwards, in the multi-axial knee and intervertebral disc bioreactors, the experiments will be performed to study other complex material properties such as the regeneration and degeneration processes of native or artificial tissues under physiological realistic conditions.

Due to the complex mechanobiological evolutions in living tissues, it is worth to develop numerical models by means of the finite element method. This should support the understanding of loading histories, stress and strain distributions in knee joints and spine segments and, moreover, the remodelling effects.

2. Methods

This study focuses on the knee joint and on spinal segments. A knee joint bioreactor was developed, which mimics the physiologically correct loading conditions in a knee joint, while the intervertebral disc (IVD) bioreactor is developed to study the de- and regeneration behaviours of the IVDs under long-term cyclic compression and torsion. However, the mechanical analysis of superposed stress distributions in different directions together with geometrical and physical nonlinearities becomes very complex in these two bioreactors. Therefore, there is a need for investigating single element samples with simplified geometry in a special type of bioreactor, especially if the pure mechanical effects are overlaid with remodelling phenomena. Experiments in the compression bioreactor, or in the tensile bioreactor as well, can be used for determining material properties under simpler boundary and loading conditions as in the knee joint bioreactor.

2.1. The tensile bioreactor

The investigation of the mechanical behaviour of tendon tissues and tendon cells requires an appropriate in vitro cultivation environment. Fig. 1 shows the tensile bioreactor. A stepper motor is mounted, which drives a spindle being connected to the clamp device. The stepper motor works with the micro stepping technology and can perform 800 steps per revolution. The clamp device does not damage the clamped sample and also reliably prevents the sample from slipping out. Moreover, the clamping force is generated by the compression of a spring with a well defined length. Thus, in every experiment, the same clamping force is generated. All these described aspects of the clamping device are essential for reproducible experimental results. Hereby, the clamping device is limited to thin structures with a thickness of less than 3 mm. Despite this limitation, a wide range of different structures, such as whole tendons or cut specimens from the IVD, can be mounted in the bioreactor. A 9.81 N load cell (Althen AUMM-K1) is connected via a rod with a high tensile, but low shear strain resistance. Thus, the influence of lateral force on the load cells is minimised. Both, the load cell and the stepper motor are controlled and read out by a custom made printed circuit board (PCB).

The PCB itself is controlled by a custom made software tool with a graphical user interface (GUI), which is running on a personal computer. With the aid of the software tool, it is possible to prepare experiments, to start and stop arbitrary stimulation cycles (e. g. periodical stimulation with sinoidal displacement shapes and integrated pauses, relaxation tests with pauses). During the experiments the bioreactor is placed in an incubator. In Fig. 1, the bioreactor is shown with a bovine tendon. A validation of this bioreactor has been presented in [24].

One interesting application of the bioreactor is the mechanical investigation and characterisation of shear forces inside of collagen containing materials. This is assumed to be an important factor, for example for the generation of tendon diseases, see [2] and [11]. However, it can be assumed that they are also an important factor in other types of tissue, such as in the IVDs. In order to provide a method for the measurement of shear forces inside of soft tissues, a special protocol has been developed and tested with tendon tissue. Therefore, a specimen with a rectangular shape was mounted in the bioreactor (initial gauge length 30 mm, width 12.1 mm) and elongated by 4 mm within 4 s. These parameters were chosen, as a strain of about 13 % and a velocity of 1 mm/s are in the physiological range [22]. The same testing procedure was repeated with an introduced cut, see Fig. 1. A comparison between the measured reaction forces reveals the acting shear stresses and strains [24].

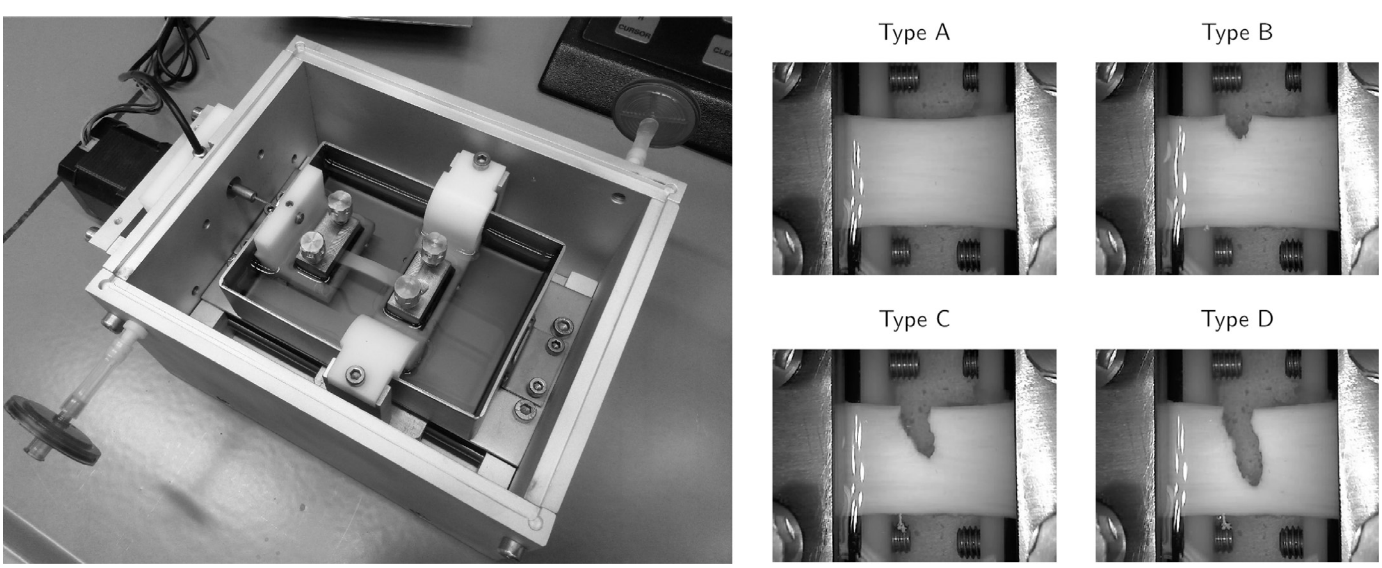

Fig. 1. Left: Tensile bioreactor with clamped sample. Right: Specimens made of tendon tissue. Due to the different lengths of the damages, the actual acting shear forces can be quantified.