Biomechanical Characterization of Cartilages by a Novel Approach of Blunt Impact Testing

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Running title

Blunt Impact Testing of Cartilage Biomechanical Properties

Summary

The present article introduces a novel method of characterizing the macromechanical cartilage properties based on dynamic testing. The proposed approach of instrumented impact testing shows the possibility of more detailed investigation of the acting dynamic forces and corresponding deformations within the wide range of strain rates and loads, including the unloading part of stress-strain curves and hysteresis loops. The presented results of the unconfined compression testing of both the native joint cartilage tissues and potential substitute materials outlined the opportunity to measure the dissipation energy and thus to identify the initial mechanical deterioration symptoms and to introduce a better definition of material damage. Based on the analysis of measured specimen

deformation, the intact and pathologically changed cartilage tissue can be distinguished and the differences revealed.

Keywords

Cartilage biomechanics • Impact testing • Poroelastic material • Impact energy dissipation • Tissue engineering

Introduction

Biomechanical properties of cartilages and other load-bearing materials are of prime importance as they form the musculoskeletal system, which enables and executes the body mechanics. They are primarily important in the science of implants and tissue engineering, as resemblance to the original tissue properties is required.

Articular cartilage is a material composed of a solid matrix and a fluid component (Garcia *et al.* 1998, McCutchen 1982, Stockwell 1979). From the mechanical point of view, the cartilage can be regarded as a so-called poroelastic material, involving viscous properties of interstitial fluid and elasticity of the matrix. Mechanical properties, viz. elasticity, strength and stiffness, are often used to characterize the physical nature of native cartilages. As the nature of cartilage tissue implies that the mechanical parameters are highly strain-rate-dependent, suitable mechanical characterization is rather complicated and it seems to be one of the principal limitations of broader application and implantation of tissue-engineered cartilage.

The key problems of a comprehensive evaluation of the cartilage mechanical parameters are the necessity of dynamic testing over a large range of strain rates, the requirement for correct interpretation of highly nonlinear material testing data but also only small volume specimens available for testing. Some of the standardized, commercially available, testing machines deal with specific features of biological specimens and can thus be used for compression as well as tension testing under certain restrictions. Limitations, unfortunately, are often very serious. Several laboratories have attempted to overcome these limitations at least partially (Duda *et al*. 2000, Grellmann *et al.* 2006, Chae *et al*. 2003, Musahl *et al*. 2003).

Recently, the cartilage mechanical properties have been mostly evaluated by both static and dynamic tests. Different strain rate (the "velocity of deformation") is usually understood as the principal difference between the static and dynamic measurement mode. In static testing the material's continuous adjustment to applied pressure is supposed, unlike in dynamic testing, when changes are too fast to adapt to. The level of the material's response is then different when different strain rates are used and hence the acquired results differ as well. For a large range of applied strain rates, a physiological basis could be found, but these tests will never cover the whole extent of physiologically possible loading of the joint cartilage. On the other hand, the so-called impact loading of joint cartilages is often a neglected feature of exceptional physiological meaning. Within the impact loading mode, applied force continuously decreases along with the strain rate (e.g. impact, jump etc.). Such a process, lasting only a few milliseconds, is common for joints and, thus, obviously important. The fact that the cartilage response to such stimulation is not sufficiently described yet can be partly attributed to the lack of adequate methods and commercially available equipment. The existing methods (Kerin *et al.* 1998, Repo and Finlay 1977) faced several limitations, such as impossibility of energy dissipation evaluation and defining of ultimate strength determination in the so-called drop-tower design. Therefore, the method for impact characterization of cartilage tissue using blunt impact is desired.

As both the native and tissue engineered cartilage material has a complex poroelastic and anisotropic structure, many attempts have been made to describe their basic mechanical properties by developing of biphasic mathematical models (Li *et al.* 1995, Mak 1986, Mow *et al.* 1980, Tan and Lim 2006, Wu and Herzog 2000). Description of both the native and artificial cartilages by only simple characteristic value (e.g. Young's modulus and Poisson's ratio) is, thus, definitely not satisfactory for adequate evaluation and comparison of their biomechanical properties and may result in the development of unsuitable implants. Any difference in mechanical properties between the implant and the surrounding original tissue would naturally hamper the implant-integration process and may lead to its rejection or disruption. As a more suitable and valuable characterization of nonlinear material there appears a stress-strain diagram. Other weak points include the comprehensive characterization of anisotropy of biological material (Donzelli *et al.* 1999, Wu and Herzog 2002) and the Poisson's ratio (Elliot *et al.* 2002, Jin and Lewis 2004), which are indispensable for numerical modeling.

Practical need of joint cartilage properties determination results from efforts for cartilage degenerative disease treatment. Osteoarthritis and rheumatoid arthritis are the most frequent joint diseases of different etiology but with a similar feature: progressive degradation of articular cartilage that leads to joint dysfunction. In both pathologies, erosion of the cartilage matrix is thought to be primarily due to increased synthesis and activation of proteinases involved in the degenerative process. Cartilage breakdown due to disease results in severe pain and disability. Except for the commonly performed conservative and operative therapy, a new approach has recently appeared, viz. implantation of autologous chondrocytes grown on a suitable scaffold support (Marlovits 2006).

Particular scaffold designed for chondrocyte cultivation has to meet several major requirements, such as biocompatibility, adequate degree of biodegredability and proper mechanical characteristics. Implantation of material that is too stiff will result in its emphasized load-bearing function and imbalance in pressure distribution inside the joint. This may lead to early implant destruction or even to severe joint structure deterioration. Implant structure that is too soft will most probably result in reduced chances for its integration in surrounding cartilage tissue, as the implant will be loaded nonphysiologically mostly sideways and not tangentially. All the mentioned situations will substantially worsen the after-surgery convalescence and rehabilitation or even cause complete implant rejection. To avoid such complications, proper biomechanical properties of engineered and implanted cartilage substitutes are essential.

Methods

The designed method of dynamic biomechanical property testing employs a novel approach, based on the examination of the drop-weight-impact sample deformation. A pendulum-like apparatus setup permits tracking of material response to a single impact. Rapid increase of acting force should resemble physiological joint cartilage loading. Sample deformation is read simultaneously by a piezoelectric accelerometer (Bruel & Kjaer Type 4375) and Laser Doppler Vibrometer LDV (Polytec OFV-302). The signal acquired by both the detectors is then collected by a preamplifier and computationally processed. This measurement setup permits effective acquisition of data with a high information yield.

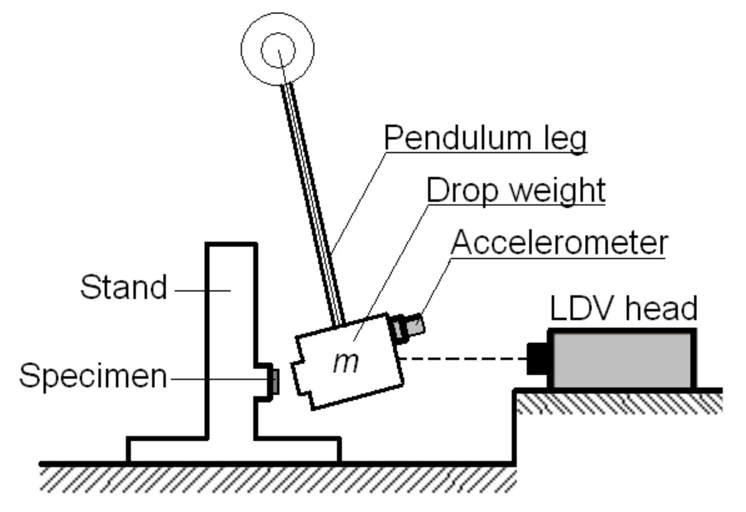

Fig. 1. Scheme of impact loading measurement setup.

Double time integration of the signal provided by the accelerometer (acceleration *a*) provides the value of actual sample deformation Δl:

$$\Delta l = \int_{t_1}^{t_2} (v(t) + v_0)dt \qquad (1)$$

where t_1 and t_2 are the times characterizing initiation and termination of the impact process, v is the actual deformation velocity and v_0 is the constant determined by LDV, specifying the critical point in the impacting object movement (change of acceleration to deceleration), while the actual velocity during the impact is evaluated as

$$v(t) = \int_{t_1}^{t_2} a(t)dt \tag{2}$$

Strain ε is then determined as the ratio of actual sample deformation Δl and initial sample thickness l_0. Strain is used due to unequal thickness of examined samples.

Acting forces as well as stresses can be evaluated simply by using Newton's force law

$$F = ma \tag{3}$$

where m is the mass of the impactor (m = 0.545 kg). Relationship of acting forces or stress vs. deformation can than be expressed by the so-called stress-strain diagram. Young's modulus is the slope of stress-strain curve in its linear part. As the cartilage material properties are non-linear, to describe the slope of all the regions of the curve a tangent modulus is defined for any point of the curve.

Laser vibrometer permits system calibration and measurement verification. The vibrometer signal serves as well for setting the integration boundaries in Eq. (1) and (2). It reveals stopping of the impacting mass (constant v_0) and its velocity v_1 at the initial and v_2 at the final point of the impact process.

For static compressive material testing MTC 858.2 Mini Bionix testing machine was used.

Biological material used in preliminary experiments came either from animal cadavers, leftovers from related *in vivo* experiments (pigs) or remaining tissue from routine human surgical cartilage transplantations. Artificial samples tested are examples of materials being developed or already used to replace the native cartilage tissue, viz. knitted chirlac fiber matrices and fibrin chondrograft.

The tested samples were not circumferentially constrained; they were loaded under unconfined compression.

As the examined samples showed slightly uneven surfaces, prior loading of 1 N was considered. This normalization approach was applied in the data-processing phase, resembling real physical preloading used in standard mechanical testing. It should partially resemble joint cartilage load at rest (Urban and Hall 1992).

Results

The loading diagram appears as a comprehensive description of material response to dynamic loading – it can either display stress-strain, force-strain or force-deformation relation.

To characterize individual native pig cartilage samples, 5 independent subsequent measurements were processed and the mean value of stress-strain curve was evaluated. Fig. 2 shows the resulting curves along with boundaries of 2 standard deviations intervals for single sample and 5 different samples. Only the ascending part of the diagram, which refers to sample compression during the loading process, is presented.

The derivative of the loading diagram represents the tangent (or differential Young's) modulus. As Young's modulus in general sense a derivative of initial, almost-linear part of the loading diagram can be considered. Even more valuable information about the inspected material is provided by maximum stiffness – slope of the stress-strain curve at the upper extremity.
Considering the tangent modulus as a slope of loading curve, its strain dependence can be drawn simply as a derivative of this function (stress-strain loading curve). Functional dependence of tangent modulus on the actual strain for one of the cartilage samples is shown as inset of Fig. 2 which thus depicts variance and reproducibility of the acquired results.

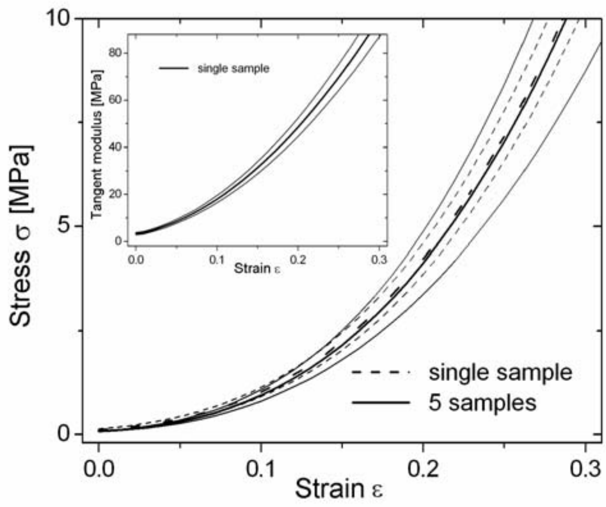

Fig. 2. Stress-strain curves of single cartilage sample and 5 different samples of native pig joint cartilage, mean values and 2 standard deviations confidence intervals. Inset: Tangent modulus nonlinear dependency on actual strain, mean value for single cartilage sample with 2 standard deviations interval.

One of the characteristic features of the instrumented impact testing based on a pendulum-type device is the possibility to observe the unloading part of the force-displacement relationship. Rebound of the impactor carries information on the material response to impact, important when analyzing the poroelastic parameters and dynamic mechanical performance. In Fig. 3 complete hysteresis curve examples are shown for intact hyaline cartilage tissue and the damaged one. The plotted records of single donor specimens illustrate the differences in shape and maximum strain values.

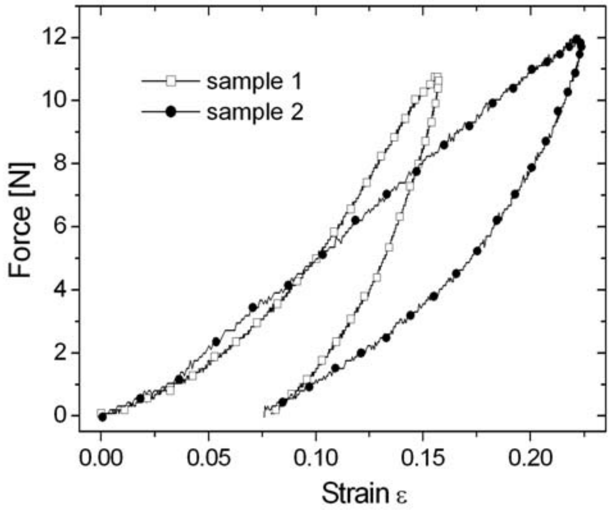

Fig. 3. Examples of loading curves for intact hyaline cartilage tissue from non-weight-bearing zone (sample 1) and cartilage from the defect zone (sample 2). Preload of 1N was considered.

Perhaps the most important value for mechanical performance is the survival characteristics of the cartilage. Structural failure of cartilage is closely associated with joint disorders, including osteoarthritis. An ordinary approach to define this limit is the ultimate stress and ultimate strain; if that is exceeded, the material will fail. It cannot be said that the material does not experience damage at stresses or strains below the ultimate values. Moreover, the ultimate values are not strictly constant; they are influenced also by the strain rate applied. Comparing the higher and lower strain rates of dynamic loading, in the latter case the boundary strain of damage is larger according to our experience. Another important factor is loading repetition rate. This effect is illustrated in Fig. 4. If the recurrence interval lasts seconds or even less the cartilage material is not able to restore its original properties due to incomplete reabsorption of the expelled interstitial fluid. Then, the cartilage matrix structure is not protected enough for repeated dynamic loads. In such a case the resistance vanishes step by step, even if the impact energy remains at the previous values. In Fig. 4 the impact velocity (i.e. also energy; see below) for the last three impacts was almost the same (Fig. 4, curves c, d, e), but the cartilage material gradually degraded from curve *c* through *d* to curve *e*. The time intervals between the impacts were 5–10 seconds at unconfined dynamic compression.

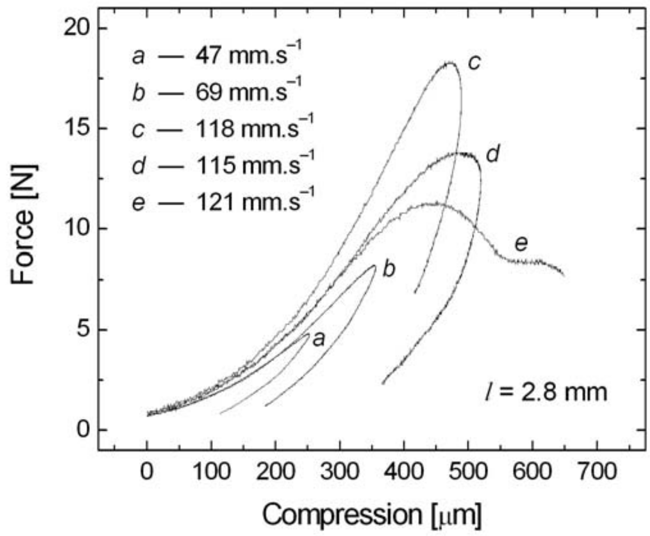

Fig. 4. Force-compression loading curves of single hyaline joint cartilage sample - consequent measurements *a* to *e* taken in intervals of 5–10 s. The initial impact velocities v_1 are denoted for each measurement. Uncompressed sample thickness *l* = 2.8 mm.

The ultimate compressive strength, understood as an acting force causing irreversible change of material, can still be assessed using the loading diagram. That would presume irreversible sample deformation by single impact. As the impact velocity (i.e. impact energy) can be gradually increased and material response monitored, boundary impulse causing irreversible deformation can be extrapolated.

As already mentioned, the reaction of poroelastic material to static, dynamic and impact loading differs substantially. An example of cartilage biomechanical characteristic comparison obtained by both the proposed impact dynamic method and static loading test using strain rates of 5 mm min^{-1} and 10 mm min^{-1} is shown in Fig. 5.

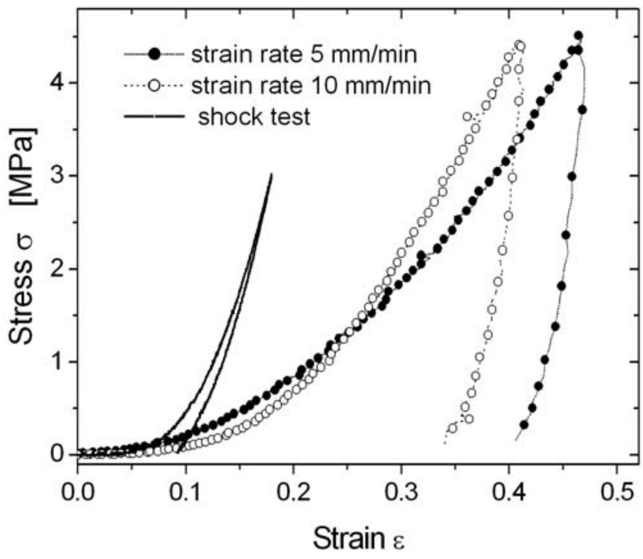

Fig. 5. Comparison of single pig joint cartilage sample stress-strain curves for static (strain rate 5 mm/min and 10 mm/min) and impact (shock test) loading.

The precise deformation velocity (loading mass movement velocity) evaluation makes it possible to interpret the energy balance. The overall energy of impact E_1 can be considered to be equal to kinetic energy of striking body just before (or at the very moment of) its contact with the sample

$$E_1 = \int_0^{l_{\max}} F_1(l)dl = \frac{1}{2} m v_1^{\;2} \tag{4}$$

where $l_{\max}$ is the maximum deformation of the sample, F_1 is the force acting during the sample compression and v_1 is the velocity of striking body at the moment of the first contact. The area under the ascending part of the loading diagram curve graphically represents the impact energy as defined by (4).

The mechanical energy lost within the process of deformation – dissipated energy ΔE, can then be evaluated as the difference between kinetic energy of the striking body at the very beginning (E_1) and very end (E_2) of the deformation process. This is graphically represented by the area under the loading curve - the hysteresis loop

$$\Delta E = E_1 - E_2 = \int_0^{l_{\max}} F_1(l)dl - \int_{l_{\max}}^{0} F_2(l)dl = \frac{1}{2} m (v_1^{\;2} - v_2^{\;2}) \tag{5}$$

where v_1 is again the initial striking body velocity, v_2 is the velocity of striking body just rebounded, F_1 is the force acting during the compressive part of the impact process – ascending curve, while F_2 is the force during the reaction (decompression) phase – descending curve.

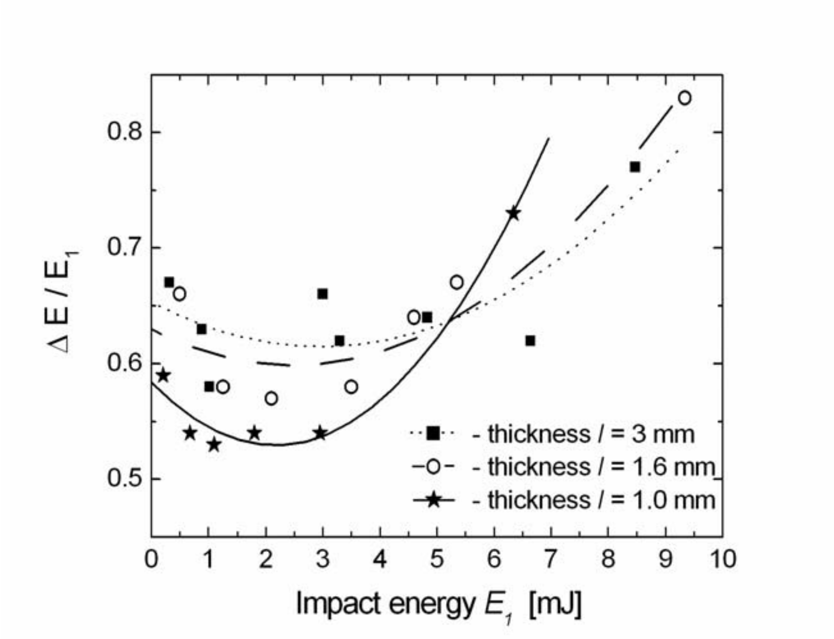

Fig. 6. Dissipation of the impact energy vs. overall impactor energy for three native cartilage specimens. Quadratic fit is shown for each sample data set. Thickness *l* of individual samples is denoted.

Knowing the material loading curve hysteresis, the relationship between the dissipation energy ΔE and the overall energy of impact E_1 can be plotted as shown in Fig. 6. For the sake of figure clarity only the measured values and deterministic quadratic fit are depicted. As seen, the ratio of dissipation energy to the impact energy $\Delta E/E_1$ remains nearly constant after small initial decrease when related to E_1. In this initial interval the cartilage structure does not show symptoms of mechanical deterioration. The increase of the value $\Delta E/E_1$ at the higher impact energies indicates that the material suffered a certain degree of mechanical structure deterioration. The thicker the cartilage sample, the smaller the differences in $\Delta E/E_1$ – a thicker sample appears more durable. This factor should be taken into account when the mechanical damage limits need to be defined.

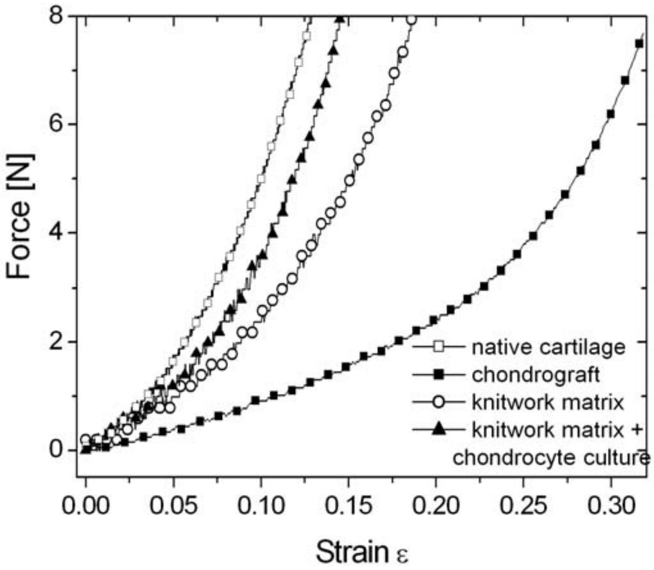

Fig. 7. Loading curves of native cartilage and some of the materials tested: native cartilage sample (the same as in Fig. 2), fibrin chondrograft used for autologous chondrocyte transplantations, knitted chirlac fiber matrix – blank damped in physiological solution and the same chondrocyte-seeded matrix after a ten-day cultivation.

Fig. 7 shows an example of native and tissue-engineered cartilage comparison. The presented graphs show mean force-deformation curves for native cartilage (same as sample 1 in Fig. 3), chondrograft used in standardized autologous chondrocyte transplantation surgery, knitted chirlac fiber matrix in two samples – once only damped in physiological solution and once with chondrocytes after ten days of cultivation. Our results prove insufficient material stiffness (excessive deformation at low acting forces) and higher maximum strain in chondrograft sample. Tested chirlac knitted matrix better resembles the course of the ascending part of the native cartilage loading curve.

Chondrocytes cultivated on artificial matrix will affect the overall mechanical properties of the material – by forming extracellular collagen network as well as by boosting up the biodegradable scaffold material degradation. Influence of chondrocyte culture can be examined using impact testing; an example is shown in Fig. 7 (knitted chirlac matrix with 10-day chondrocyte culture).

Discussion

Compared to many different experiments that have been performed to assess the mechanical properties of native hyaline cartilages *in vitro*, blunt impact response evaluation seems to be the most promising method to obtain comprehensive mechanical data. With regard to the distinct poroelastic and viscoelastic material properties of cartilages, the dynamic testing in the large extent of strain rates and acting forces has to be realized. The feasibility of simple laboratory realization, repeatability and sufficient accuracy (as demonstrated in Fig. 3) are the main features of this approach. Despite the non-ideal shapes and millimeter dimensions, the data were only minimally affected by the exact knowledge of specimen size. Still, the conventional problem of precisely defining the initial contact point of loading remains real. It can be overcome by choosing a certain level of “pre-loading” on the recorded force-deformation curves, following the idea of preload used in testing experiments as usual under static conditions.

Healthy joint cartilage response consists of nearly linear part characterizing elastic matrix, followed by steep non-linear part caused mainly by viscous fluid. In damaged cartilage diagram changes usually occur in favor of linearity (damaged porous structure, lack of fluid content), as seen in Fig. 2, or when an almost immediate deformation to certain extent occurs, followed by sudden diagram course change to viscous curve (loss of matrix elasticity). Similar drawbacks and limitations are typical of designed artificial tissue material. Generally speaking, while also the viscoelastic behavior of the material in dynamic load is visible, the interstitial fluid flow seems to be dominant for short-time response even for relatively small strain rates of 10 s^{-1} to 100 s^{-1}. The strong influence of the poroelasticity is confirmed also by the shape of stress-strain curves measured at different strain rates applied to the same specimen, as the initial ascending parts of these curves coincide to a large extent (see Fig. 4). It was also approved experimentally by the repeated loads of the same specimens that the native articular cartilage material has restored its initial mechanical performance by reabsorption of the expelled fluid within the time interval of several tens of seconds. This reversible process is repeated, until the compression stress reaches a definite level when irreversible defects are induced. Destructive processes do not appear suddenly, the native cartilage loses its bearing capability progressively (Fig. 4). This observation seems to have a crucial meaning for the rehabilitation process of patients.

The deterioration of the cartilage bearing capacity becomes evident also in the material harvested from the degraded zone (see Fig. 2). However, the partial loss of poroelastic properties involves a risk of larger compression deformation at dynamic loads, possibly resulting in structural integrity damage. Dissipated energy, understood here as part of the impact mechanical energy transformed to different energy form or so-called energy losses, is another important quantity characterizing the mechanical properties of the tested material. When there is no dissipated energy, we deal with ideal elastic material, while when all the energy appears to dissipate no real elasticity is observed. The extent of mechanical energy lost by dissipation also depends on strain rates and acting forces (as seen in Fig. 6). Dissipated energy thus appears crucial in cartilage biomechanics evaluation. Our findings coincide with the statements of Kerin *et al.* (1998) where also the enlargement of the hysteresis loop area was observed at higher levels of loading at nearly ultimate values.

For the testing with circumferentially unconstrained and unconfined specimens there is a characteristic feature of interstitial fluid escape, as was noted by several authors (McCutchen 1982, Kerin *et al.* 1998, Repo and Finlay 1977, Wu and Herzog 2000). Under these conditions (quasistatic compression), the unloading part of the loading curve sharply falls – that means no rebound of the impactor mass is present. Such a short-time irreversibility of the process is caused by a squeeze of the fluid from the material elastic matrix. The dynamic test curve is always shifted compared to that of static one. It has to be noted that the nonlinear shape of each loading curve results predominantly from large strains of unconfined compression, as the interstitial fluid flow within the tissue is sufficient for its escape from the material structure at low loading rate. The poroelasticity of tissue, which is due to viscous fluid flow inside the material, is reflected mainly in the hysteresis curve shift with respect to strain scale. Hysteresis loop of the quasistatic test curve (Vrána *et al.* 2003) is then wider and, consequently, its area is larger than that of the dynamic test curve (see Fig. 5). The loop gets even narrower as the strain rate increases, and the area under the shock test curve tends to be smaller, as was proved by impact experiments. From this it emerges that energy losses in the case of articular cartilage tissue impact loading are smaller in comparison with the low loading rates. It implies that the material reacts to impact loads in a more elastic manner and its compression deformation changes are to some extent reversible.

The main goals of the study were to evaluate the strain-rate-dependent features, such as development of compressive stress during dynamic loading as well as unloading hysteresis, characterization of the interstitial fluid flow function and the definition of initial mechanical damage. As was outlined by the present results, intact and pathologically changed cartilage tissue can be distinguished and revealed the differences described. It should be possible to attribute individual changes in mechanical behavior of the material to specific defects. Different materials can be compared with respect to the course of the loading curve or its derivative – usually in ther initial or culminating part. Dissipated energy appears to be another key characteristic, which is achievable from the loading diagram.

All of the above-mentioned features, yielded by the introduced impact testing method, should serve mainly for native cartilage tissue characterization (in combination with so far available methods) and subsequent artificial tissue quality evaluation. As our results show a suitable scaffold with adequate biomechanical properties, seeded with chondrocytes, seems to be a crucial step in the production of artificial cartilage.

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